Salivary Leptin, pH and Flow Rate Among a Group of Patients with Rheumatoid Arthritis In Iraq

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic multisystemic disease which can lead to significant deformities and functional disability.**Objectives:**is to estimate level of salivary Leptin, flow rate and pH of saliva among group of patients with rheumatoid arthritis according to treatment.**Patients and methods:** Study design ;a case control study included Fifty women patients with rheumatoid arthritis; twenty-five on Methotrexate treatment and twenty-five on combination treatment of Methotrexate and Etanercept selected as study groups with an age range (30-40) years old and twenty-five gender, age and Body Mass Index matched healthy looking persons were selected as control. Collection of unstimulated salivary samples was carried out under standard conditions then salivary flow rate, pH in addition salivary Leptin was estimated**Results** after adjustment for age, gender and body mass index; mean salivary flow rate was highest among rheumatoid arthritis cases on combination treatment (Etanercept and Methotrexate) =0.4±0.202 ml/min and least significant difference test between groups was statistically significant (p< 0.05). The mean salivary pH was highest among rheumatoid arthritis cases on combination treatment (Etanercept and Methotrexate) =7.1±0.29; however the differences between groups failed to reach the level of statistical significance. The median of salivary Leptin was highest among rheumatoid arthritis cases on Methotrexate =0.65ng/ml without statistically significant difference between groups, furthermore the salivary levels of Leptin in this study reveals weak correlation with disease activity score 28 r=0.127/p=0.42 among rheumatoid arthritis patients. **Conclusions:**the whole unstimulated salivary flow rate was higher among rheumatoid arthritis cases on combination treatment (Etanercept and Methotrexate) revealing improvements in salivary gland functions, the study also concluded that salivary Leptin level is of low importance to assess disease activity to rheumatoid arthritis patients .

Keywords: rheumatoid arthritis, salivary flow rate, salivary Leptin

Introduction ; Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that affects all ethnic groups throughout the world [1, 2]. The extra-articular organ involvements include the skin, eye, heart, and lung, renal, nervous and gastrointestinal systems   [7, 8]. oral dryness and salivary gland swelling also in patients with RA. These patients can also develop secondary Sjögren's syndrome which is relatively common in 6 to 10% of patients[8 and 22]. The normal unstimulated salivary flow rate ranges from 0.25 to 0.35 mL/min, low 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 [9.] The main factors responsible for a decreased flow rate are therapeutic drugs and radiation treatment for cancer [10]. Hyposalivation is attributed to destruction of the gland and dysfunction on the residual glandular tissue [11]. Dryness of the mouth causes discomfort and a stinging sensation with difficulty in chewing and swallowing dry food, resulting in increase in gingival disease, dental caries and difficulty in wearing dentures [12, 13, and 14]. Hadi et al. [15] found that Xerostomia was the most prominent oral manifestations of RA among study group of Iraqi rheumatoid arthritis patients. Leptin is a peptide hormone that initially thought to regulate body weight by inhibiting food intake and by stimulating energy expenditure [16]; it also seems to play a role in autoimmune diseases such as RA [7]. In RA, circulating leptin levels have been described as either higher or unmodified in comparison to healthy controls; and leptin may be directly implicated in the pathogenesis of RA. but, results of other studies assessing leptin concentrations in patients with RA have been controversial [17]. Saliva is a body fluid that contain biomarkers within its components making it an attractive diagnostic tool that could be used as an alternative to blood in tests measuring biomarkers.

2. Patients and Methods

2.1 Study design

A case–control study was conducted in the outpatient consultation clinic of rheumatology Unit in Baghdad Teaching Hospital during the period between December 2012 and May 2013. Patients with RA, diagnosed on base of American College of Rheumatology Criteria for the classification of RA [10].This study was granted full ethical approval from the local ethics committee and all the patients have given informed written consents prior to the commencement of our study.

Patients were excluded from the study if there were evidences ofoverlapping connective tissue disease, or pregnancy.

2.2 Clinical, laboratory, and radiological evaluation

Patients data were obtained via face-to face interview with the researcher. Focused history was taken including: age, sex, duration of RA ,smoking , patients self-assessment of disease activity or Visual Analogue Scale for the patient (Patient s VAS), functional status was assessed according to the criteria for classification of functional status in rheumatoid arthritis [11] and drug related history (corticosteroids, disease modifying antirheumatoid drugs (DMARDS),and biologic agents).

Each patient was examined for detecting tender and swollen joints, rheumatoid nodules, hand deformities,and mouth examination .

