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ESTIMATION OF SALIVARY ALPHA-AMYLASE, SALIVARY TOTAL PROTEIN IN SECONDARY SJOGREN SYNDROME PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT
Secondary Sjogren syndrome is a peculiar disorder presented in individuals with rheumatoid arthritis. The extra-articular manifestation affected salivary glands function & in turn salivary composition. This study was aim to evaluate the differences in salivary (alpha-amylase & total protein) in secondary Sjogren syndrome in relation to rheumatoid arthritis. Sixty one rheumatoid arthritis patients of either gender with age range (25- 60) y, of them (31 patients with Secondary sjogren syndrome diagnosed according to American-European Consensus Group criteria (AECC) & 30 Rheumatoid arthritis patients diagnosed clinically by rheumatology specialists) & 30 healthy volunteer of both gender selected as control group. Unstimulated salivary flow sample were collected from all groups. Estimation of salivary total protein & salivary alpha-amylase was done according to assay procedure using a spectrophotometer with a specific wave length. The present study revealed that no significant differences between rheumatoid arthritis & control groups regarding alpha-amylase, while there was a significant differences in secondary Sjogren disordered group and controlled at $P<0.05$. The results also showed that no significant differences regarding salivary total protein between any of studied groups. differences in salivary (total protein & alpha amylase) between rheumatoid arthritis & secondary sjogren groups reveal low oral defense mechanism in secondary Sjogren groups despite statistically non significant.

KEYWORDS*:* Sjogren syndrome,rheumatoid arthritis, salivary α-amylase, salivary total proteins.

INTRODUCTION

Sjögren's syndrome "is a chronic autoimmune disorder of the exocrine glands characterized by lymphocytic infiltrates of the affected gland & dryness of the eyes & mouth that results from involvement of the lachrymal & salivary glands" (Norheim *et al*., 2012) Sjögren's syndrome might be manifestated as "primary or secondary disease when it is coupled with other autoimmune disease such as rheumatoid arthritis (RA) & scleroderma" (Antero *et al*., 2011) & (Gomes, P.D. *et al*., 2012). The type I interferon & B cells has a role in primary SS. (Fox, R.I. & Liu, A.Y. 2006) & (Dörner, 2006). In the other hand the pathophysiology of RA is multifactorial involving, B cells, T cells in addition to the complicated interaction of many pro-inflammatory cytokines, including IL-6 & TNF-a, (Choy, 2012) So the pathophysiology of these two disordered was different, assumed that "RA secondary SS" has a different physiopathology than the primary type or patients with RA and secondary SS have two different diseases (Antero *et al*., 2011).

The main etiology of SS is not well understood till now, in spite of that (Bayetto, K. & Logan, R.M. 2010) & (Konttinen Y. & Kasna-Ronkainen, 2002) proved that the following events occur in all the patients with SS: Initiation by an exogenous factor, followed by disruption of salivary gland epithelial cells, after that T-lymphocyte migration and lymphocytic infiltration of exogenous glands, then B-lymphocytic hyper reactivity and production of rheumatoid factor and antibodies to Ro (SS-A) and La (SS-B). Xerostomia considered as a constant oral symptoms in SS (Orellana *et al.*, 2006). The signs & symptoms of xerostomia resulted from changes in the quantity & quality of saliva. (Clair, 2013) Many researchers evaluate the composition of saliva in patients suffering from xerostomia in ordered to ameliorate the xerostomic state of those patients.

Salivary α-Amylase "is one of the most plentiful components in saliva, accounting for 10–20% of the total protein content". Salivary α-amylase is produced locally by the specialized epithelial acinar cells of the exocrine salivary glands mainly of the parotid glands; also it contributes in food digestion via the hydrolysis of starch to glucose and to maltose (Arhakis *et al*., 2013). Consequently, salivary α-Amylase has been proposed to prevent the attachment of bacteria to oral surfaces and to permit the clearing of bacterial from the mouth (Bosch *et al*., 2003). Total salivary protein considered to be essential component of saliva, composing of mainly mucin, proline rich proteins, statherin, amylase, immunoglobulins & antibacterial factors & these are responsible for most function of saliva (Panchbhai *et al*., 2010) Salivary proteins also have protective effects against dental caries, these proteins act either directly &/or indirectly through different methods on plaque and bacteria via changing tooth susceptibility to dental caries (Vibhakar *et al.*, 2013).

