Rheumatology International (2019) 39:1191–1200
https://doi.org/10.1007/s00296-019-04306-8

Rheumatology
INTERNATIONAL

OBSERVATIONAL RESEARCH

Epicardial adipose tissue thickness in systemic sclerosis patients without overt cardiac disease

Duygu Temiz Karadag[1] · Tayfun Sahin[2] · Senem Tekeoglu[1] · Ozlem Ozdemir Isik[1] · Ayten Yazici[1] · Ayse Cefle[1]

Received: 28 January 2019 / Accepted: 15 April 2019 / Published online: 25 April 2019

Abstract

Systemic sclerosis is associated with an increased prevalence/incidence of coronary artery disease. The aim of this study was to investigate epicardial adipose tissue (EAT) thickness which may contribute to cardio-metabolic risk in systemic sclerosis (SSc) patients without overt cardiac disease. EAT thickness was measured by transthoracic conventional Doppler echocardiography and compared in SSc patients ($n=47$) and age- and sex-matched healthy controls ($n=36$). The relationships between EAT thickness and markers of cardio-metabolic risk in SSc were examined. EAT thickness was significantly greater in patients with SSc compared to healthy controls (6 [7–5] vs 5 [6.75–3.25], $p=0.041$). Compared to controls, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), leukocyte, neutrophil, B-type natriuretic protein (BNP), fasting plasma insulin and HOMA-IR were elevated (18 [31–10] vs 8.5 [18–4], $p<0.001$; 0.4 [0.67–0.18] vs 0.21 [0.48–0.09], $p=0.012$; 7510 [8731–5990] vs 6435 [7360–5195], $p=0.002$; 4350 [5440–3570] vs 3390 [4168–2903], $p<0.001$; 111 [185–74] vs 70 [127–70], $p=0.010$; 6.7 [10.5–4.7] vs 4.7 [6.8–4.1], $p=0.008$; 1.7 [2.6–1] vs 1.1 [1.7–0.9], $p=0.015$, respectively). The total and low-density lipoprotein (LDL)-cholesterol were decreased in SSc patients (197 ± 45 vs 284 ± 36, $p=0.005$; 118 [148–84] vs 140 [180–115], $p=0.003$, respectively). In patients with SSc, the EAT thickness correlated positively with age, ESR, CRP, insulin, hemoglobin A1c and total and LDL-cholesterol ($r=0.574$, $p<0.001$; $r=0.352$, $p=0.015$; $r=0.334$, $p=0.022$; $r=0.290$, $p=0.048$; $r=0.317$, $p=0.030$; $r=0.396$, $p=0.006$ and $r=0.349$, $p=0.016$, respectively). Our study confirms that EAT thickness is greater in SSc patients compared to healthy controls using echocardiographic measurements. The results of our study suggest that EAT thickness is a candidate for atherosclerotic risk assessment in SSc.

Keywords Cardiovascular disease · Echocardiography · Epicardial adipose tissue thickness · Systemic sclerosis

✉ Duygu Temiz Karadag
dr_dtemiz@hotmail.com

Tayfun Sahin
tayfunsa@yahoo.com

Senem Tekeoglu
senemtekeoglu@gmail.com

Ozlem Ozdemir Isik
luska80@hotmail.com

Ayten Yazici
burakdefy@hotmail.com

Ayse Cefle
acefle@hotmail.com

[1] Division of Rheumatology, Department of Internal Medicine, Kocaeli University School of Medicine, Umuttepe-İzmit 41380, Kocaeli, Turkey

[2] Division of Cardiology, Kocaeli University School of Medicine, Umuttepe-İzmit, Kocaeli, Turkey

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by vascular abnormalities and immune dysfunction, leading to fibrosis of the skin and internal organs [1]. Based on the results from epidemiological studies, in SSc patients, approximately one-third of deaths not directly attributable to the SSc are known to be caused by cardiac disease and this occurs more than one decade earlier compared to the general population.

Cardiac involvement in SSc is variable and silently progressive until overt clinical manifestations occur. The prevalence of cardiac disease varies depending on its definition and the methods used for diagnosis. Although cardiac involvement is often clinically occult, myocardial involvement is estimated to affect almost all of the patients with SSc depending on the sensitivity of the diagnostic method [2].

The main etiologic factors of cardiac involvement are usually identified as myocarditis, fibrosis, pulmonary hypertension and blood vessel abnormalities [3]. The patchy myocardial fibrosis with contraction band necrosis, which is reported to develop as a response to an ischemia–reperfusion insult from microvascular involvement, increases the risk of conduction abnormalities. An increase in the prevalence of atherosclerotic vascular disease affecting coronary arteries, carotid arteries, cerebrovascular vessels, and peripheral arteries has been reported in SSc [4]. The factors which may contribute to atherosclerosis in SSc are not well-understood [5]. However, chronic inflammation, microvasculopathy, altered lipid profile, treatment agents (corticosteroids, etc.) and age have been reported to be related to increased risk of cardiovascular disease [6, 7]. In autoimmune diseases, chronic inflammation is a well-known and widely reported cause of accelerated atherosclerosis, mainly related to the secretion of inflammatory cytokines or oxidative stress [8–10].