A blood sample was taken for the measurement of Erythrocyte sedimentation rate (ESR) wasmeasured (usingWestergren's method, in mm/ hour) and rheumatoid factor (using latex fixation test) .

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

(VAS) , and ESR according to the following formula [12]:

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

X-rays of both hands in postero-anterior view (PA view) were obtained ,and the progression of rheumatoid arthritis patients was classified according to steinbrocker classification [13]. study and control groups were examined gathering a total sum of seventy five women with an age range (30 to 40) years old; fifty of them were (study group) were diagnosed as established RA patients. RA patients were divided into two subdivisions groups: the first group (twenty five) under conventional treatment disease-modifying antirheumatic drugs DMARD (Methotrexate MTX) and the second group (twenty five) under combination treatment with anti-TNF-α (Etanercept) and DMARDs (MTX).

The control group composed of twenty five subjects and they were apparently healthy according to their medical history matching with age, gender and body mass index BMI of the study group. The body mass index (BMI) was calculated as (body weight/height ^{2}) (kg/m^2).

Unstimulated saliva collected after subjects instructed to relax and to swallow all saliva present in their mouths; while seated and leaning forward, they were told to spit all the saliva they produce into a graduated sterile labeled screw capped bottle test tube. The un stimulated whole saliva collected for 5 minutes was then measured by volume and expressed as milliliters per minute (ml/min). Each salivary sample was separated in two parts, one for the measurements of pH of saliva by using an electronic pH meter, and other part was centrifuged at 3000 r.p.m for 10 minutes then the clear supernatants was separated by micropipette and then stored at (-20^{0}C) in a deep freeze till the time of biochemical analysis. To obtain the most critical consistency of diagnostic criteria inter and intra examiner calibrations were performed.

2.3 Statistical analysis

Data of recruited patients in this study were checked for any errors or inconsistencies and transferred into computerized database software with analytic facilities; Statistical package for social sciences (SPSS) was used in all statistical analysis and procedures. Statistical analysis data were translated into a computerized database structure. The database was examined for errors using range and logical data cleaning methods, and inconsistencies were remedied. Compliance of quantitative random variables with Gaussian curve (normal distribution) was analyzed using the Kolmogorov-Smirnov test. Age, BMI, flow rate of saliva and pH where shown to be normally distributed quantitative continuous outcome variables and described by mean, SD (standard deviation) and SE (standard error). The statistical significance of difference in mean between 2 groups was assessed using the independent samples t-test, while between more than 2 groups ANOVA test was used. When ANOVA model shows a statistically significant difference, further exploration of the statistical significance of difference in mean between each pair of groups was assessed by LSD (least significant difference). Salivary Leptin was non-normally distributed quantitative variables. Such variable can be described by median, which is not sensitive for differences in group average. In such a condition the mean rank is useful in comparing the central tendency (group center) of compared groups. The difference in mean rank between 2 groups was assessed by non-parametric test (Mann-Whitney), while between 3 groups Kruskal-Wallis test was used. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 20 computer software (Statistical Package for Social Sciences).

The correlation coefficient tests between the variables were done by using Spearman's rank linear correlation coefficient. P value less than 0.05 and 0.01 was considered statistically significant.

3. Results

Age and body structure represented in Table**1**, in which study and control groups were comparable.

Table 1: Age and Body Mass Index among Study Groups.

Age in years	Apparently healthy controls n=25	Rheumatoid arthritis treated with Methotrexate n=25	Rheumatoid arthritis treated with Etanercept n=25	P value (NS) (NS)
Range	(30-40)	(30-40)	(30-40)	
Mean	33.8	34.1	35.8	
SD	3.2	3.78	3.57	
BMI (Kg/m^2)	(18.3-38.6)	(16.9-44.4)	(17.3-45)	
Range	28.4	30.1	28.5	
Mean	5.66	7.7	5.73	
SD				

* d.f (between groups/within groups) = 2/72.
SD standard deviation
NS non significant
BMI Body Mass Index
Kg kilogram
m^{2}square meter

Body Mass Index of study groups ranged from (16.9 to 45) Kg/m^2 with a mean of (28.4 to 30.1) Kg/m^2 with no statistically significant difference between three groups. The mean salivary flow rate was highest among RA cases on Etanercept treatment (0.4 ml/min) and lowest in RA cases on MTX (0.26 ml/min). The difference observed in mean salivary flow rate between three groups was statistically significant. The mean salivary flow rate was significantly lower (0.26 ml/min) in RA on MTX compared to both RA on Etanercept and controls (0.4 ml/min and 0.36 ml/min respectively). There was no statistically significant difference in mean salivary flow rate between RA cases on Etanercept and controls (Table **2**).

