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Research article

Diagnostic value of Endothelin 1 as a marker for diagnosis of pulmonary parenchyma involvement in patients with systemic sclerosis

Masoud Nazemiyeh[1], Mehrzad Hajalilou[2], Mohsen Rajabnia[3], Akbar Sharifi[1] and Sabah Hasani[4,*]

[1] Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Connective Tissue Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran
[4] Lung Diseases and Allergy Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran

* **Correspondence:** Email: s.hasani@muk.ac.ir.

Abstract: *Background:* Systemic sclerosis (SSc) is a connective tissue disease and one of the manifestations associated with this disease is pulmonary involvement. Studies showed that endothelin 1 (ET-1) plays a role in pulmonary dysfunction due to SSc. The aim of this study was to determine the level of ET-1 in SSc patients and to evaluate its association with the early stages of pulmonary fibrosis. *Methods:* In this cross-sectional study, 48 patients with SSc were enrolled in the study. The stiffness of the skin was evaluated based on the modified Rodnan skin score. CT records, lung function tests and echocardiography were done. The samples were divided into two groups of patients with and without pulmonary parenchymal involvement. Blood sample was taken to measure the serum levels of ET-1 by ELISA method. ANOVA and Tukey HSD tests were used for statistical analysis. *Results:* According to pulmonary CT scan, 68.2% of patients had pulmonary involvement. There was no significant difference in the presence of honeycomb lesions and fibrosis in lung CT scan of the patients in case of gender (P = 0.819 and P = 0.356, respectively). There was no statistical difference between the results of spirometry in patients with anti-Scl-70 and anti-centromere test. The mean serum level of ET-1 in the studied population was 0.55 ± 0.232 pg/mL, in the men was 0.47 ± 0.14 pg/mL and in the women was 0.55 ± 0.24 pg/mL. There was no significant difference between the two groups in the case of gender and type of the disease (P = 0.475 and P = 0.150, respectively). The ROC diagram of serum level of ET1 in patients based on the type of the disease shows that the serum level of ET-1 of 55% has 50% sensitivity and 52.2% specificity. *Conclusion:* Serum level of ET-1 could not be used for screening of pulmonary involvement in SSc because its sensitivity and specificity is related to

pulmonary fibrosis, honeycomb lesions and disease form. Anti Scl-70 with low levels, DLCO, FVC, FEV1, TLC can be used to assess the possibility of pulmonary involvement and response to treatment.

Keywords: Endothelin Type 1; systemic sclerosis; pulmonary fibroses

1. Introduction

Systemic sclerosis (SSc) is a connective tissue disease with unknown etiology which its clinical manifestations are very wide and unpredictable. In systemic sclerosis, recurrent vasospasms and free radicals related endothelial dysfunction may eventually cause local thrombotic complications. The hallmark of SSc is autoimmunity, inflammation, extensive vasculopathy, progressive interstitial and perivascular fibrosis [1,2]. It is more common in women, and although it has a genetic background, it does not follow the Mendelian model [3]. Although the clinical outcome of SSc has improved due to medical progress, it remains an incurable disease, and its diffuse cutaneous type is associated with the highest risk of mortality among connective tissue diseases with a 10-year survival of 55% [4].

One of the manifestations associated with scleroderma is pulmonary involvement that is associated with poor prognosis and can even lead to death, which is because of right heart failure due to increased vascular resistance of lung [5]. Systemic pulmonary manifestations of SSc appear as idiopathic pulmonary fibrosis, organizing pneumonia, isolated peripheral pulmonary artery, aspiration pneumonia, and chest constriction [2]. Paranchymal lung involvement and pulmonary fibrosis generally show up in the first 5 years after the diagnosis by clinical symptoms such as coughing, dyspnea and impaired pulmonary function tests, and because of the slow progress and gradual manifestations of the disease, can diagnosed only by pulmonary function test as screening at early stages of the disease [6,7].

According to the National Institute of biomarkers, a biomarker is a characteristic that is a measurable indicator of normal biological process, pathogenic processes or pharmacologic response to therapeutic intervention. An ideal biomarker should reflect the underlying biological process, predict the clinical events and could be easily performed and replicated. Biomarker may contribute to identification of the pathological mechanisms responsible for the disease, and may be a screening method for predicting disease severity and determine the efficacy of treatment [8,9].

There are great evidences that show endothelin (ET) is important in the pathogenesis of SSc [10]. ET is a 21 amino acid peptide that is potent mitogen affecting smooth cells and fibroblasts and is associated with inflammatory processes [11]. Among the three endothelin isoforms, ET-1 is the main isoform in humans [12]. Increased levels of ET-1 are observed In SSc [13]. Also, increased expression of ET-1 in the skin of patients with premature active SSc is observed, and there is an increase in the binding of radiolabeled ET-1 to superficial vessels and dermal/epidermal junction in SSc skin compared with normal tissue [14]. It has been reported that ET receptor antagonists can improve the symptoms of severe pulmonary hypertension associated with SSc, and in less severe cases can delay the onset of symptoms, which indicate that ET plays a role in pulmonary dysfunction due to SSc [15,16].