MATERIALS & METHODS

This comparative study was performed in Baghdad Teaching Hospital/ Department of Rheumatology. This study was conducted on patients after approval of the research protocol by the Research Scientific Committee. The study samples consist of ninety patients, 60 Rheumatoid arthritis (RA) patients of either gender with age range (25- 60), of them (30 patients with secondary Sjogren syndrome (sSS)diagnosed according to American-European Consensus Group criteria (AECC) (Vitali *et al.*, 2002) & 30 RA patients diagnosed clinically by rheumatology specialists) and both of them evaluated by disease activity depending on DAS 28 "Disease Activity Score in 28 Joints (DAS28)" 30 Healthy control subjects.

The study sample divided into three groups:

Group I: (n=30) sSS patients with RA.

Group II: (n=30) RA patients.

Group III: (n=30) healthy control subjects.

Exclusion criteria

Past head and neck radiation treatment; hepatitis C infection; acquired immunodeficiency syndrome (AIDS); preexisting lymphoma; sarcoidosis; graftvs.-host disease; use of anticholinergic drugs (since a time shorter than fourfold the half-life of the drug).

Diagnoses of the secondary Sjogren syndrome patients according to American-European Consensus Group criteria (AECC) all included patients subjected to Schirmer test "Cutoff for abnormally low tear production is 5 mm distance or less usually"(Clair WS.2013), whole unstimulated salivary flow rate "a less than 1.5mL is considered abnormal or xerostomia" (Vitali *et al.*, 2002). Also those patients should have (+ve ocular symptoms & oral symptoms).

Saliva collection

The method of saliva collection is done according to the procedure suggested by (Wu-Wang *et al*., 1995), so in order to avoid any circadian variation salivary sample collected between 9 am & 12 am. Before collecting saliva all subjects were instructed not to eat or drink (except water) for at least one hour, then asking the subjects to wash their mouth then sit down in comfortable position & collect saliva by spit into sterile graduated tube .Then the salivary sample kept in ice until centrifuged at 3500 rpm for 15 min., the supernatants store at -20 ℃ freezer until analyzed.

Salivary total protein analysis

Let the salivary samples to thaw at room temperature then the concentration of salivary total protein estimated using (SPINREACT, SPAIN) kit.

Principle of the method: Proteins react in acid solution with pirogallol red and molybdate to form a colored complex, the intensity of the formed color is corresponding to the protein concentration in the sample, the measurement is done according to the assay procedure using spectrophotometer at 598nm wave length (Orsonneau *et al*., 1989).

Salivary α-amylase analysis

After thawing of the salivary samples at room temperature the estimation of salivary α-amylase was using (SPINREACT, SPAIN) kit.

Principles of the methods: Alpha amylase hydrolyzes the 2-chloro-4-nitrophenyle-α-D-maltotrioside (CNPG3) to release 2-chloro-4-nitrophenol (CNP) and form 2-chloro-4-nitrophenyl -α-D-maltoside (CNPG2), maltotriose (G3) y glucose (G) according to following reaction

$$10\ \text{CNPG3} \xrightarrow{\text{Amylase}} 9\text{CNP} + 1\text{CNPG2} + \text{G3} + \text{G}$$

The catalytic α-amylase concentration found in the sample is proportional to the rate of 2-chloro-4-nitrophenol generation which measured photometrically at 405 nm wave length according to the assay procedures in the kit (Foo AY & Bais R 1998).

RESULTS & DISCUSSION

Demographic characteristic variable: Demographically this study in table 1, demonstrated that, vast majority of a diseased groups are reported at (40 – 49) yrs, and at (60 – 70) yrs for the RA, and sSS with mean and standard deviation (48.30 ± 10.02) yrs., and (52.65 ± 9.88) yrs. respectively, and highest percentages of the diseased groups were found at females, and they are accounted 28(93.3%), and 31(100%), in RA, and sSS groups respectively.

