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Research Article

Saliva: Can it be a Supportive Marker for Oxidative Stress among Rheumatoid Arthritis Patients?

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Abstract

Objective: The aim of the present study is to assess the diagnostic capacity of saliva in measuring oxidants and antioxidants levels in Rheumatoid Arthritis (RA) patients there by evaluating the role of oxidative stress in the pathogenesis of RA and its comparison with healthy controls.

Material and Methods: 30 Rheumatoid factor positive arthritis patients were compared with the 30 age and gender matched healthy controls. Unstimulated salivary flow rate was assessed by spitting method in the morning between 9 am and 12 noon. Saliva was centrifuged and analyzed for Malondialdehyde (MDA), Uric acid (UA) and Total Antioxidant Status (TAS).

Results: The mean salivary flow rate was 0.28 + 0.08 ml/min and 0.44 + 0.09 ml/min, MDA levels were 5.67 + 1.12 nm/ml and 3.67 + 0.65nm/ml, UA levels were 2.82 + 0.48 mg/dl and 4.32 + 0.42 mg/dl and TAS levels were 3.14 + 0.20 mmol/lit and 0.52 + 0.17 mmol/lit in RA patients and controls respectively. All these levels were statistically significant between the RA patients and controls ($p<0.001$).

Conclusion: The present study showed free radicals plays an important role in the pathogenesis of Rheumatoid Arthritis. Salivary analysis is helpful for the diagnosis of the oxidative stress levels and to monitor the disease which is easy, non-invasive, cheaper and patient-friendly rather than going for invasive plasma analysis.

Keywords: Rheumatoid arthritis; Saliva;Malondialdehyde; Total antioxidants; Uric acid

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints in which small diarthrodial joints of the hands and feet were commonly affected; accounting for approximately 1.0% of world population with 3 times higher in women than in men [1-3]. The pathogenesis of RA is characterized by prolonged inflammation of the synovial tissue accompanied by morphological alterations and the recruitment of mononuclear and polymorphonuclear cells into the synovial fluid [4].

Although RA is considered as one of the entities of autoimmune disorders, the causes of which are still incompletely known.[1] In recent years, increasing experimental and clinical data has provided compelling evidences for the involvement of free radicals/reactive oxygen species in large number of pathophysiological states including RA [5]. Several pathways can lead to increased formation of reactive oxygen metabolites in inflamed joints. This enhanced oxidative stress may play a significant role in the tissue-damaging and inflammation perpetuating process in rheumatoid synovium [6].

Activated oxygen intermediates together with highly reactive radicals, such as the hydroxyl radicals are able to destroy membrane lipids, proteins, deoxyribonucleic acid, hyaluronic acid, and cartilage. The intermediate products of lipid peroxidation such as malondialdehyde (MDA), serves as an index of lipid peroxidation among RA patients [7]. The harmful effect of reactive oxygen species is neutralized by a broad class of protective agents termed antioxidants which reacts with the free radicals thereby minimizing the oxidative damage. They include enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and non-enzymatic antioxidants like beta-carotene, alpha-tocopherol, ascorbic acid, glutathione, ceruloplasmin, albumin, bilirubin, ferritin, transferrin, uric acid and lactoferrin [2,5].

The ability to evaluate physiological conditions, trace disease progression, and monitor post- treatment therapeutic effects through a noninvasive method is one of the primary objectives in the field of healthcare research. Saliva, a multi-constituent oral fluid has considerable potential for the surveillance of general health and disease. The reason why saliva can potentially be used as a specimen for diagnosis is because of its exchange with substances existing in human serum. One of the principal advantages of using saliva as a diagnostic fluid is that its sampling is easy and noninvasive thus eliminating any discomfort and pain associated with the blood collection [8].

Saliva is also rich in antioxidants such as uric acid, albumin, ascorbic acid, glutathione and various enzymatic antioxidants. Uric acid appears to be the dominant antioxidant present in saliva. Total Antioxidant Status includes all salivary antioxidants and presents clinical significance in the evaluation of the antioxidant status of

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saliva under normal and pathological situations. Thus saliva may replace plasma for analysis of antioxidants, which in turn may be of importance in monitoring the severity of the condition and prognosis [9].

The aim and objectives of the present study is:

1. To assess the diagnostic capacity of saliva in measuring oxidants and antioxidants levels in RA patients.
2. To discern the role of oxidative stress in the pathogenesis of RA.
3. Assessment of oxidants and antioxidants levels in saliva of Rheumatoid Arthritis patients and its comparison with healthy controls.

Materials and Methods

Study centre

RA patients were recruited from the Department of Orthopedics, Bapuji Hospital and Chigateri General Hospital, Davangere and controls were recruited from the outpatient Department of Oral Medicine and Radiology, College of Dental Sciences, Davangere during the period June – December 2013, after obtaining written informed consent. The necessary Ethical Committee clearance for the study was obtained from the Institutional Review Board of College of Dental Sciences, Davangere. All the biochemical analysis was carried out in Department of Biochemistry, JJM Medical College, Davangere.

