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Evaluation of Anti-Scl-70, Anti-Ro (SSA) Levels in Serum and Saliva of Patients with Systemic Sclerosis

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Abstract
Systemic sclerosis (SSc) is one of autoimmune diseases that affected multiorgans, clinically showed that the extensive skin and internal organs fibrosis. Systemic sclerosis showed that the three basic sorts: autoimmunity, inflammation, vasculopathy. This study was design to investigate the changes of anti-topoisomerase I antibody (Scl-70) and Anti-Ro (SSA) in the whole saliva and serum of patients with SSc. There were highly significant ($p< 0.001$) elevation in Scl-70 antibody in serum and non-significant difference in saliva of patients with systemic sclerosis with median and mean rank (215.91, 51.80, 228.43 and 37.13 ng/ml respectively) in compares with control subject. The mean level of serum SSA in SSc patient (140.4 ± 22.67) ng/ml that it was significant increased ($p< 0.001$) as well as the mean level of salivary SSA in SSc patient (145.5 ± 19.98) ng/ml was highly significantly increased in compares with control subject.
Keywords: Anti topoisomerase antibody (Scl-70), SSA (anti-Ro), SSc.

1. Introduction

Systemic sclerosis (SSc) is a multiorgan illness with immune-mediated of unidentified cause considered by progressive fibrosis of skin and internal organs, with occurrence of a set of specific auto reactive antibodies (Varga and Abraham, 2007). The most important autoantibodies appeared significantly in SSc patients are anti-topoisomerase I autoantibody (Scl-70), anti-centromere autoantibody (ACA), and anti-RNA polymerase III autoantibody (RNAP3) (Steen, 2005). SSc was classify into two main types, according to the extent of skin involvement. (Khanna and Denton, 2010).

1. Limited cutaneous systemic sclerosis (lcSSc)
2. Diffuse cutaneous systemic sclerosis (dcSSc)

Systemic sclerosis was classified in subdivision by LeRoy *et al.,* 1988 as firstly limited type SSc when the skin involved up to the elbow and knees with the face – and in diffuse form SSc – with skin envelopment also including the trunk. There are three main features of SSc, which integrated by 2013 SSc criteria but not all patients, come with these feature: autoantibodies, vascular injury, and finally with fibrotic changes. Raynaud phenomenon (RP) is a feature included in SSc criteria if distinguished from other disease associated with RP, but because SSc without RP is so infrequent, so raynaud phenomenon augmented statistical significance to this criteria (Van den hoogen *et al.,* 2013). The criteria of SSc in 2013 can be demarcated principles and classify more patients with SSc such as a patient with Raynaud phenomena, sclerodactyly, anticentromere antibody, and established pulmonary arterial hypertension would be categorized as having SSc by criteria of rheumatism in 2013 but not by the 1980 criteria (Van den hoogen *et al.,* 2013).

There are some features associated with SSc patient such as sclerodactyly, anticentromere antibodies, Scl-70, Raynaud phenomena, dilated nailfold capillaries, dysphagia, and calcinosis. However, a patient with only sclerodactyly, gastroesophageal reflux disease, dysphagia, RNA polymerase III and renal crisis would not encounter either usual of SSc criteria, but as soon as the scleroderma advanced beyond the fingers, the patient would fulfil both classifications. The 2013 SSc classification criteria may need some description and clarification (Van den hoogen *et al.,* 2013).

SSc pathophysiology is very multifaceted and incompletely clear. It's branded by various histological and cellular abnormalities, such as endothelial cells, fibroblasts, and cells of the immune systems such as "monocytes/macrophages, dendritic cells, and lymphocytes". Endothelial dysfunction is early appears to be the inducing factor, with endothelial activation triggered by anti-endothelial cells antibodies. Vasoconstriction is promoted by endothelial dysfunction due to endothelin-1 synthesis as an endogenous vasoconstrictors and decreasing endothelial vasodilators like nitric oxide and prostacyclin. Vasoconstriction modifies tissue oxygenation and the resulting hypoxia stimulates VEGF production, charted by reduction of progenitor endothelial cells. Cytokines derived from endothelial dysfunction (e.g. endothelin-1 and thrombin) exert direct fibrogenic effects by enhancing the proliferation and transformation of fibroblasts into myofibroblasts (*Hua-Huy and Dinh-Xuan, 2016).*

The real cause of systemic sclerosis (SSc) disease remains unidentified, but it is showed that the abnormalities in B cell characterized by autoantibody creation (Okano, 1996).

Abnormalities in blood homeostasis and B cell have been considered by activation and expansin of naive cells, but memory B cells reduced, as well as hyper-globulinemia and polyclonal B cell hyperactivity are thought

to be dramatic role in SSc illness furthermore, autoantibody production controlled by appearance of CD19, a serious B cells transduction signal molecule, that it is suggestively increased production and hyperactivity of naïve B cells in SSc patients (Sato *et al.,* 2000; Sato *et al.,* 2004). Thus, B cells was considered a significant influence on the fibrotic changes and growth development as labelled in scleroderma mouse models (Yoshizaki *et al.,* 2008).