TABLE 1: Demographical Characteristics variables (age & gender) of the studied groups

DCv.	Groups	Groups					
		RA		sSS		Control	
		No.	%	No.	%	No.	%
Age	20 _	0	0.00	1	3.2	10	32.3
	30 _	5	16.7	2	6.5	12	38.7
	40 _	12	40	7	22.6	4	12.9
	50 _	8	26.7	10	32.3	5	16.1
	60 _ 70	5	16.7	11	35.5	0	0.00
	Total	30	100	31	100	31	100
Mean ± SD		48.30 ± 10.02		52.65 ± 9.88		36.03 ± 10.89	
Gender	Male	2	6.7	0	0.00	18	58.1
	Female	28	93.3	31	100	13	41.9
	Total	30	100	31	100	31	100

Salivary Total protein (Sa - TP)

Table 2 shows a descriptive statistics of (Sa–Tp) parameter for the studied groups, such that, mean values, standard deviation, standard error, 95% confidence interval for the population mean, and the two extreme values (minimum, and maximum). Related to Sa–Tp parameter, Controlled, and sSS of diseased group are recorded similarly responding according to mean values, while RA group had increased almost by ten percent in light others groups Figure 1.

TABLE 2: Summary Statistics of (Sa – Tp) Parameter in the studied groups

						95% C.I. for Mean			
Parameters	Groups	No.	Mean	S.D.	S.E.	L.B.	U.B.	Min.	Max.
Sa – Tp	RA	30	154	74	14	126	181	72	361
	sSS	31	143	50	9	124	161	65	293
	Control	31	144	46	8	127	161	64	245

The present study revealed that no significant differences throughout probable pair wise comparisons of equal means in Sa – Tp parameter are accounted at P>0.05 among the studied groups Table 3.

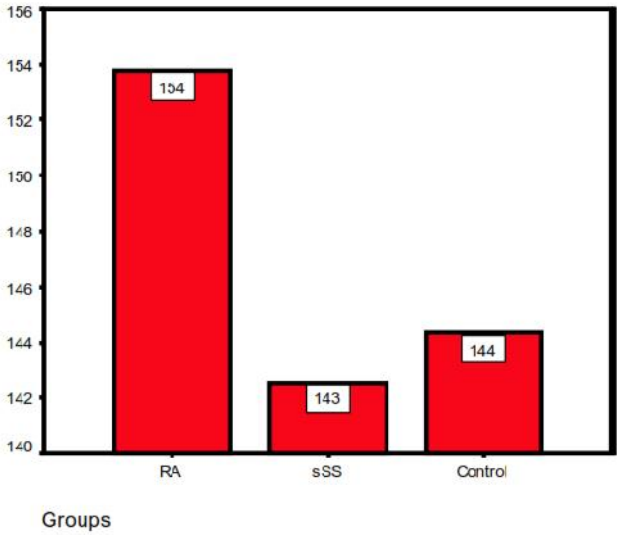

FIGURE 1: Bar charts plot of mean values of (Sa – Tp) Parameter for studied group's disorders and control group

TABLE 3: Pair wise Comparisons by (GH) tests concerning (Sa – Tp) parameters

	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD
Parameters					L.B.	U.B.	Diff.
		sSS	11.22	0.770	-28.04	50.48	39
Sa – Tp	RA	Control	9.44	0.824	-28.96	47.84	38
	sSS	Control	-1.77	0.988	-31.18	27.63	29

(*) S: Sig. at P<0.05; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD).

Salivary α-amylase (Sa - AM)

Table 4 showed a descriptive statistics of (Sa–AM) parameter for the studied groups, such that, mean values, standard deviation, standard error, 95% confidence interval for the population mean, and the two extreme values (minimum, and maximum).

Regarding to Sa – AM parameter, RA, and sSS of diseased groups are recorded too low levels of responding compared with controlled group, rather than RA group are increased by one third percent in light of sSS group. Figure2.

The present study in table 5 showed that with respect to (Sa – AM) parameter no significant differences throughout probable pair wise comparisons of equal means are accounted, except between sSS disordered group and controlled at P<0.05.