Inclusion and exclusion criteria

Subjects of either sex were included in the study with the inclusion criteria of RA factor positive with the age above 18 years. 50 patients were screened for rheumatoid factor, out of which 30 patients who met the inclusion criteria were included. Thirty subjects who were age and gender matched, free of any systemic disease and not on any medication considered as controls.

Examination protocol

All the subjects underwent routine general clinical examination which included demographic variables: name, age, sex, occupation, address, any adverse habits like cigarette smoking, gutkha chewing, alcohol consumption and any relevant medical history. Intraoral examination was carried out to rule out presence of any mucosal lesions.

Severity of rheumatoid arthritis

The degree of severity of RA was determined by Health Assessment Questionnaire (HAQ) and classified the rheumatoid arthritis subjects into mild, moderate and severe categories [10]. The duration of rheumatoid arthritis and history of medication was recorded.

Saliva collection

Unstimulated whole saliva was collected by spitting method in accordance with Navazesh et al. [11] in the morning between 9 am and 12 noon. Subjects were asked to refrain from eating, drinking and smoking for at least 90 minutes before the procedure. The patient was asked to sit in an upright position with the head tilted downwards and saliva was allowed to accumulate in the floor of the mouth in closed lip position. Patient was asked to spit in a pre weighed container usually every 60 seconds for 10 minutes and then the container was again weighed. The difference between pre weight and post weight to time period of collection will give salivary flow rate. The flow rate will be calculated in gm/min, which is almost equivalent to ml/min.

Biochemical investigations

Collected saliva was centrifuged at 1000 rpm for 10 minutesand supernatant was analyzed for the Malondialdehyde (MDA), Uric acid (UA) and Total Antioxidant Status (TAS). Salivary levels of Malondialdehyde was assessed by method developed by Nadiger et.al. [12] in which auto oxidation of unsaturated fatty acidlead to the formation of semistable peroxides, which then undergo a series of reactions to form short chain aldehydes like Malondialdehyde. The method is consisted of treating salivary Malondialdehyde with Thiobarbituric acid to form pink color and the intensity of color was measured spectrophotometrically at 530 nm.

Salivary concentration of Uric Acid was measured with a kit supplied by the Lab-Care Diagnostics (INDIA) by means of enzymatic calorimetric method in which the uric acid is converted by uricase to allantoin and the hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to from a red-violet quinoeimine compound and the color intensity is read at the wavelength at 505 nm by spectrophotometer and the intensity of color is proportional to the concentration of uric acid.

Salivary Total Antioxidant Status was determined by estimating the capacity of saliva to inhibit the production of thiobarbituric acid derivative substance from sodium benzoate under the influence of free radical derived from Fenton reaction. The reaction will be measured spectrophotometrically at the wavelength of 532nm [13].

Statistical Analysis

SPSS statistical software version 16.0 was used for analysis. Unpaired't-test' was used to compare the means between cases and controls. Results were presented as mean + SD. Pvalue of <0.05 was considered as significant.

Results

Patients characteristics

This study comprised of 60 subjects: 30 subjects were known RA patients and 30 subjects were age and gender matched controls. The mean age of the RA patients was 43.2 + 7.36 years where as in controls it was 43.1 + 7.3 years. The number of males in each group was 3 and females were 27. The mean duration of RA was 32 months. Among 30 subjects, 26 subjects were on treatment for RA at the time of enrollment in our study. RA patients were on the drugs like methotextrate7.5 mg once weekly andAceclofenac100mg + Paracetamol 650 mg twice daily. Five among 30 subjects had the habits of smoking, gutkha chewing and pan chewing and controls were free of any adverse habits. Subjects in either group were free of mucosal lesions.

Classification of RA subjects based on the HAQ questionnaire

The patients were divided into three groups according to well established HAQ criteria: mildly affected accounted for 37 % (n=11), moderately affected 26 % (n=8) and severely affected 37 % (n=11).

Table 1: Salivary Parameters between RA patients and controls:

Parameter	RA patients	Controls	p value
Salivary Flow Rate (ml/min)	0.28 ± 0.08	0.44 ± 0.09	<0.001**
MDA (nm/ml)	5.67 ± 1.12	3.67 ± 0.65	<0.001**
Uric Acid (mg/dl)	2.82 ± 0.48	4.32 ± 0.42	<0.001**
TAS (mmol/lit)	3.14 ± 0.20	0.52 ± 0.17	<0.001**

** Highly significant. ml/min – milliliter per minute, nm/ml- nanomoles per milliliter, mg/dl – milligram per deciliter, mmol/lit – millmoles per liter.

Sialometry

The mean salivary flow rate in RA patients was 0.28 + 0.08 ml/min where as in controls was 0.44 + 0.09 ml/min ($p<0.001$). The mean salivary flow rate in mildly, moderate and severely affected RA groups was 0.32 ml/min, 0.29ml/min and 0.22ml/min respectively.