Adipose tissue has been recognized as an endocrine and paracrine organ, producing a variety of molecules that play a role in energy metabolism, inflammation and immunologic responses [11, 12]. Based on previous studies, EAT has been suggested to be a promising marker for increased cardio-metabolic risk [13]. EAT is the inner layer of the pericardium located on the free wall of the right ventricle and is also found extending from the surface of the myocardium to the adventitia of the coronary arteries [14]. Echocardiography has been previously validated for the assessment of EAT thickness [15].

The purpose of this study was to investigate EAT thickness by echocardiography in SSc patients without overt cardiovascular disease. We also aimed to assess its association with organ involvement due to SSc and laboratory and metabolic parameters related to cardiovascular risk.

Materials and methods

Study population

Forty-seven patients who fulfilled the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) SSc classification criteria [16] and 36 gender- and age-matched healthy subjects were selectively enrolled in the study. The patients were classified into limited or diffuse cutaneous SSc (lcSSc) according to LeRoy's criteria [17]. The extent of the skin involvement was evaluated by using the modified Rodnan skin score (mRSS) by a single rheumatologist [18]. The severity of disease was assessed by Medsger severity scale [19] and activity with the European Scleroderma Trials and Research Group (EUSTAR) activity index [20]. The disease duration was defined from the onset of first non-Raynaud's phenomenon (RP) symptom related to SSc. Patients with known diabetes mellitus (i.e., patients with a history of diabetes who were on a diabetic diet or on treatment with oral hypoglycemic drugs or insulin, with fasting blood glucose > 126 mg/dl), chronic renal disease (creatinine > 1.3 mg/dl), liver disease, thyroid dysfunction, respiratory disorders (asthma, chronic obstructive pulmonary disease), hypertension (i.e., patients who were on treatment with antihypertensive drugs, with systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg [21]), overt cardiac disease (a history of angina pectoris, coronary artery disease, acute coronary syndrome or coronary revascularization, rhythm and conduction disorders, valvular heart disease, pacemaker, prosthetic heart valves, tachycardia, stroke, left ventricular systolic dysfunction with LV ejection fractions < 55%), pulmonary arterial hypertension diagnosed by right heart catheterization, peripheral artery disease or patients on treatment with glucocorticoid treatment were excluded. Patients with a pulmonary artery pressure (PAP) greater than 45 mmHg indirectly calculated by measuring the Doppler flow of the tricuspid regurgitant jet on echocardiography were excluded because of the strong correlation between right heart catheterization and this estimated cut-off level [22].

The study was approved by the local ethics committee and conducted in accordance with the principles of the World Health Organization-Declaration of Helsinki. Written informed consent was obtained from all patients and controls.

Metabolic and laboratory parameters

The biochemical, haematologic and immunologic test results including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), plasma fasting glucose and insulin, hemoglobin A1c (HbA1c), cholesterol, uric acid, B-type natriuretic peptide (BNP), homocysteine, anti-nuclear antibodies (ANA), anti-centromere antibodies (ACA) and anti-topoisomerase I antibodies (Scl-70) autoantibodies were obtained from blood samples after 12 h of fasting in both groups. ANA indirect immunofluorescent (IIF) testing was performed and evaluated by two experienced physicians at our laboratory.

Insulin resistance was estimated using the homeostasis model assessment of insulin resistance index (HOMA-IR) formula [(fasting insulin (μU/l) × fasting glucose (mmol/l)]/22.5] [23]. Metabolic parameters including waist circumference, weight and height were measured and body mass index (BMI) was calculated. Those patients with a BMI of < 18.5 kg/m^2 were considered underweight, 18.5–24.9 kg/m^2 normal, 25–29.9 kg/m^2 overweight and ≥ 30 kg/m^2 obese [24].

Metabolic syndrome was defined based on the National Cholesterol Education Program Adult Treatment Panel

(NCEP) III criteria. Three or more of the following NCEP criteria needed to be met in order to be classified as having Metabolic syndrome: waist circumference > 102 cm in men and > 88 cm in women, triglycerides ≥ 150 mg/dl, HDL < 40 mg/dl in men and < 50 mg/dl in women, high blood pressure ≥ 130/85 mmHg or use of antihypertensives, and fasting glucose ≥ 110 mg/dl [25].

