Evaluation of Anti-Centromere Antibodies, Anti-SSA and Anti-SSB in Serum and Saliva of Patients with Systemic Sclerosis

Mohassad H Al-Mudhaffar B.D.S, M.Sc. (1)
Taghreed F. Zaidan B.D.S, M.Sc., Ph.D. (2)

ABSTRACT

Background: Systemic sclerosis (SSc) is a chronic autoimmune illness, which is consider by three main features: Sclerotic changes in the skin and internal organs, Vasculopathy of small blood vessels, Particular autoantibodies [1]. 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) [2]. Anti-centromere antibodies (ACA) are infrequent in rheumatic conditions and in healthy persons but occur commonly in limited systemic sclerosis (CREST syndrome), and rarely appeared in the diffuse form of systemic sclerosis [3]. Anti-Ro/SSA and antiLa/SSB, antibodies directed against Ro/La ribonucleoprotein complexes, can serve as a diagnostic hallmark of autoimmune disease specially Sjogren's syndrome [4].

Materials and methods: This study was carried out during the period from the middle of November 2015 until the end of November 2016 in Baghdad city. The sample of this study was divided into two groups : Forty systemic sclerosis patients: Those patients were treated at Rheumatology department in Baghdad teaching hospital in Baghdad city as well as Forty healthy control subjects, age matched with no signs and symptoms of any systemic diseases.

Results: The serum anti-SSA in SSc patient was significant increased as well as the salivary anti-SSA in SSc patient was highly significantly increased than in the control subjects by using t-test. The present study found that there no statically difference in salivary ACA, anti-SSB and serum anti-SSB while serum ACA was significantly increased.

Conclusions: autoantibodies play a role in pathogenesis of SSc patients represented by increased serum (ACA and anti-SSA) that it considered reliable indicator for SSc patients while unpredicted marker in saliva except anti-SSA. Anti-La/SSB is unreliable marker in both serum and saliva SSc patients. The presence of Anti-Ro/SSA antibodies in serum and saliva of SSc patient has been predictive marker for SSc overlapped Sjogren's syndrome.

Key word: systemic sclerosis. ACA, anti-SSA and anti-SSB. . **(J Bagh Coll Dentistry 2018; 30(3): 17-20)**

INTRODUCTION

Systemic sclerosis (SSc) is a chronic multi-organ complex autoimmune disease that causing the own tissue strike dawn by body's immune system; SSc was classified into two main types, according to the extent of skin involvement [5].

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

LeRoy et al., 1988 classified systemic sclerosis in subdivision 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 as shown in table 1 [6]. There are three main features of SSc, which have been integrated by 2013 SSc criteria but not all patient come with these features: 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, therefore RP increased statistical value to these criteria [7]. There are some features associated with SSc patients such as sclerodactly, anticentromere antibodies, Scl-70, Raynaud phenomena, dilated nailfold capillaries, dysphagia, and calcinosis.

(1) College of Dentistry, University of Kufa, Iraq
(2) Professor, Department Oral Diagnosis, College of Dentistry, University of Baghdad.

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 [7].

Changes caused by progressive systemic sclerosis extent from mouth to anus. In the mouth, the changes are in the following [8]. Microstomia (decreased mouth opening). It is produced due to skin sclerosis on the face that stretches the look of "the bird's face" which is one of the most characteristic features of patients with progressive systemic sclerosis. This may interfere considerably with eating, speaking, oral hygiene measures, and dental treatment, thus deteriorating the quality of life of these subjects [9]. Fibrosis of salivary glands may related to dryness in mouth of SSc patient (xerostomia).

1. Telangiectasia.
2. Trigeminal neuralgia.
3. Histological alterations are due to fibrotic changes in lamina propria, layer of submucosa, and muscular layer. Each portion of alimentary canal that containing smooth muscle can be attacked by progressive systemic sclerosis.
4. Blanching of the mucosa involving buccal mucosa, soft and hard palate [10].