Sialochemistry

The mean salivary MDA level in RA patients was 5.67 + 1.12 nm/ml where as in controls it was 3.67 + 0.65nm/ml ($p<0.001$). The mean salivary MDA levels in mildly, moderate and severely affected RA groups was 4.79nm/ml, 5.77nm/ml and 6.35nm/ml respectively. The mean salivary UA level was 2.82 + 0.48 mg/dl and 4.32 + 0.42 mg/dl in RA patients and controls respectively ($p<0.001$). The mean salivary UA level in mildly, moderate and severely affected RA groups was 2.91 mg/dl, 2.89mg/dl and2.62 mg/dl respectively. The mean salivary TAS level was 3.14 + 0.20 mmol/lit and 0.52 + 0.17 mmol/lit in RA patients and controls respectively ($p<0.001$). The mean salivary TAS level in mildly, moderate and severely affected RA groups was 3.30 mmol/lit, 3.09 mmol/lit and 2.98 mmol/lit respectively.

Table1 shows Salivary flow rate, MDA, UA and TAS levels in RA patients and controls.

Discussion

The present study revealed mean age of occurrence of RA was 43.2 years and 90 % of RA patients were females which coincides with the fact that predilection of RA to middle aged females.

Salivary flow rate was significantly lower in RA patients than in controls which was similar to the results of Hadi et al. [1] and Nagler et al. [4],which imply that the salivary glands are the major target organs of RA. Morphological studies revealed that the minor salivary glands of RA patients were highly infiltrated with lymphocytes, B cells predominating over T cells and with a higher ratio of helper T cells to suppressor T cells. Alterations like fibrosis, acinar atrophy and lymphoplasma cell sialadenitis which leads to decreased salivary flow [14,15].

Inflammatory disorders like RA will generate the free radicals in the course of the disease and several pathways will generate reactive oxygen metabolites in inflamed joints. These reactive oxygen metabolites constitute the oxidative stress which enhances the tissue damage process in already inflamed joints. MDA being the product of the lipid peroxidation, by measuring these levels, we can measure the levels of oxidative stress indirectly in an individual. Our study results showed the increased salivary levels of MDA than in healthy controls which were similar to the results of Hadi et al. [1] and Nagler et al. [4]. which signifies that oxidative stress plays a definitive role in the pathogenesis of RA.

Salivary Uric Acid levels were significantly lower in RA patients compared to that of the controls which was in contrast to the results of Hadi et al. [1] and Nagler et al. [4]. The previous studies showed increase in the levels of UA in RA patients, which infers that salivary glands response to RA is by up-regulation of the production of specific salivary antioxidants [4]. In contrast to the previous studies, our study showed lower UA levels in RA patients, the reason might be that active secretion system for salivary uric acid rather than passive diffusion from the circulation. Uric acid is the principal salivary antioxidant accounting for almost 70 % of total antioxidants present in saliva which would have been consumed earlier by free radicals rather than other antioxidants. It can also be attributed to difference in the dietary habits among the study population and most of the patients were on treatment which might show altered uric acid levels.

Salivary TAS was significantly higher in the RA patients compared to the controls which were similar to the results of Hadi et al. [1] and Nagler et al. [4]. This suggests that body is trying to cope up the oxidative stress. This finding may be of paramount importance in the light of the presumably major importance of the salivary antioxidant capacity in fighting various pathologies in the oral cavity.

As the severity of the disease increases, the free radical generation is more due to the active pathological process, thereby causing more damage to the individual, which was evident in our study that severely affected RA patients showed more levels of MDA levels than mildly and moderately affected individuals. In the similar manner, body's own defense system is trying to defend these harmful effects of oxidative stress by consuming the antioxidants to maintain the normalcy of the individual. The present study also showed a similar pattern i.e., antioxidants levels were lower in severely affected RA patients rather than in mildly or moderately affected individuals.

Our study results were compared to the previous studies where serum was used for analysis of oxidative stress levels in RA. Usage of saliva as a diagnostic fluid minimizes the patient apprehension and increases the patient compliance towards the diagnostic procedure thereby we can monitor the patient condition regularly whenever is required rather than going for invasive plasma analysis. From our study results we can say that the saliva can be routinely used as diagnostic fluid to measure the oxidants and antioxidants levels in RA patients. Further studies with the larger sample size can be carried out to establish saliva as a routine diagnostic fluid in these conditions.

Conclusion

This is the first study in which the levels of oxidants and antioxidants were measured solely by using saliva till now reported in the literature. Effects of rheumatoid arthritis on salivary glands and salivary analysis of oxidants and antioxidants from our study establishes the fact that free radicals play an important role in the pathogenesis of RA. Salivary analysis can be used for the diagnosis of the oxidative stress levels and to monitorthe disease which is easy, non-invasive, cheaper and patient-friendly rather than going for invasive plasma analysis.

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