The attendance of serum autoantibodies attacked to many intracellular antigens is a serological hallmark of SSc, more than 95% of these autoantibodies are presented in SSc patients as a supportive biomarkers for instituting an accurate early diagnosis of such disease with interesting that they are also associated with individual clinical divisions, exact patterns of organ envelopment, and different predictive features (Arnett *et al.,* 2010; Mehra *et al.,* 2013).

Anti-topoisomerase 1 (Scl-70), anti-centromere (ACA), and anti-RNA polymerase III are considered highly specific for SSc, and these were added to League against Rheumatism (ACR/EULAR) SSc classification criteria of the 2013 American College of Rheumatology/European (Van den hoogen *et al.,* 2013).

Anti-topoisomerase antibodies (Scl-70) are auto reactive antibodies focused toward topoisomerase enzyme that it is initiate in some diseases, most prominently in SSc as well as any diseases with Scl-70 positive. SSc is an autoimmune disease because of they reacted with self-proteins. They are also represented to as anti-DNA topoisomerase I antibody as a types of anti-nuclear autoantibody realized mainly in diffuse systemic sclerosis, but it is also seen in the more limited form of systemic sclerosis (CREST syndrome). Anti Scl-70 antibodies are related with more severe scleroderma disease (Mitchell *et al.,* 2007). Classification of anti-topoisomerase antibodies can be rendering to their immunoglobulin class (IgM, IgG, and IgA). IgG class is commonly present in scleroderma, with IgA being fairly mutual but IgM very rare (Hildebrandt *et al.,* 1990).

The strain on DNA relaxed by topoisomerase I enzyme that nicking and ligating the DNA. Anti-topoisomerase I antibodies (Scl-70) prevents the enzyme activity, because of this action happens in the cell nucleus, therefore Scl-70 is anti-nuclear antibody. SSc is determined by increasing collagen deposition in affected tissues, some studies prerogatives that there are an increasing topoisomerase I concentration in sites of the collagen genes, and that the transcription at these loci may be changes. Scl-70 associated with fast disease development as well as Scl-70 autoantibody in systemic lupus erythematosus are allied with nephritis (Hamidou *et al.,* 2006; Perera *et al.,* 2007).

Scl-70 autoantibodies are strappingly related to dcSSc and an unfortunate prognosis. Nonetheless, Scl-70 have also been described in patients with lcSSc. Systemic sclerosis patients with Scl-70 positive have a higher risk of cardiac and severe pulmonary envelopment. Scl-70 autoantibodies also related to joint involvement, digital ulcers, and tendon rubbing. The renal crisis has been reported but not reliably found in all SSc allies. Additionally, the existence of Scl-70 autoantibodies with Raynaud's phenomenon is prognostic in that they are concomitant with SSc development (Steen, 2005).

At least two diagnostic criteria of connective tissue diseases is established to diagnosis overlap syndrome. The most common association of systemic sclerosis with "Sjogren's syndrome, dermatomyositis or polymyositis, rheumatoid arthritis, and systemic lupus erythematosus". Primary Sjogren's syndrome is mutual with SSc about "68%", but the criteria of Sjogren's syndrome is achieve only in 14% of SSc patients (Balbir-Gurman and Braun-Moscovici, 2011).

One hundred and eighteen SSc patients were included in previous study. All SSc patients were questioned with respect to sicca symptoms. Levels of rheumatoid factor (RF), anti-nuclear antibodies (ANA), and anti-Ro and anti-La antibodies were measured; non-stimulated saliva amounts were recorded and Schirmer test and break-up time were applied to all patients. Minor salivary gland biopsy samples were obtained from those patients giving ≥ 3 positive answers to sicca symptom questions, patients with positive xerostomia/xerophthalmia test results, and patients with at least one antibody being positive. Patients presenting with grade 3 and/or grade 4 sialoadenitis based on Chisholm criteria were considered pathological (Kobak *et al.,* 2013).

2. Material and Methods:

This study carried out during the period from the middle of November 2015 until the end of March 2016 in Baghdad city, the sample of this study divided into two groups:

1- Forty systemic sclerosis patients: Those patients treated at Rheumatology department in Bagdad teaching hospital in Baghdad city.

2- Forty healthy control subjects, age matched with no signs and symptoms of any systemic diseases.

All patients diagnosed by a Rheumatology specialist as systemic sclerosis patients depending on the criteria of the ACR, 2013. All the subjects answered a written questionnaire regarding their name, age, gender, occupation, dental and medical histories, regularity of tooth-brushing, feeling of dry mouth and bad breath, any oral, dental and systemic diseases, complete medical history with clinical and physical examinations for each individual included in this study was performed by Rheumatologist.