Table 2: Comparison of mean salivary flow rate among three groups (Control, RA on MTX and RA on Etanercept).

Variable Salivary flow rate	Control group (mean ±SD) N=25 0.36±0.134	Methorexate group (mean ±SD) N=25 0.26±0.117	Etanercept group (mean ±SD) N=25 0.4±0.202	P1 Control + Methotrxate 0.024	P2 Control + Etanercept 0.37(NS)	P3 Methotrxate + Etanercept 0.002	F value 5.474	P Value for Difference Between 3 groups 0.006

* d.f (between groups/within groups) = 2/72.
SD standard deviation
RA rheumatoid arthritis
MTX methotrexate

Regarding salivary pH; its mean was obviously highest among RA cases on Etanercept (pH =7.1) followed by control group (pH =7) and lowest among RA cases on MTX (pH =6.9). The differences observed however, failed to reach the level of statistical significance (Table **3**).

Table 3: Comparison of mean salivary pH among three groups (Control, RA on MTX and RA on Etanercept).

Variable Salivary pH	Control group (mean ±SD) N=25 7±0.2	Methorexate group (mean ±SD) N=25 6.9±0.34	Etanercept group (mean ±SD) N=25 7.1±0.29	P1 Control + Methotrxate **	P2 Control + Etanercept **	P3 Methotrxate + Etanercept **	F value (NS)	P Value for Difference Between 3 groups (NS)

* d.f (between groups/within groups) = 2/72.
** Not calculated, because the overall P values for difference between three groups didn't reach the level of statically significance.
N number
NS non significant
The median salivary Leptin was obviously highest among RA cases on MTX (0.65ng/ml). The median of salivary Leptin among RA cases on Etanercept and controls were (0.42ng/ml and0.42ng/ml respectively) (Table **4**).

Table 4: The difference in: median and mean rank of salivary Leptin between two RA cases groups and control.

Salivary CRP (mg/1)	Apparently healthy controls N=25	RA treated with Methotrexate N=25	RA treated Etanercept N=25	P value (NS)
Range	(0.42-1.8)	(0.42-2.04)	(0.42-2.62)	
Median	0.42	0.65	0.42	
Mean Rank	36.7	43.9	33.4	
Inter-quartile range	(0.42-0.65)	(0.42-0.88)	(0.42-0.65)	

CRP c-reactive protein

N number

NS non significant

RA Rheumatoid arthritis

mg/1 milligram/milliliter

The differences observed however, failed to reach the level of statistical significance. there was statistically non significant difference between high and low of disease activity in salivary flow rate and salivary pH(Table **5**).

Table 5: Comparison of mean salivary flow rate, salivary pH and plaque index among RA cases with high disease activity (DAS=5.1) and those with low disease activity.

High disease activity (DAS=5.1+)			
Salivary variables	Low activity (mean ±SD) N=23	High activity (mean ±SD) N=20	P Value between two groups
Salivary Flow rate (ml/min)	0.34 ± 0.177	0.32 ± 0.207	(NS)
pH	7.00 ± 0.2	7.00 ± 0.34	(NS)

* d.f = 41.

DAS disease activity score

N number

SD standard deviation

RA Rheumatoid arthritis

There was statistically not significant difference between positive and negative of high disease activity in salivary Leptin. (Table 6)

Table 6: The difference in median and mean rank salivary biomarkers between RA cases with high disease activity (DAS=5.1) and those with low disease activity.

High disease activity (DAS=5.1+)			
Salivary biomarkers	Low activity Median (Mean rank). N=23	High activity Median (Mean rank). N=20	P Value NS
Salivary Leptin (ng/ml)	0.42 (21.3)	0.42 (22.8)	

* d.f = 41.

DAS disease activity score

N number

Ng nanogram

NS non significant

4. Discussion

Rheumatoid arthritis (RA) is a chronic systemic, inflammatory disease affecting the adult population. Affected individuals experience significant morbidity, including loss of function, joint destruction, and permanent deformity, and have higher mortality than the general population [18]. This study enrolled 50 patients and 25 apparently healthy subjects, to estimate salivary biomarkers (Leptin) with evaluation of some salivary parameters (salivary flow rate and salivary pH) In relation to the activity of the disease, the group of rheumatoid arthritis patients subdivided according to treatment type into conventional treatment Methotrxate (MTX) and new combination treatment (anti-TNF-α (Etanercept) and MTX) subgroups. Through modulating the ecosystem within the oral cavity. Alterations, quantitatively and/or qualitatively in salivary secretions may lead to deleterious effect on many aspects of oral function and general wellbeing [19, 20]. In this study unstimulated salivary flow rate was measured for RA cases; patients on MTX and patients who received Etanercept, in addition to the control subjects for comparison; the study of unstimulated salivary secretion is an accurate

