The study of oral manifestations, oxidative stress marker and antioxidants in serum and saliva of rheumatoid arthritis patients

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a heterogeneous disease with a spectrum of clinical severity ranging from mild arthritis to a crippling joint disorder with internal organ involvement. Besides the immunological reaction, there is another biological process, based on the injurious activity of free radicals, playing a major role in the pathogenesis; an increase in the generation of oxidants and lipid peroxidation products was demonstrated in the serum of RA patients, which correlated with the antioxidant levels.

Subjects, Materials and Methods: Seventy six individuals were enrolled in this study; Fifty one of them were patients having RA; twenty five were healthy control individuals matching the patients in age and sex. Serum and saliva samples have been taken from each subject for biochemical analysis.

Results: The highest number of RA patients was of mild disease activity, while the lowest number was of inactive disease activity. Xerostomia was the most prominent oral manifestations of RA patients. The mean of serum and saliva MDA and UA in RA patients was significantly higher than of the healthy controls. The mean of serum and saliva caeruloplasmin in RA patients was higher than that of the healthy controls.

Conclusion: Rheumatoid arthritis patients were associated with increased oxidative stress, antioxidants and inflammatory markers. Patients with RA have different oral manifestations xerostomia were the most prominent. The highest number of RA patients was of mild disease activity.

Key words: rheumatoid arthritis, oxidative stress, DAS, saliva flow rate. (J Bagh Coll Dentistry 2011;23(3): 74-79)

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The process produces an inflammatory response of the synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints (1). Rheumatoid arthritis is a form of autoimmunity, the causes of which are still incompletely known. It is a systemic (whole body) disorder principally affecting synovial tissues (2). Rheumatoid arthritis affects women three times more often than men, and it can first develop at any age. The risk of first developing the disease (the disease incidence) appears to be greatest for women between 40 and 50 years of age, and for men somewhat later (3).

The most important diagnostic criteria for RA patients are morning stiffness, arthritis and soft tissue swelling (4). There is no known cure for rheumatoid arthritis, but many different types of treatment can alleviate symptoms and/or modify the disease process.

Pharmacological treatment of RA can be divided into disease-modifying antirheumatic drugs (DMARD), Cortisone therapy, biological agents and anti-inflammatory agents and analgesics. Treatment also includes rest and physical activity (5). The course of the disease varies greatly. Some people have mild short-term symptoms, but in most the disease is progressive for life. Around 20%-30% will have subcutaneous nodules (known as rheumatoid nodules); this is associated with a poor prognosis (6). Oral manifestations of RA patients include TMJ disorders, xerostomia and Sjogren's syndroms. Also RA patients have oral manifestations as a side effect of treatment includes: Aphthous stomatitis, Glossitis, Oral ulceration, Lichenoid eruptions, oral pigmentation, Angular Cheilitis and Candidal infection (7).

Oxidative stress represents an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents (8). Oxidative stress is thought to contribute to the development of a
wide range of diseases including rheumatoid arthritis, it is unclear if oxidants trigger the disease, or if they are produced as a secondary consequence of the disease and from general tissue damage. In the case of rheumatoid arthritis, rheumatoid factor binds IgG when it is exposed to free radicals. This binding stimulates the production of more free radicals which then attack the cartilage matrix (9).

MATERIALS AND METHODS
Seventy six subjects were enrolled in this study, they were divided into 2 groups:- Fifty-one patients were diagnosed clinically, by rheumatologist specialist as rheumatoid arthritis depending on the seven criteria of the American Rheumatism Association (ARA) that are mentioned before, their ages ranged between 17-65 years, they were 12 males and 39 females. Rheumatoid arthritis patients with other systemic diseases such as diabetes mellitus, hypertension or other cardiovascular disease should be excluded; also patients with periodontitis (because periodontitis may increase the saliva oxidative stress) were excluded. Rheumatoid arthritis patients were subdivided into subgroups:-a- Newly diagnosed patients. The duration of the disease was less than 6 weeks. (Patients without any type of treatment; they were 30 patients).b- Old diagnosed patients (patients mainly on methotrexate treatment the dose was 1cc/week; they were 21 patients).

Twenty-five individuals were age and sex matched healthy looking volunteers with no signs and symptoms of any systemic disease and with no periodontitis. Including 9 males and 16 females, their age ranges between (20-60) years.