Whole resting (Unstimulated) saliva was gathered under inactive circumstances ranged from 8.0-11.0 a.m. Any oral hygienic procedure should be avoided and patients were asked to clean their mouth with water, create

saliva in their oral cavity and spit into a test tube **(Navazesh, 1993)**.

The resulting supernatant from centrifuged saliva was stored at -20 °C in polyethylene tubes until analyzed. Saliva was centrifuge at "3000 rpm" for 10 minutes.

Serum sample:

About 5 ml of venous blood samples permitted to clot at room temperature for 2 hours, from each subject before centrifugation for 15 minute at 1000 rpm. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin

The quantitative determination of Human anti-SSA/Ro ELISA Kit used from Kono biotech Co. LTD company.

Proposed use:

For content determination in "serum, plasma, cell culture supernatant, tissue homogenate and any other biological fluid".

The quantitative determination of Human Scl-70 (anti-topoisomerase) ELISA Kit used from Kono biotech Co. LTD Company.

Proposed use: For content determination in "serum, plasma, cell culture supernatant, tissue homogenate and any other biological fluid".

2.2 Statistical analysis

The documents data transformed into a computerized record structure. A professional statistical guidance was sought for statistical analyses and computer aided by using "SPSS version 21.0 Statistical Package for Social Sciences". The statistical analysis includes:

- Descriptive statistic: The quantitative result was tested by Kolmogorov-Smirnov Z test to determine whether these data was parametric or non-parametric. The normally distributed data (parametric data) was suitably described by "mean, standard deviation (SD)" while non-parametric data was suitably described by "median and mean Rank"
- Inferential statistic: These were used in order to accept or reject the statistical hypothesis.
  - Independent sample t-test was used to test statistical differences between two parametric groups.
  - Mann-Whitney test was used to test statistical differences between two non-parametric groups.

3. Results and Discussion

In present study, the median and mean rank level of serum Scl-70 in SSc patients (215.91and 51.80) ng/ml using Mann-Whitney U test the data was reveal highly significantly increased (P<0.001) than that control group.

Serum autoantibodies are reflected the main biomarkers for the early and accurate diagnosis of SSc as well as related to individual clinical subsets and diverse prognostic features. Although SSc-related autoantibodies were historically considered to be commonly exclusive.

Serum Scl-70 increased in patients with SSc. This comes in agreement with (Reveille and Solomon, 2003).

The median and mean rank level of salivary Scl-70 in SSc patient (228.43 and 43.88) by using Mann-Whitney U test the data revealed non significantly difference (P<0.001) than that control group as shown in table (1, 2).

Table 1: The median and mean rank values of Serum Scl-70 in both SSc patients and control subjects

	Groups	Median	Mean Rank	P value
Serum Scl-70 ng/ml	patients	215.91	51.80	<0.001 **(HS)**
	Control	169.27	29.20	

HS: Highly significant
P< 0.001

Table 2: The median and mean rank values of Salivary Scl-70 in both SSc patients and control subjects.

	Groups	Median	Mean Rank	P value
Saliva Scl-70 ng/ml	patients	228.43	43.88	0.194
	Control	220.96	37.13	

The mean level of serum anti-SSA in SSc patients (140.4 ± 22.67) which was significantly increased (P<0.05) than that control group (113.1 ± 18.01) ng/ml by using t-test. The mean level of salivary anti-SSA in SSc patient (145.5 ± 19.98) ng/ml was significantly increase by using t-test than that control group (111.5 ± 15.47) ng/ml, as shown in table (3, 4) and figure (1).

In present study serum and salivary Anti Ro/SSA significant increase. In previous great, multicenter cohort study, were found that anti-Ro52 antibodies detected in "20% of 963" patients, making them the second

most common autoantibodies in systemic sclerosis, as well as overlapped with other disease specific auto reactive antibodies (Hudson *et al.,* 2012).

In present study was found highly significantly increased Scl-70, anti-SSA antibodies in saliva SSc patient that parallel with previous studies were showed that the presence of "circulating IgG class antibodies to citrullinated peptides (IgG-ACPA)" was extremely specific for rheumatoid arthritis (RA) (Van der Woude *et al.,* 2010).

Table 3: The mean values of different Serum anti-SSA in both SSc patients and control subjects.

	SSc (N=40)		Control (N=40)			
	mean	**SD**	**mean**	**SD**	**t-test**	**P-value**
Serum anti-SSA ng⁄dl	140.4	22.67	113.1	18.01	2.569	0.011 **(S)**

S: Significant
P< 0.05

Table 4: The mean values of different Salivary SSA in both SSc patients and control subjects.

	SSc (N=40)		Control (N=40)			
	mean	**SD**	**mean**	**SD**	**t-test**	**P-value**
Saliva anti-SSA ng⁄dl	145.5	19.98	111.5	15.47	3.459	0.014 **(S)**

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