method to analyze salivary gland status [19].The salivary flow rate was found to be significantly lower among RA cases on MTX (0.26 ml/min) in comparison to both RA on Etanercept and controls (0.4 ml/min and 0.36 ml/min respectively). This decreasing of salivary function in RA is assumed to be related to the lymphocytic infiltrate of affected glands resulting in decrease salivation and chemical changes, Methotrxate is among chemotherapies agents that reduced salivary flow rate [21]. Decrease salivary flow rate among RA cases also were obtained by Hadi et al. 2011and Zalewska et al., 2011[15, 20], revealing similarities with results of present study, although Hadi et al. 2011 recorded higher mean salivary flow rate for study and control groups which may be attributed to variation in age, gender BMI, fasting condition, duration of disease and duration of treatment. The unstimulated flow rate averages 0.25 to 0.35ml per minute [23]. Etanercept group in present study reported mean salivary flow rate to be near mean of that among controls. Etanercept is one of several TNF inhibitors approved for rheumatoid arthritis (RA) and a variety of other immune-mediated inflammatory conditions [24]. Results of present study reports an improvement in amount of salivary gland secretion as treatment with Etanercept has proven successful in reducing chronic inflammation [25], another explanation for this improvement is effectiveness of Etanercept in blocking TNF-α-mediated apoptosis in salivary gland epithelial cells of Sjögren's syndrome: secondary manifestation of RA [26], however results of present study disagree Sankar et al., 2004[27] who found no evidence to suggest that treatment with Etanercept was clinically efficacious in Sjögren's syndrome. This may explained by short duration time of treatment (12 weeks). The capacity of human saliva to stabilize acids is essential for maintaining pH in the oral environment above critical levels [28]. The current study revealed an elevation in pH among RA cases on Etanercept (pH =7.1) followed by control group (pH =7) and lowest among RA cases on MTX   (pH =6.9), however the results don't reach the significant difference. Corvo et al in 2012[29] found that no statistically significant difference in the salivary pH of individuals with secondary Sjögren s syndrome and healthy individuals, both in the non-stimulated total saliva as well as in the total stimulated saliva, these findings come in agreement with results of current study. In present study the salivary pH among study and control groups remain within physiological range pH = 6.0–7.0 [30 and 31] with slightly alkaline pH, these results are not contrary to expectations since the physiological salivary pH range increases as the flow rate of saliva increases and vice versa ([19 and 32]) since the pH values come in coordination with salivary flow rates among groups. The slight alkaline pH among study groups, can be explain by evidences obtained from other studies; urea was found to be higher among patients with chronic periodontal diseases, urea is metabolized quickly by bacterial urease, producing ammonia and carbonic gas causing an elevation in the pH of the saliva [33].

Estimation of levels of salivary Leptin among RA patients were one of aims of current study, Levels of salivary Leptin were measured in this study to be obviously higher among RA patients on MTX (median 0.65 ng/ml) while median of salivary Leptin for both RA on Etanercept and controls (median 0.42 ng/ml), though the difference failed to reach the level of statistical significance, also levels of Leptin in this study reveals weak correlation with DAS 28 among RA patients (0.127/p=0.42). Findings of current study agree with Hizmetly et al., in 2007[34] who demonstrated that there wasn't any significant difference at plasma Leptin levels between RA patients and control group . Other studies reported higher serum leptin level in RA patients in comparison to healthy controls [35]; the reasons for this discrepancy between results of studies may be related to the effects of medications used for treatment or to the differences in body mass indices of patients with RA. Also chronic periodontitis affect Leptin concentration in saliva because chronic inflammation induces a suppression of Leptin synthesis [36]. No study regarding Etanercept (anti-TNF biological treatment) effect on Leptin level among RA patients could be found, but Popa et al., 2009[73] found similar Leptin concentrations compared with the controls and anti-TNF therapy (Inflaximap) have limited influence on plasma Leptin concentrations.

5. Conclusion

the whole unstimulated salivary flow rate was found to be higher among rheumatoid arthritis cases on combination of treatment (Methotrexate and Etanercept) revealing improvements in salivary gland functions. Also salivary Leptin level is of low importance to assess disease activity to rheumatoid arthritis patients.

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