5. Tongue rigidity and limited movement of tongue as shown in Figure [10].

Anti-Ro/SSA and antiLa/SSB, antibodies directed against Ro/La ribonucleoprotein complexes, can serve as a diagnostic hallmark of autoaimmune disease especially Sjogren's syndrome. Depending on the method applied for their identification, anti-Ro/SSA and anti-La/SSB antibodies detected in approximately 50 to 70% of pSS patients [4]. It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and is especially valuable in the young, old and in large scale screening and epidemiologic studies. it has found that saliva was used as a diagnostic aid in an increasing number of systemic diseases that can affect salivary gland function and composition. Therefore, a correct diagnosis will always require a full clinical and laboratory investigation. However, sialochemistry is a useful means of chronologically, monitoring qualitative and quantitative changes [11].

Table 1: Classification of Scleroderma [6].

Localized Scleroderma (Localized cutaneous fibrosis)
Limited or generalized morphea: Circumscribed patches of sclerosis Linear scleroderma: Linear lesions seen in childhood Encoup de sabre: Linear lesions of the scalp or face
Systemic Scleroderma (Cutaneous and noncutaneous involvement)
Limited cutaneous systemic sclerosis (lcSSc), formerly called CREST syndrome (*c*alcinosis of the digits, *R*aynaud's phenomenon, *e*sophageal dysmotility, sclerodactyly, and *t*elangiectasias) Diffuse cutaneous systemic sclerosis (dcSSc): Sclerosis of proximal extremities, trunk, and face Systemic sclerosis sine scleroderma (ssSSc): Organ fibrosis only; no skin thickening.

MATERIALS AND METHODS

This study carried out during the period from the middle of November 2015 until the end of November 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. Each subject was informed about the study purpose and her consent was obtained. Case sheet contained the information about name, gender, age, medical history, family history, and some oral manifestation that occurs in systemic sclerosis patients and related investigation was filled.

All patients with systemic sclerosis and control subjects were examined by using dental mirror and probe with artificial light. The examination would begin in systems according to W.H.O. that started from "upper and lower lip, upper and lower sulcus, retro-molar area, upper and lower labial mucosa, buccal mucosa, then hard and soft palate, dorsal margin and inferior surface of the tongue, floor of the mouth were also examined [12].

Detection of oral mucosal lesions and their features such as "duration, size, clinical description, location of lesion", and lastly the clinical diagnosis was identified.

The quantitative determination of Human ACA (anti-centromere), Human anti-SSA/Ro and anti-SSB/La ELISA Kits used from Kono biotech Co. LTD company.

The proposed use was for content determination in serum, plasma, cell culture supernatant, tissue homogenate and any other biological fluid.

The inclusion criteria were patients with diffuse cutaneous systemic sclerosis.

The exclusion criteria included:

1. Limited cutaneous systemic sclerosis
2. Patients previously diagnosed overlap syndrome.
3. Smoking patients
4. Patients with renal failures.

RESULTS

Serum and salivary ACA level:

The result in the present study showed that the median and mean rank level of serum ACA in SSc patients (361.59 and 49.28 ng/ml) was highly significant increased ($P<0.001$) than that the control subjects.

The median and mean rank level of salivary ACA in SSc patients were 411.19 and 41.63 ng/ml which was no significant difference ($P>0.05$) by using Mann-Whitney U test than that control subjects as shown in tables 2 and 3.

Serum and salivary Anti-SSA level:

The mean level of serum Anti-SSA in SSc patients (140.4 ± 22.67 ng/ml) which was significant increased ($P<0.05$) using t-test than that control subjects (113.1 ± 18.01 ng/ml).

The mean level of salivary Anti-SSA in SSc patients (145.5 ± 19.98) which was significant increased (P<0.05) by using t-test than that control subjects (111.5 ± 15.47 ng/ml), as shown in tables 4 and 5.

Serum and salivary Anti-SSB level:

The median and mean rank level of serum Anti-SSB in SSc patients (38.86 and 38.05 ng/ml) which showed no significant difference than that in the control subjects.

The median and mean rank level of salivary Anti-SSB in SSc patients (42.06 and 38.05 ng/ml), also showed no significant difference than that in the control subjects as in table (6 and 7).

Table 2: The median and mean rank difference of serum ACA (ng/ml) in SSc patients.

Groups	Median	Mean Rank	P value
Patients	361.59	49.28	0.001 (HS)
Control	313.38	31.73	

Table 3: The median and mean rank difference of saliva ACA (ng/ml) in SSc patients.