Each patient was examined by rheumatologist and each tender and or swollen joint was calculated according to the CDAI. According to this index the patients were subdivided to inactive or remission if the CDAI < 2.8, mild or low disease activity if the CDAI was 2.8-10, moderate disease activity if the CDAI was 10-22, and sever disease activity if the CDAI was more than 22. Intraoral examination was done for each individual using sterile dental mirrors, sterile dental probes and artificial light. Examination of oral mucosal changes: - The examination was begun with the lips proceeding to the right buccal mucosa, including the upper and lower sulcus, retro molar area, the upper and lower labial mucosa, the left buccal mucosa, the palatal mucosa and the surface, margins of the tongue with the inferior surface of the tongue and the floor of the mouth were examined. All clinically evident mucosal alteration (redness, swelling, ulcer …etc) were determined and recorded, to find any oral manifestations. Examination of the temperomandibular joint also done and all clinically evident changes (clicking, limitation, dislocation…etc) were determined and recorded. About 6 ml of venous blood sample were aspirated from anticubital vein of each individual, using plastic disposable syringes with 21 gauge stainless steel needle; 2ml of blood was immediately transferred to a tube containing 0.4 ml sodium citrate. The remaining blood was transferred to polyethylene plain tube. Serum was obtained by centrifugation at 3000 rpm for 10 minutes, transferred immediately into another tube and frozen at (-20°C) for subsequent analysis. Haemolyzed samples were discarded.

Unstimulated (resting) whole saliva was collected after an individual was asked to rinse his mouth thoroughly with water to allow removal of debris. The first mouth –full of saliva was discarded to allow clearance of water. Then they were asked to spit all the saliva during 10 minutes into plastic graduated tubes, saliva flow rate was immediately calculated as the volume of saliva in ml divided by the time in minutes required for collection of saliva.

\[
\text{Saliva flow rate (ml/min)} = \frac{\text{Volume (ml)}}{\text{Time (min)}}
\]

The collected saliva was centrifuged at 3000 rpm for 10 minutes; the clear supernatants were separated and stored frozen at about (-20°C) until assayed.

RESULTS
Age and Gender: Fifty one patients with rheumatoid arthritis (RA) were incorporated in this study, 12 patients (23.5%) were males and 39 patients (76.5%) were females. Twenty five healthy control subjects were included in this study, they were 9 (36.0%) males and 16 (64.0%) were females. The mean age of healthy controls was 36.4 ± 11.8 years (table 1)

CDAI: According to the CDAI the RA patients were classified into inactive (remission), mild (low disease activity), and moderate disease activity and sever disease activity depending on number of swelling and tender joints.

Table (2) showed that the highest number 18 (35.3%) of RA patients were of mild disease activity, while the lowest number 5 (9.8%) of RA patients were of inactive (remission) disease activity.
Saliva flow rate: The results showed that the salivary flow rate in healthy controls was significantly higher (0.8 ml/min) than that in RA patients (0.4 ml/min) ($P < 0.001$) as shown in figure 1.

**Oral manifestations and TMJ disorders:** Each patient was examined extraorally and intraorally, the results revealed that the main oral manifestations of RA patients were angular chilitis and candidal infection, TMJ disorder, oral ulceration, and xerostomia. Xerostomia was the most prominent oral manifestations of RA patients which was found in 25 (49%) patients followed by TMJ disorders in 23 (45%) patients, then oral ulceration and angular cheilitis and candidal infection which was found in 15 (29.4%) patients. (Figure 2).

Oxidative stress marker Malondialdehyde (MDA): The mean of serum MDA in RA patients (5.6 µmol/L) was significantly higher ($P < 0.001$) than that of the healthy controls (2.7 µmol/L), also the mean of saliva MDA in RA patients (4.8 µmol/L) was significantly higher ($P < 0.001$) than that of the healthy controls (1.8 µmol/L). (Figure 3).

Caeruloplasmin (CP): The mean of serum caeruloplasmin in RA patients (0.3 g/mL) was significantly higher ($P < 0.001$) than that of the serum healthy controls (0.1 g/mL). Although the mean of saliva caeruloplasmin in RA patients (0.06 g/mL) was higher than that of the healthy controls (0.05 g/mL) (Figure 4), but it didn’t reach the significant level ($P > 0.05$), so no significant differences was found in saliva caeruloplasmin between RA patients and healthy controls.

Uric acid: The results showed that the mean of serum uric acid in RA patients was (6.1 mg/dl) which was significantly higher ($P < 0.001$) than that of the healthy controls (3 mg/dl), it has been shown that the mean of saliva uric acid in RA patients (5 mg/dl) was also significantly higher ($P < 0.001$) than that of the healthy controls (1.9 mg/dl) (figure 5).

