An Impairment of Salivary Gland Function in Rheumatoid Arthritis: Association with Change in Salivary Biomarkers and Disease Activity

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ABSTRACT

Background: Rheumatoid arthritis is a chronic inflammatory autoimmune disease characterized by joint inflammation, involvement of exocrine salivary and lacrimal glands may occur as extra-articular manifestations in this disease. This study aimed to provide evidence of altered in function and composition of salivary gland in patients with rheumatoid arthritis by determine salivary flow rate and some biochemical parameters(total protein, amylase, peroxidase) and to investigate the relationship between disease activity and changes in function and composition of salivary gland.

Materials and Methods: Fifty five patients with RA (7 males and 48 females) were enrolled in this study with age range (20-69) years. The patients were separated into two groups in proportion to their salivation: normal salivation group (37) and hypo salivation group (18). Thirty five (9 male and 26 female) apparently healthy volunteers were also participated in the study. Three ml of unstimulated saliva was collected from all patients and control to determine salivary flow rate on one hand and salivary total protein, α-amylase and peroxidase by colorimetric method on other hand.

Results:Resultsshowed that there is highly significant decrease (P< 0.01; p< 0.001) in the median salivary levels of (flow rate, total protein, α-amylase and peroxidase) among RA patients when compared to control. There was highly significant reduction (P< 0.01) in median salivary levels of flow rate, total protein, α-amylase and peroxidase in two study groups (normal salivation and hypo salivation) as compared to that in control group. Also the levels of all these parameters (sialometry and sialochemistry) were significantly decrease (P =0.00) in RA patients with hypo salivation as compared to that in patients with normal salivation. There was strong positive correlation between total protein and salivary flow rate (r= 0.651, P=0.000), in one hand, and on the other hand, there was strong positive correlation between α-amylase and both salivary flow rate (r=623, P= 0.000) and total protein r=658, P=0.000).

Conclusion: These findings indicate that the changes in salivary composition may represent involvement of salivary glands in patients with rheumatoid arthritis.

Key words: Rheumatoid arthritis, salivary gland, sialometry and sialochemistry. (J Bagh Coll Dentistry 2016; 28(2):165-170).

INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic inflammatory disorder that is characterized by jointswelling, joint tenderness, and also destruction of synovial joints, which lead to severe disability and premature mortality [(1,2)].

RA may present with extra-articular manifestations which includes involvement of exocrine salivary and lacrimal glands. The reduction of salivary gland function in RA is possibly related to the infiltration of lymphocytes in the affected glands and presented as decrease of saliva production and chemical changes of saliva [(3)]. It has been known for a long time that salivary gland involvement occur in RA patients; however it does not take attraction.

In 1978 Sullivan and colleagues stated that 58 of 100 unselected RA out-patients had decreased salivary and/or lacrymal secretion [(4)]. Many morphological studies showed that the minor salivary glands of patients with RA were heavily invaded with B lymphocytes, which are more predominant than Tlymphocytes, and also the helper T cells are higher thansuppressor T cells, and that the changes also involved atrophy of acinar cells with fibrosis and also sialadenitis[(5,6)].

The diagnosis of salivary gland involvement is based on the detection of functional impairment or anatomical changes resulting from an autoimmune inflammatory process in which the salivary glands are the main target [(7)]. Even thoughinvolvement of salivary gland in RA has been noted for a long period, it did not attract much interest, eitherfrom researchers orclinicians. Therefore this study was established to provide evidence on alteration in salivary gland function by salivary flow rate and chemical composition by measuring salivary (total protein, peroxidase and α-amylase).

MATERIALS AND METHODS

Fifty five patients with RA (7 males and 48 females) were enrolled in this study with age range (20-69) years. The patients were diagnosed clinically by rheumatology specialists and assessment of disease activity depending

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ondisease activity score-28(DAS 28);they were from attendance to the Baghdad Teaching Hospital seeking for treatment. The patients group was divided into two groups according to their salivary flow rate: normal salivation group (37 patients) and hypo salivation group (18 patients).

Data were collected from patients including name, age, gender, whether smoker or alcoholic or not, onset of disease and duration, family history, other systemic diseases, and medications. The control group consist of 35 participants (9 males and 26 females) and they were in healthy conditions (not suffering from systemic diseases and not taking any medication), with age range from (20- 69) years. Three ml of unstimulated (resting) whole saliva samples were collected under resting conditions between 9.0-12.0 A.M.