Groups	Median	Mean Rank	P value
Patients	411.19	41.63	0.665 (NS)
Control	411.31	39.38	

Table 4: The Mean and SD of serum Anti-SSA (ng/ml) in SSc patients.

Groups	Mean and SD	t-test	P-value
Patients	140.4 ± 22.67	2.569	0.011 (S)
Control	113.1 ± 18.01		

Table 5: The Mean and SD of saliva Anti-SSA in SSc (ng/ml) patients.

Groups	Mean and SD	t-test	P-value
Patients	145.5 ± 19.98	3.459	0.014 (S)
Control	111.5 ± 15.47		

Table 6: The median and mean rank difference of serum Anti-SSB (ng/ml) in SSc patients.

Groups	Median	Mean Rank	P-value
Patients	38.86	38.05	0.346 (NS)
Control	40.79	42.95	

Table 7: The median and mean rank difference of saliva Anti-SSB (ng/ml) in SSc patients.

Groups	Median	Mean Rank	P-value
Patients	42.06	38.05	0.364 (NS)
Control	42.85	42.95	

DISCUSSION

Serum and salivary ACA:

The present study showed that the ACA significantly increased in serum SSc patients than in the control subjects but salivary level remains not significant than in the control subjects.

Chung et al. stated that ACA significantly increased in serum SSc patients [13].

Anti-centromere antibodies are highly specific for scleroderma with limited cutaneous type "CREST syndrome"; therefore, the patients with primary Raynaud's phenomenon alone or with other connective tissue diseases can develop ACA autoantibodies [14-15].

There are no previous studies showed that the level of ACA in saliva SSc patients.

Serum and salivary Anti Ro/SSA and anti La/SSB:

In present study, serum and salivary anti-Ro/SSA were significantly increased.

In present study, serum anti-Ro/SSA was significantly increased. This came into agreement [16-19].

In previous great, multicenter cohort study, found that anti-Ro52 antibodies detected in "20% of 963" in serum patients, making them the second most common autoantibodies in systemic sclerosis, as well as overlapped with other disease specific auto reactive antibodies [17].

Anti-Ro antibodies found also in 3–11% of serum patients with SSc [18].

Brouwer et al. described the occurrence of anti-Ro52 antibodies in "dermatomyositis /Polymyositis and Scleroderma" [16].

Anti-Ro/SSA and anti-La/SSB antibodies are commonly in systemic lupus erythromatosis and Sjögren's syndrome patients and their presence is one of the criteria for the diagnosis and classification of Sjögren's syndrome. They are rare in the general population and in diseases other than Sjögren's syndrome and systemic lupus erythromatosis, although they can be detected in scleroderma, polymyositis, mixed connective tissue disease, and rheumatoid arthritis [19]

In present study, serum and salivary anti-La/SSB antibodies were non-significant difference than in the control subjects.

In present study, serum anti-La/SSB antibodies was non-significant difference than in the control subjects. Disagree with a previous study [20].

There are no previous studies showed that the level of anti-La/SSB antibodies in saliva patients with SSc but previous studies on other autoimmune disease as Sjogren's syndrome detected anti-La/SSB antibodies in saliva [21, 22].

The presence of anti-La/SSB antibodies in saliva has been predictive marker for Sjogren's syndrome as well as in the control of disease progression. [22]. The present study was suggested that Sicca syndrome in SSc predictive that patients overlapped with Sjogren's syndrome. This finding was in agreement with previous reported studies [23-25].

CONCLUSION

1. The levels of ACA, and anti-SSA significant increased in serum of SSc patients.
2. The levels of anti-SSA significant increased in saliva of SSc patients and predicted to SSc overlap Sjogren's syndrome.