According to the importance of SSc disease and the complications it causes, and because lung involvement is one of the major causes of morbidity and mortality in these patients, examination of pulmonary function and proper assessment of the condition of lung fibrosis is very important. Since ET-1 is a marker associated with fibrosis and its levels have undergone a change in SSc, the aim of

this study was to determination of the level of ET-1 in patients with systemic sclerosis and to evaluate its association with the early stages of pulmonary fibrosis in this patient.

2. Materials and methods

2.1. Studies population

In this cross-sectional study, 48 patients with SSc were diagnosed by a rheumatologist and enrolled in the study by a census method. A written letter of consent of inclusion in the plan was taken from all patients.

2.2. Inclusion and exclusion criteria

Patients with pulmonary arterial systolic pressure above thirty-five centimeters of water in echocardiography and had a history of smoking were excluded from the study.

2.3. Ethical issue

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences. Also, a written letter of consent of inclusion in the plan was taken from all patients.

2.4. Clinical evaluation

All patients were examined, and the stiffness of the skin was evaluated based on modified Rodnan Skin Score. In this method, the stiffness of the patient's skin was examined by clinical touch and the score was given between 0–3 (0 = normal stiffness, 1 = mild stiffness, 2 = moderate stiffness, 3 = severe stiffness so that skin is not wrinkled by pinching it). In this study, seventeen anatomic areas were examined, and the obtained scores were summed up and calculated. The maximum score in this method was 51 [17].

The presence of pulmonary crackles was evaluated by auscultation of the lungs. Lung CT records and lung function tests and echocardiography were evaluated. In previous cares, pulmonary respiratory testing, DLCO and 6MWD were performed for patients with dyspnea, cough, palpitations and crackles in the lung bottom areas. Lung HRCT scan was requested for patients with disorders in lung examinations including FVC less than 80 percent, DLCO less than 60%, 6MWD less than 300 meters, and the patients were divided into two groups of scleroderma patients with pulmonary parenchymal involvement in the form of pulmonary fibrosis and patients without pulmonary parenchymal involvement. Involvement percentage in pulmonary CT was classified based on the overall percentage of pulmonary involvement, including reticulation, ground glass pattern, violence of reticulation in two degrees including diffused (involvement ≥20%) and limited (involvement ≤20%). From all patients, 5 ml of blood sample was taken and the clotting was sent to measure levels of endothelin-1. Endothelin-1 was measured by ELISA method with monoclonal antibodies against endothelin-1. The normal range is 0.1–3 pg/ml. It should be noted that patients with pulmonary arterial systolic pressure above thirty-five centimeters of water in echocardiography and had a history of smoking were excluded from the study.

2.5. Statistical analysis

Data were analyzed by SPSS version 23 software. Descriptive methods (frequency, percentage, mean ± standard deviation) were used for statistical analysis. ANOVA and Tukey HSD tests were used for comparison.

3. Results

The demographic data showed that the mean age of the patients was 48.06 ± 9.5 years, mean height 157.7 ± 7.27 m, mean weight 66.4 ± 11.4 kg, mean BMI 27.02 ± 3.8, and mean duration of the disease was 7.45 ± 3.75 years. According to pulmonary CT scan, 68.2% of patients had pulmonary involvement. 58% of patients had impaired FVC and 85% had impaired TLC disorder. DLCO and 6MWD were less than 350 m in 77% and 27% of patients, respectively. In the CT scan, 68.8% of the patients had paranchymal pulmonary involvement, of which 50% had a limited type of pulmonary involvement, and 18.8% of the patients had diffuse involvement as fibrosis or GGO of more than 20%. 52.1% of patients had rale in lung auscultation and 6.2% of patients had heart murmur. 24 of the patients had diffuse cutaneous form, and 23 cases had limited cutaneous form of the disease.

There was no significant difference in the form of the disease between in case of gender ($P > 0.05$) (Table 1). Honeycomb lesions in lung CT scan, in 13 patients were limited and in 5 cases were diffuse, and in 30 cases, honeycomb lesions were not seen in lung CT scan. There was no significant difference between the presence of honeycomb lesions in lung CT scan of the patients based on gender ($P = 0.819$). In 18 patients, fibrosis in lung CT scan was limited and in 5 cases was diffuse, and in 21 cases there was no evidence of fibrosis in the lung CT scan. There was no significant difference in fibrosis in the lungs CT scan of the patients, based on gender ($P = 0.356$).