TABLE 4: Descriptive Statistics of (Sa – AM) Parameter in the studied groups

	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
Parametrs						L.B.	U.B.		
	RA	30	88273	113376	20699	45938	130608	3068	395600
Sa – AM	sSS	31	56135	55602	9986	35739	76530	8320	223200
	Control	31	108778	98920	17767	72494	145063	7810	448700

TABLE 5: Pair wise Comparisons by (GH) tests concerning (Sa – AM) parameters

Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD Diff.
					L.B.	U.B.	
Sa – AM	RA	sSS	32138	0.351	-23704	87981	55842
		Control	-20505	0.734	-86138	45127	65632
	sSS	Control	-52644	0.034*	-101960	-3327	49316

(*) S: Sig. at P<0.05; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD)

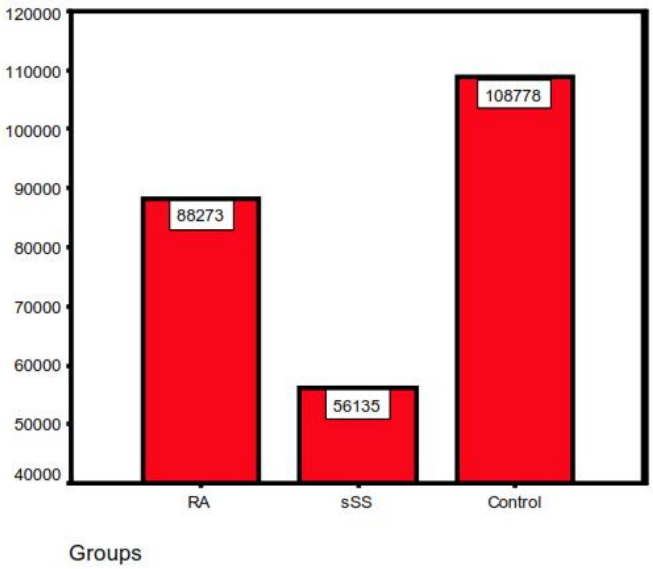

FIGURE 2: Bar charts plot of mean values of (Sa – AM) parametersor studied group's disorders and control group

DISCUSSION

Salivary total protein

Data of the present study showed that no significant differences are accounted at P>0.05 between the two diseased groups & control group. However controlled, and sSS of diseased group are recorded similarly responding according to mean values, while RA group had increased almost by ten percent in light of others groups. On the other hand Helenius *et al.*, 2005 mentioned that there was an increase in salivary total protein concentration in patients with RA & sSS than control, while Abdulla, W.L. *et al*., 2016 found a decrease in level of salivary total protein within RA patients in compared with control, and there was lower level of salivary total protein in patients with hyposalivation .In contrast to these finding Reijden W.A. *et al.*, 1996 showed that absolute concentrations of total protein were" increased significantly in both primary and secondary Sjögren's syndrome". These differences in those finding might be due to different methodology in measuring SA-TP& /or deterioration in immune regulation.

This finding probably suggests that the secretary epithelium in the initial inflammatory stage is "stimulated by the cellular inflammation or by Lymphokines" so produce more substances. While in patients with mild inflammation the increased levels can be related to the fact that the "metaplastic epithelial cells that replace the normal ductal cells" in those patients not able to reabsorb those substances sufficiently. Alternatively during the late inflammatory stage, though, decreasing of the "salivary constituents" can be related to the "significant decrease of the secretory epithelium or to the replacement of these cells by non-functional collagen tissue" (Tsianos *et al.*, 1985).

Salivary α-amylase

The current study revealed that there is a significant differences in concentration of Sa – AM between sSS disordered group and controlled at P<0.05, so the control group showed increase Sa – AM concentration than sSS group, while no significant differences in concentration of Sa – AM between neither (RA & control) group nor (RA & sSS), additionally RA group registered low concentration compared to control agreed with our study Kim *et al*., 2013 proved that there was no difference in salivary α-amylase levels between the RA and the control group. However Abdulla *et al*., 2016 estimated that a significant decrease in level of amylase in RA patients when compared to control. Subsequently Greabu *et al.*, 2009 & Malamud, 2011 suggested decreased in levels of salivary amylase and carbonic anhydrase in sjogren syndrome patients. This finding perhaps suggests that in the initial inflammatory stage the secretary epithelium is stimulated by the cellular inflammation or by lymphokines to produce more substances. Alternatively the increased levels in patients with mild inflammation can be attributed to the fact that the metaplastic epithelial cells that replace the normal ductal cells in these patients are not capable of reabsorbing these substances effectively. In the late inflammatory stage, however, the decrease of the salivary constituents can be attributed to the significant decrease of the secretary epithelium or to the replacement of these cells by non-functional collagen tissue. Tsianos *et al*., 1985.

CONCLUSION

The occurrence of secondary Sjogren syndrome is affected by salivary α-amylase & not affected by salivary total protein.

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