REFERENCES

1. Ismet HB, Kryeziu A, Sherifi F. Oral manifestations of systemic sclerosis and correlation with anti-Topoisomerase I Antibodies (SCL-70). Med Arh. 2015; 69(3): 153-6.
2. Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum 2005; 35(1): 35-42.
3. Imboden JB, Hellmann DB, Stone JH. Current rheumatology diagnosis and treatment. 2nd Ed. New York: Lange Medical Books/McGraw-Hill. 2007
4. Routsias JG, Tzioufas AG. Sjogren's syndrome—study of autoantigens and autoantibodies. Clin Revi Allerg Immunol 2007; 32(3): 238–51.
5. Khanna D, Denton CP. Evidence-based management of rapidly progressing systemic sclerosis. Best Pract Res Clin Rheumatol. 2010; 24(3): 387-400.
6. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): Classification, subsets and pathogenesis. J Rheumatol 1988; 15(2): 202-5.
7. Van den Hoogen F, Khanna D, Fransen J, et al. Classification criteria for systemic sclerosis: an American College of Rheumatology/European league against rheumatism collaborative initiative. Arthritis Rheum 2013; 65(11): 2737-47.
8. Bajraktari IH, Kryeziu A, Sherifi F, Bajraktari H, Lahu A, Bajraktari G. Oral manifestations of systemic sclerosis and correlation with anti-topoisomerase I Antibodies (SCL-70). Med Arh. 2015; 69(3): 153-6.
9. Wada T, Ram S. Limited mouth opening secondary to diffuse systemic sclerosis. Case Reports Dentist. 2013; 2013, 3 pages.
10. Singh P, Kapoor S, Bither S. Oral manifestations in progressive systemic sclerosis: Case report. J Dentist Oral Hyg 2011; 3(7): 89-94.
11. W.H.O. Oral health survey, Basic methods. 4th ed. World Health Organization, Geneva, Switzerland 1987.
12. Sunil R, Nupur P, Kishore MA, Alok A. Sialochemistry – An Emerging Oral Diagnostic Tool. J Dent Sci Oral Rehab. 2013; 1-3.
13. Chung L, Lin J, Furst DE. Systemic and localized scleroderma. Clin Dermatol 2006; 24:374-92.
14. Schachna L, Wigley FM, Morris S, et al. Recognition of granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. Arthritis Rheum 2002; 46:1873-84.
15. Careta MF, Romiti R. Localized scleroderma: clinical spectrum and therapeutic update. An Bras Dermatol. 2015; 90 (1):62-73.
16. Brouwer R, Hengstman GJ, Vree Egberts W, Ehrfeld H, Bozic B, Ghirardello A, et al. Autoantibody profiles in the sera of European patients with myositis. Ann Rheum Dis 2001; 60:116-23.
17. Hudson M, Pope J, Mahler M, Tatibouet S, et al. Clinical significance of antibodies to Ro52/TRIM21 in systemic sclerosis. Arthr Res Therap. 2012; 14: 50.
18. Yoshimi R, Ueda A, Ozato K, Ishigatsubo Y. Clinical and Pathological Roles of Ro/SSA Autoantibody. Clinical and Developmental Immunology 2012; 2012, 12 pages.
19. Birtane M. Diagnostic role of anti-nuclear antibodies in rheumatic diseases Turk. J Rheumatol 2012; 27(2): 79-89.
20. Kobak S, Oksel F, Aksu K, Kabasakal Y. The frequency of sicca symptoms and Sjogren's syndrome in patients with systemic sclerosis. Int J Rheum Dis. 2013; 16:88–92.
21. Margaix-Muñoz M, Bagán JV, Poveda R, Jiménez Y, Sarrión G. Sjögren's syndrome of the oral cavity. Review and update. Med Oral Patol Oral Cir Bucal. 2009; 14: 325-30.
22. Deepa TN. Saliva as a potential diagnostic tool. Indian J Med Sci. 2010; 64(7): 293-306.
23. Avouac J, Sordet C, Depinay C, Ardizonne M, et al. Systemic sclerosis–associated Sjogren's syndrome and relationship to the limited cutaneous subtype. Arthrit Rheum 2006; 54(7): 2243-9.
24. Balbir-Gurman A, Braun-Moscovici Y. Scleroderma overlap syndrome. IMAJ. 2011; 13: 14-20.
26. Savarino E, Furnari M, Bortoli N. Gastrointestinal involvement in systemic sclerosis. Presse Med. 2014; 43: 279-91.
25. Baron M, Hudson M, Tatibouet S, et al. The Canadian systemic sclerosis oral health study III: Relationship between disease characteristics and oro-facial manifestations in systemic sclerosis. Arthritis Care Res 2015; 67(5): 681-90.