In 24 patients, anti-Scl-70 was positive. Based on the statistical test, there was no statistical difference in the spirometry finding based on positive/negative anti-Scl-70 ($P > 0.05$) (Table 2). In 2 patients, the anti-centromere was positive. Based on the statistical test, there was no statistical difference in the spirometry finding based on positive/negative anti-centromere (Table 3).

The mean serum level of ET-1 in the studied population was 0.55 ± 0.232 pg/ml, in the men was 0.47 ± 0.14 pg/ml and in the women was 0.55 ± 0.24 pg/ml. There was no significant difference between the two groups in the case of gender ($P= 0.475$). The mean serum level of ET-1 in patients with limited type of the disease was 0.60 ± 0.27 pg/ml and in patients with diffuse type of the disease was 0.50 ± 0.18 pg/ml and in this case, there was no significant difference between the two groups ($P = 0.150$). The mean serum level of ET-1 in patients with limited type honeycomb lesions in lung CT scan was 62.2 ± 0.26 pg/ml and in diffuse type was 0.43 ± 0.14 pg/ml and there was no significant difference between the two groups ($P = 0.067$). The mean serum level of ET1 in limited type patients with fibrosis in lung CT scan was 0.59 ± 0.21 pg/ml and in diffuse type was 0.41 ± 0.15 pg/ml, which was not significantly different ($P = 0.325$).

The ROC diagram of serum level of ET-1 in patients based on the type of the disease shows that the serum level of ET-1 of 55% has 50% sensitivity and 52.2% specificity (Figure 1).

Table 1. Results of the patient's spirometry in case of gender.

Variable	Gender		P value
	Male	Female	
FVC	96.76 ± 16.84	85.40 ± 19.71	0.223
FEVI	99.02 ± 15.91	83.08 ± 16.77	0.049
FEV1/FVC	85.40 ± 2.65	86.84 ± 11.51	0.783
RV	31.66 ± 19.59	64.46 ± 23.38	0.004
RV/TLC	43.32 ± 14.86	87.12 ± 27.01	0.001
TLC	70.22 ± 16.11	73.84 ± 14.68	0.607
DLCO	77.60 ± 37.29	70.05 ± 25.87	0.558

Table 2. Results of the patient's spirometry in case of anti-Scl-70.

Variable	Anti SCL-70		P value
	Positive	Negative	
FVC	79.40 ± 16.01	92.92 ± 15.42	0.013
FEVI	79.84 ± 14.89	87.69 ± 10.29	0.082
FEV1/FVC	89.39 ± 11.58	84.92 ± 11.98	0.254
RV	57.54 ± 20.89	69.65 ± 32.87	0.166
RV/TLC	81.40 ± 32.75	86.98 ± 28.91	0.592
TLC	69.50 ± 11.67	79.78 ± 14.25	0.019
DLCO	68.54 ± 24.21	72.87 ± 27.10	0.607

Table 3. Results of the patient's spirometry in case of anti-centromere.

Variable	Anti-centromere		P value
	Positive	Negative	
FVC	95.00 ± 2.83	84.04 ± 17.22	0.380
FEVI	93.50 ± 7.78	82.28 ± 13.79	0.266
FEV1/FVC	98.00 ± 5.66	87.11 ± 11.81	0.207
RV	83.00 ± 18.38	61.07 ± 26.49	0.258
RV/TLC	95.00 ± 21.21	82.93 ± 31.59	0.599
TLC	91.00 ± 1.41	72.50 ± 13.23	0.058
DLCO	75.00 ± 7.07	69.95 ± 25.73	0.786

