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Assessment of salivary composition and detection of immunological markers in serum and saliva of patients with systemic sclerosis in relation to oral findings

A thesis

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By

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Abstract

Background: Systemic sclerosis is a multi organs disorder with hyperactive immune system reflected by excessive sclerosis of skin and other affected organs. Cytokine released as basic procedures in systemic sclerosis pathogenesis as they convoluted in T and B cell activation leading to inflammation, autoantibodies, and microvascular injury, this entire event leading to fibrosis. The most important autoantibodies appeared significantly in those patients are anti-topoisomerase I autoantibody, anti-centromere autoantibody, and anti-RNA polymerase III autoantibody. 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.

Aims of the study: This study was designed to evaluate the oral findings of systemic sclerosis patients and correlate it to salivary composition changes as potential markers of autoimmune salivary gland dysfunction to assist in early diagnosis of sjogren syndrome. Investigate the changes of the whole saliva composition through measuring the concentrations of (calcium, sodium, potassium, inorganic phosphorus, and chloride, albumin and total protein) in systemic sclerosis patients and comparing the results with clinically healthy individuals. Investigate the changes of Interlukine-6, Anti-centromere antibodies, anti-topoisomerase I antibody, Anti-Ro(SSA), Anti-La(SSB) and Transforming growth factor beta 1 (TGF- β1) in saliva and serum of patients with systemic sclerosis.

Subjects, 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:

1- Forty diffuse cutaneous systemic sclerosis female patients: Those patients were treated at Rheumatology department in Baghdad teaching hospital in Baghdad city with age ranged 20-49 years.

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

The whole resting (Unstimulated) saliva was gathered under inactive circumstances ranged from 8.0-11.0 a.m. patients asked to create saliva in their oral cavity and spit into a test tube for 10 minutes**.** Saliva flow rate and pH were measured. The resulting supernatant from centrifuged saliva was stored at -80 °C in polyethylene tubes until analyzed.

About 5 ml of venous blood samples permitted to clot at room temperature for 2 hours from each subject before centrifugation then centrifuged at 3000 rpm. Serum was aspirated and stored at -80 °C until analysis.

The quantitative determination of Interlukin-6 and transforming growth factor beta 1 using kits that determined these human markers concentrations in serum, plasma and other biological fluids by ELISA technique.

The quantitative determination of Human anti-centromere, anti-topioisomerase, anti-SSA/Ro and anti-SSB/La, for content determination in "serum, plasma, cell culture supernatant, tissue homogenate and any other biological fluid".

Results: The present study stated that the main clinical findings was sclerodactyly which found in (97.5%), followed by history of Raynaud's phenomena which found in (92.5%), also digit deformity in (10%) and digit ulceration in (7.5%) of systemic sclerosis patients. Loss of wrinkles and tight skin were the main orofacial manifestations found in (92.5%) of systemic sclerosis patients, followed by microstomia and peak nose which were found in (87.5%) of SSc patients, while orofacial telangiectasia which found in (7.5%) and temperomandibular joint pain (0.25%) of those patients. The

mean value of interincisal distance at maximum mouth opening in systemic sclerosis patients was (28.7± 6.3) mm, while for the control subjects was (44.1±6.6) mm, which was highly significantly decreased than in the control subjects. Salivary flow rate and pH were highly significantly decreased in systemic sclerosis patients than in the control subjects. The median and mean rank level of salivary calcium in patients with systemic sclerosis (0.77 and 30.54) μmol/L was highly significantly decreased than in the control subjects. While concerning chloride was highly significantly increased among systemic sclerosis patients, than in the control subjects. Other salivary elements (sodium, potassium, inorganic phosphorus, albumin and total protiens) showed no significant difference than in the control subjects. The median and mean rank of serum and mean level of salivary interlukin-6 in systemic sclerosis patients were highly significantly increased ($P<0.001$) (18.27 and 55.93; 7.08 ± 1.98) pg/ml than in the control subjects. The median and mean rank levels of serum and salivary transforming growth factor beta 1 in systemic sclerosis patients were highly significantly increased ($P<0.001$) than in the control subject. Anti-topioisomerase antibody (anti-Scl-70) was highly significantly increased ($p<0.001$) in serum systemic sclerosis patients but was not significant in saliva samples with median and mean rank (215.91, 51.80, 37.13 and 43.22 respectively) ng/ml than in the control subjects. The mean level of serum anti-SSA in systemic sclerosis patients (140.4 ± 22.67) ng/ml that it was significant increased ($p<0.001$) as well as the mean level of salivary anti-SSA in systemic sclerosis patients (145.5 ± 19.98) ng/ml was highly significantly increased than in the control subjects. The present study found no significant difference in salivary anti-centromere, anti-SSB and serum anti-SSB while serum anti-centromere was significantly increased.

Conclusions: Cytokines may play a role in pathogenesis of systemic sclerosis patients represented by increased serum and salivary interlukine-6 and transforming growth factor beta 1. Autoantibody biomarker (anti-Scl-70,

anti-centromere) in serum is reliable indicator for systemic sclerosis patients, while unpredicted marker in saliva. Anti-La/SSB is unreliable marker in both serum and saliva of systemic sclerosis patients. The presence of Anti-Ro/SSA antibodies in serum and saliva of systemic sclerosis patients considered a predictive marker for systemic sclerosis overlapped Sjogren's syndrome. There were no correlation between above marker and salivary flow rate and interincisal distance at maximum mouth opening.