Patients were asked to rinse their mouth with water and to generate saliva in their mouth and drool into a wide test tube. And calculate the time for collection by divided the volume of saliva on time obtain salivary flow rate.After that the saliva was centrifuged at (3000 rpm) for 10 minutes. The resulting supernatant was stored at – 20 °C in polyethylene tubes until assayed.Salivary total protein, α-amylase and peroxidase were measured by colorimetric method,and performed as recommended in leaflet with kits (Spinreact, Spain).

Statistical analysis was assessed using one way ANOVA test, Mann-Whitney-test and Kruskal-Wallis H-test. Correlation between the different parameters was calculated by the Spearman rank coefficient test. P-value less than the 0.05 was considered statistically significant.

RESULTS

This study revealed that there is no significant difference ($p>0.05$) in mean of DAS-28 score among three groups of patients [total RA patients group (4.79 ± 0.86), normal salivation group (4.81 ± 0.95) and hypo salivation group (4.73 ± 0.69)] as in table (1).

The present study showed that there is significant decrease ($P<0.01$) in median salivary flow rate among patients (0.35 ml\min) when compared to control group (0.6 ml\min) as shown in table (2).

In regard to the differences in median salivary flow rate between two groups of patients (hypo and normal) and controls group, the current result showed that there are highly significant differences ($P<0.001$). Median salivary flow rate among control group was (0.6 ml/min) whereas in normal salivation group was (0.56 ml/min) and in hypo salivation group was (0.17 ml/min). On the other hand, salivary flow rate was significantly decreasing ($P<0.001$) in RA patients with hypo salivation as compared to that in patients with normal salivation(Table 3).

Table1: Difference in DAS-28 in three patients groups

DAS-28	Normal n= 37	Hypo n= 18	Total n= 55	(ANOVA-test) P-value
Range	(2.14 – 6.57)	(3.62 - 5.90)	(2.14 – 6.57)	0.622^{NS}
Median	4.79	5.01	4.89	
Mean	4.81	4.73	4.79	
SD	0.95	0.69	0.86	
SE	0.156	0.159	0.12	

Table2: Difference in salivary flow rate in study group and control group

Salivary flow rate	Control group n= 35	Study group n= 55	P (Mann-Whitney) test
Range	(0.31- 1.9)	(0.08- 2.5)	0.004^{**}
Median	0.6	0.35	
Mean	0.64	0.52	
SD	0.32	0.44	
SE	0.05	0.06	

****: Highly significant**

Table 3: Differences in salivary flow rate between two study groups and control group

Salivary flow rate	Healthy control n= 35	Normal salivation n= 18	Hypo salivation n= 37	P (Kruskall-Wallis H)
Range	(0.31-1.9)	(0.3- 2.5)	(0.08- 0.26)	0.001**
Median	0.6	0.56	0.17	
Mean	0.64	0.68	0.17	
SD	0.32	0.45	0.06	
SE	0.05	0.07	0.01	
P (Mann-Whitney)				
Normal X Hypo = P<0.001				

Table (4) illustrated that there are highly significant decrease (P<0.01) in median salivary levels of total protein among study group (689.7 mg/L) as compared to median level in control group (890.5 mg/L).Moreover, there was highly significant reduction (P<0.001) in median salivary level of total protein in two study groups; normal salivation (834.8 mg/L) and hypo salivation (345.7mg/L) as compared to that in control group (890.5mg/L) as shown in table (5). However, the median salivary total protein was significantly decreasing (P< 0.01) in patients with hypo salivation group than that in patients group with normal salivation. The median salivary α-amylase level in study group was lower when compared with control group (123100unit\L vs.140930unit\L) as shown intable (6).In addition there are high significant differences (P<0.01) in median salivary α-amylase level among three study groups (normal salivation patients, hypo salivation patients and control group). The median salivary α-amylase level was decrease in hypo salivation patient (49665 unit\L) as compared to those in normal salivation patient (146300 unit\L) and in control group (140930 unit\L) as seen in table (7). Furthermore, there was highly significant decrease (P<0.01) in the level of salivary α-amylase in hypo salivation group in comparison with normal salivation group.Salivary peroxidase level was significantly decrease (2.8 unit\L) in patients when compared to control (4.44 unit\L), (p< 0.001), as clearly shown in table (8).Also this study observed that the median salivary level of peroxidase among two study groups hypo salivation (2.07 U/L) and normal salivation (2.98 U/L) was significant decrease (p< 0.001) in comparison to controls (4.44U/L), as clarified in table (9) this in one hand , on the other hand salivary peroxidase level was significantly decrease in patients with hypo salivation (2.07) than those patients with normal salivation (2.98 U/L), (p< 0.001).