**DISCUSSION**

Age and Gender: In the present study the mean age of RA patients was $40.4 \pm 14.9$ years and the age range was 17-65 years. The number of female RA patients was higher than male RA patients, $76.5\%$ of RA patients were females and $23.5\%$ were males which were agreed with other studies (10-11). Rheumatoid arthritis is a chronic, progressive multisystemic inflammatory disorder with a prevalence of approximately 0.5-1%. It usually involves middle aged adults with a females being affected more than males (12).

CDAI: The results showed that the number of RA patients with mild disease activity according to the CDAI was significantly higher. Joint damage begins early in the course of the disease as a consequence of the active inflammation, and can lead to progressive and irreversible disability. To allow physicians to evaluate the indication and effect of particular therapies, accurate assessment of disease activity is necessary. Some of these (and additional) measures are used in composite indices to assess disease activity or a disease activity state at any point in time and can inform the physician (and patient) about improvement (or deterioration) in disease activity from or states at a particular level at baseline, to that seen at any specific time point. The accurate assessment of disease is, therefore, an important part of the care of patients with RA (13).

Salivary flow rate: It has been found that saliva flow rate was significantly higher in the healthy controls than in the RA patients. The reduction in salivary gland function as measured by saliva flow rate in the RA patients result from that the salivary glands are major target organs of RA.

**Oral manifestations:** Xerostomia was the important oral manifestations of the RA patients which was present in 49% of those patients. It was shown as a decrease in saliva flow rate. The results of the present study also showed that the percentage of RA patients with xerostomia was higher in the newly diagnosed patients than in the old diagnosed patients this is may be due to the effect and benefit of methotrexate treatment in those patients. Angular chilitis and candidal

**Table 2: Distribution of RA patients accord to clinical disease activity index.**

<table>
<thead>
<tr>
<th>Disease Activity</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive (remission)</td>
<td>5</td>
<td>9.8</td>
</tr>
<tr>
<td>Mild (low disease activity)</td>
<td>18</td>
<td>35.3</td>
</tr>
<tr>
<td>Moderate disease activity</td>
<td>15</td>
<td>29.4</td>
</tr>
<tr>
<td>Sever disease activity</td>
<td>13</td>
<td>25.5</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 1: Distribution of the study samples according to gender and age.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Healthy Control</th>
<th>Rheumatoid arthritis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age in years</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>20-60</td>
</tr>
<tr>
<td>Mean</td>
<td>36.4</td>
</tr>
<tr>
<td>S.D.</td>
<td>11.8</td>
</tr>
</tbody>
</table>

**Table 2: Distribution of the study samples according to gender and age.**

**Table 3: Distribution of the study samples according to age range.**

<table>
<thead>
<tr>
<th>Age Range</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-60</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>61-65</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

**Table 4: Distribution of the study samples according to age range.**

<table>
<thead>
<tr>
<th>Age Range</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-60</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>61-65</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

**Table 5: Distribution of the study samples according to age range.**

**Table 6: Distribution of the study samples according to age range.**
Infection was found in 29.4% of RA patients and it was found to be higher in old diagnosed patients (on methotrexate treatment) which were found in 30% of those patients. Patients with rheumatoid arthritis (RA) have been shown to have an increased susceptibility to the development of infections. The exact causes of this increased risk are unknown, but may relate to immunologic disturbances associated with the disease or to the immunosuppressive effects of agents used in its treatment. Oral candidiasis that are presented in RA patients on methotrexate may be due to the use of immunosuppressive drugs or to lower salivary flow rates that usually affect RA patients. Oral ulceration was also found in 29.4% of RA patients and was higher in the old diagnosed RA patients (patients on MTX treatment) which were found in 33.3%.

Serum and saliva MDA: In the presented study it has been shown that serum and saliva MDA were significantly higher in RA patients than in the healthy controls, this result was agreed with the results of other studies (10-14).

Serum and saliva Caeruloplasmin: CP is a highly significant event in the course of rheumatic disease such as rheumatoid arthritis, ankylosing spondylitis and systemic sclerosis. There is a significant correlation between serum levels of CP and disease activity in RA patients.

Serum and saliva uric acid: It has been shown that serum and saliva UA were significantly increased in RA patient than in the control subjects (10).

REFERENCES

![Figure 1: Saliva flow rate in healthy control and RA patients.](image)
The study of oral manifestations

Figure 2: Oral manifestations and TMJ disorders

Figure 3: The mean of serum and saliva MDA in healthy controls and RA patients.

Figure 4: The mean of serum and saliva ceruloplasmin in healthy control and RA patients
Figure 5: The mean of serum and saliva uric acid in healthy control and RA patients