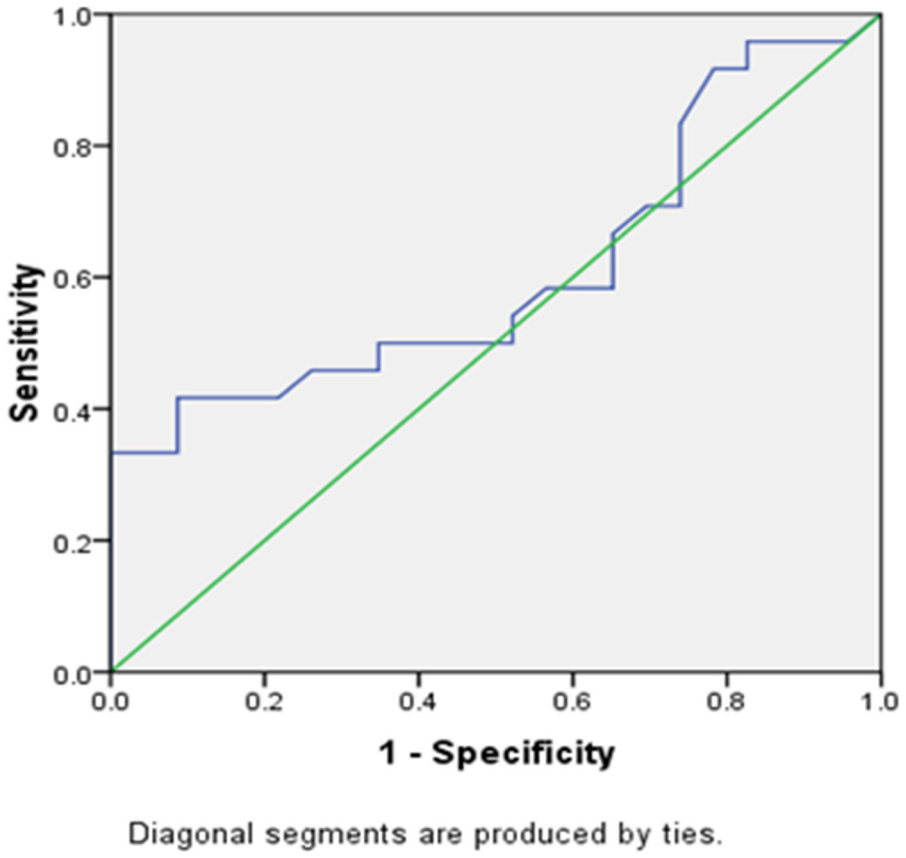

Figure 1. ROC diagram of serum level of ET1 in patients based on the type of the disease.

4. Discussion

In the current study, the mean age of patients was 48.06 ± 9.5 years. However, some studies such as Codullo et al. study [18] and the study of Szamosi et al. [19] reported the mean age of patients over 50 years. The cause of this difference can be an environmental and genetic factor in the disease, because researches have shown that these factors lead to the age variation of the onset of the disease, but in most studies the incidence of the disease is over 35 years old [20,21].

In this study, based on CT scan, 68.2% of patients had pulmonary involvement, of which 50% had a limited type of lung involvement, which was consistent with Mottaghi et al. study [22]. The prevalence of the lung parenchyma involvement is estimated between 35–53% [23,24], but the cause of the prevalence of more than 50% in our study can be due to the climate and race of the studied patients, which indicates the need for follow-up and more investigations of this complication.

Positive anti-Scl-70 was associated with decreases in FVC, FEV1, TLC and DLCO in comparison with negative patients, and its results were significant for TLC and FVC, which is consistent with the study of Guillen-Del et al. [25]. Previously, various medical reports have reported lower values of DLCO in patients with positive anti-Scl-70, that suggests a greater incidence of lung parenchyma involvement in patients with this antibody [26].

In the present study, the distance of 6MWD was reduced in patients with limited pulmonary involvement in CT scan. But it was not statistically significant which its reduction was consistent with

Whalley's study. In that study, ET-1 and its products were associated with cardio-pulmonary hemodynamics and 6MWD [27]. The use of CT scan to examine the involvement of the lung in patients is a selective and non-invasive method, but sometimes there are also non-specific and non-related results, which is due to the low sensitivity of this method.

In this study, 18.8% of patients had diffuse type of pulmonary involvement, which is consistent with Aghaei et al. study [28]. In a cohort study in 30 European and non-European countries of 120 centers, 3656 patients were enrolled in the study, of which 36.9% had diffuse form and others had limited form of the disease [29]. Yamane's study also showed that ET-1 serum levels in cases with diffuse form was significantly higher than those with limited form [30]. Pulmonary involvement leads to an increase in mortality and morbidity in patients with scleroderma, in which 84–88% of deaths in these patients were reported after pulmonary complications.

In the present study, the serum level of ET-1 in the group with limited pulmonary involvement increased compared to patients without pulmonary involvement, which was consistent with the study of Vancheeswaran et al. [31], but was not consistent with the study of Horowitz et al. in the United States, which was done with the aim of measurement the level of ET-1 in patients with lung fibrosis. In the mentioned study, the level of ET-1 was significantly higher in patients with lung fibrosis [32]. Also, Cozzani et al. found that ET-1 levels in patients were not associated with skin ulcers, and decreased with anti-endothelin therapy that was not consistent with the present study [33]. The difference between the results of this section and the two mentioned studies can be related to the duration of systemic sclerosis in patients.

5. Conclusion

The present study showed that there was no significant relationship between ET-1 level and pulmonary parenchyma involvement, severity of the pulmonary parenchyma involvement and the severity of DLCO and 6MWD involvement, and the serum level of ET-1 could not be used for screening. Because its sensitivity and specificity is related to pulmonary fibrosis, honeycomb lesions and disease form. Anti Scl-70 with low levels, DLCO, FVC, FEV1, TLC can be used to assess the possibility of pulmonary involvement and response to treatment.

Conflict of interest

The authors declare no conflict of interest.

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