Table4: Difference in salivary total protein in study group and control group

Salivary total protein	Control group n= 35	Study group n= 55	P (Mann-Whitney)
Range	(111.3-1382.6)	(120-1910.7)	0.009**
Median	890.5	689.7	
Mean	873.68	714.70	
SD	242.75	382.98	
SE	41.03	51.64	

Table 5: Differences in salivary total protein between two study groups and control group

Salivary total protein	Healthy control n= 35	Normal salivation n= 37	Hypo salivation n= 18	P (Kruskall-Wallis H)
Range	(111.3-1382)	(412.3-1910.7)	(120-467.9)	0.000 **
Median	890.5	834.8	345.7	
Mean	873.68	907.02	319.38	
SD	242.75	313.44	109.00	
SE	41.03	51.53	25.69	
P (Mann-Whitney)				
Normal X Hypo = P<.01				

Table 6: Difference in salivary amylase in study group and control group

Salivary α-amylase	Control group n= 35	Study group n= 55	P (Mann-Whitney)
Range	(94280- 199040)	(21610- 198750)	0.007**
Median	140930	123100	
Mean	140377.43	107849.69	
SD	29843.89	51568.51	
SE	5044.54	6953.50	

Table 7: Differences in salivary amylase between two study and control group

Salivaryamylase	Healthy control n= 35	Normal salivation n= 37	Hypo salivation n= 18	P (Kruskall-Wallis H)
Range	(94280-199040)	(21610-198750)	(31102-77500)	0.000 **
Median	140930	146300	49665	
Mean	140377.43	135623.54	50759.00	
SD	29843.89	38416.81	13853.46	
SE	5044.54	6315.68	3265.29	
P (Mann-Whitney)				
Normal X Hypo = P<0.01				

Table 8: Difference in salivary peroxidase in study group and control group

Salivary peroxidase	Control group n= 35	Study group n= 55	P (Mann-Whitney)
Range	(2.72- 5.9)	(0.72- 4.32)	0.000**
Median	4.44	2.8	
Mean	4.41	2.67	
SD	0.86	0.94	
SE	0.15	0.13	

Table 9: Differences in salivary peroxidase between two study groups and control group

Salivary peroxidase	Healthy control n= 35	Normal salivation n= 37	Hyposalivation n= 18	P (Kruskall-Wallis H)
Range	(2.72-5.9)	(1.2-4.32)	(0.72-2.96)	0.000 **
Median	4.44	2.98	2.07	
Mean	4.41	2.98	2.02	
SD	0.86	0.83	0.82	
SE	0.15	0.14	0.19	
P (Mann-Whitney)				
Normal X Hypo = P<0.001				

There was strong positive correlation between salivary total protein and salivary flow (r= 0.651, P=0.000), in addition there was strong positive correlation between salivary amylase and both salivary flow (r=623, P= 0.000) and total protein r=658, P=0.000). Moreover there was positive correlation between salivary peroxidase and salivary amylase (r=0.352, P= 0.008). Surprisingly, this result failed to show any significant correlation between changes in salivary composition and DAS-28 as shown in table (10).

Table 10: Correlation between the variables in study group

Variables		DAS-28	Salivary flow	Total protein	Amylase	Peroxidase
Salivary flow	r	0.131				
	p-value	0.339				
Total protein	r	0.008	0.651			
	p-value	0.956	0.000			
Amylase	r	0.167	0.623	0.658		
	p-value	0.223	0.000	0.000		
Peroxidase	r	-0.086	0.263	0.327	0.352	
	p-value	0.532	0.053	0.015	0.008	

DISCUSSION

The current results denoted that the median levels of salivary flow rate are significantly decreased in RA patients as compared to control group; this result is consistent with other studies[8,9]. Mignonga and colleagues reported that in the early phase of this disease, the infiltration of lymphocyte might cause via secretion of cytokine salivary gland function alteration, clinically which might be appeared as increased salivary flow. Only destruction of the glandular acinar units may occur afterwards, which cause a salivary secretion reduction. It is thought that infiltration of T and B lymphocytes in salivary glands, cause cell-mediated destroy of glandular elements; cytokines secretion; autoantibodies production which is cause interference with muscarinic receptors; and metalloproteinases secretion that interfere with efficient glandular functions [10].When comparing between two study groups, the present study noticed that highly significant reduction in salivary flow rate was observed in RA hypo salivation group than those in RA normal salivation group and control group, also the salivary flow rate have tendency to decrease in normal salivation group in comparison to control group, this finding coinciding with previous reports [7,9,11]. Zalewska and associates at 2011showed that there is decrease in salivary flow in the RA with group of hypo salivation, it was 5.49 and was 5.29 times lower as compared to the RA with normal salivation group and control group.

On the other hand, Helenius attributed the cause of decrease salivary flow rate among RA patients to that similar to the situation in another organs, the salivary glands can functionally compensate for modest loss of parenchyma. In the early stages of disease, stimulated flow rates may therefore be only slightly reduced or normal. In later stages, however, there is loss of tissue so much and stimulated flow rates may be significantly reduced [7]. Contradictory, Mignogna*et al* showed 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 saliva composition may have been significantly changed by autoimmune inflammation [8,12]. Nevertheless, Alosami and colleagues pointed out to that there is increase in unstimulated salivary flow rate among patients with RA on combination treatment (Methotrexate and Etanercept) showing improvements in functions of salivary gland [13].

This study found decrease in level of salivary total protein among RA patients as compared to control, and patients with hypo salivation had lower level of salivary total protein, this result is agreed by other study conducted by Zalweska andco-workers who stated that RA patients with xerostomia had significantly reduction in level of salivary total protein, and they denote that xerostomia in RA patients may be a harbinger of decrease production of saliva respecting quality and quantity, and may be indicative of the salivary immune system impairment of the oral cavity in RA patients with xerostomia[12]. On the other hand, this is contrary to the findings of other studies[7,14].

Helenius and colleagues mentioned that there was increase in salivary total protein level among RA patients than that in control group. Similarly other study on other autoimmune disease (systemic lupus erythromatous) also found that salivary total protein level was significantly higher among patients than in the control subjects [15]. They concluded that rheumatic diseases patients, independent of specific diagnosis, so had various alterations in salivary flow and saliva composition and general oral health. So their findings may represent mirror to the autoimmune inflammation of the salivary glands which frequently noticed in these patients [7].

Amylase is one of main components of parotid saliva and is the most important digestive enzyme present in saliva. Concentration of amylase is a marker of synthesis of protein in the acinar cell [16].The current study demonstrated a significant decrease in level of amylase in patients when compared to control, and this confirms previous finding indicated by Helenius*et al* who indicate the impaired function of the acini of salivary gland in these patients [7], since concentration of amylase is a marker of synthesis of protein in the acinar cell [17]. Whereas this result was at variance with other studies which revealed that there was no difference in salivary amylase levels between the RA patients and the normal control probably the cause behind this result is due to method of measurement of amylase [18,19]. Pedersen *et al.* also found that the activity of amylase in the whole saliva or parotid saliva did not differ in other autoimmune disease as primary SS from those of the healthy controls [20].

Another important result in the current study is a significant decrease in salivary level of peroxidase enzyme in RA group as compared to control group and also the level of salivary peroxidase was significantly lower in hypo salivation group than in normal salivation patient group. However, similar finding was showed in other study done by Zalewska*et al.* which shown significantly lower salivary peroxidase level in

RA group, because salivary glands are main target in RA which cause disintegration acini salivary gland and decrease salivary parameters production involved salivary peroxidase [12].

Conversely, previous study carried out by Nagler*et al.* at 2003 shown different results, the salivary peroxidase level was higher in RA patient group than in control group, while there was no significant difference between the RA patient's subgroup (normal salivation and hypo salivation groups). This might be because of the fact that peroxidase is the most significant antioxidant enzyme present in saliva, so elevation in salivary antioxidants in patients with RA which may be due to the elevation in plasma antioxidants in general, because to a great extent saliva composition is reflected from plasma composition causes rise of peroxidase in saliva [21,22].

However, this also may reflect the same response of the salivary glands to RA that is the up-regulation of the secretion of salivary antioxidants [21,23]. In conclusion; these findings indicate that the changes in salivary composition may represent involvement of salivary glands in patients with rheumatoid arthritis.

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