Baseline echocardiography and assessment of epicardial adipose tissue thickness

All subjects were imaged in the left lateral decubitus position with a commercially available system (VIVID 7, General Electric-Vingmed Ultrasound, Horten, Norway) using a 3.5-MHz transducer. Images were stored in GE echopacs (Vingmed Ultrasound, Horten, Norway) system. Basal echocardiographic measurements including chamber diameters, wall thicknesses, and mitral velocities were measured according to previous guidelines [26]. Ejection fraction (EF) was measured with the modified biplane Simpson's method from the apical 4- and 2-chamber views [27].

A previously validated method was used to asses EAT thickness in captured images [15]. Briefly, EAT thickness was measured on the free wall of right ventricle from the parasternal long-axis view. The aortic annulus was used as the reference point. Echo-free space between the echo-dense pericardial layers on two-dimensional echocardiography was measured perpendicularly on the ahead of the right ventricle free wall at the end of diastole. After the measurement of two beats, maximum EAT thickness values were measured and the average value was obtained. This method was shown to be strongly correlated with various metabolic markers.

Intra-observer variability

All echocardiographic studies and measurements were performed by an experienced cardiologist (T.S.) who was blinded to previously obtained data. In our laboratory, the intra-observer variability was $r = 0.98$ for two-dimensional; and M-mode echocardiographic measurements, and $r = 0.97$ for Doppler measurements.

Statistics

Descriptive statistics for clinical and demographic characteristics of the patients were presented as frequency and percentage (%) for categorical variables and mean with standard deviation (mean ± SD) or median with interquartile range (median [Q3–Q1]) according to the distribution of the continuous variables.

The normality was assessed both visually and through Shapiro–Wilk test. The independent samples *t* test was used to evaluate the intergroup differences for the variables which were normally distributed (age, waist circumference, body mass index, hemoglobin, fasting plasma glucose, total cholesterol, HDL-cholesterol, LV mass index, LVESD, and TAPSE). For the parameters which were not normally distributed, Mann–Whitney *U* test (Wilcoxon rank sum test) or Kruskal–Wallis test was used as appropriate. Pearson Chi-square test was applied to analyze the categorical variables between SSc and controls. Conventional echocardiography data was tested with independent sample *t* test or the Mann–Whitney *U* test (Wilcoxon rank sum test) between SSc and controls.

Among patients with SSc, the relationships between EAT thickness and anthropomorphic measures, demographics, disease activity, inflammation, cardiovascular risk factors, and severity index were assessed. Spearman's rank correlation coefficients were used to calculate the bivariate relationships between categorical and continuous variables and EAT thickness. Independent associations were examined by multiple linear regression with adjustment for age and sex as covariates. EAT thickness was not normally distributed and was therefore corrected by log-transformation. A multivariable analysis to estimate predictors of EAT was performed using variables found to be significantly different between patients and controls on univariate analysis.

Statistical analyses were performed using "SPSS version 20.0 software package" (IBM Inc., Chicago, IL, USA). Two-sided *p* values less than 0.05 were considered statistically significant ($p < 0.05$).

Results

Baseline characteristics of the study subjects

A total of 83 participants (47 SSc patients, 36 control subjects) were included in the study. The clinical and laboratory features of SSc patients are summarized in Table 1. The mean disease duration was 8.51 ± 5.9 years and 70.2% of the patients had limited disease subset. None of the patients had severe organ involvements, including interstitial lung disease (ILD), pulmonary hypertension (PHT) or scleroderma renal crisis. During the study, a total of 13 patients (27.7%) underwent immunosuppressive treatment.

Comparison of demographics and laboratory parameters of the study subjects

The main characteristics of the study population are detailed in Table 2. There were no differences between the groups in terms of age, gender, body mass index (BMI), waist circumference, current smokers, homocysteine, uric acid, triglycerides (TG), fasting plasma glucose and HbA1c. ESR, CRP, leukocyte and neutrophil counts and BNP

Table 1 Clinical characteristics, laboratory features and severity score index of SSc patients

SSc ($n=47$)				
LcSSc	33 (70.2%)			
Disease duration, years	8.51 ± 5.9			
Raynaud's phenomenon	47 (100%)			
Sclerodactyly	35 (74.5%)			
Fingertip ulcers	12 (25.5%)			
Pitting scars	30 (63.8%)			
Telangiectasia	30 (63.8%)			
Dyspnea	13 (28.3%)			
Gastrointestinal involvement	21 (44.7%)			
Inflammatory arthritis	2 (4.3%)			
Interstitial lung disease	13 (27.7%)			
Immunosuppressive treatment	13 (27.7%)			
Cyclophosphamide	2 (4.3%)			
Methotrexate	9 (19%)			
Azathioprine	2 (4.3%)			
FVC (%)	93.4 ± 15			
DLCO (%)	67.5 ± 15.5			
Anti-nuclear antibodies (positive)	44 (93.6%)			
Anti-centromere antibodies (positive)	21 (44.7%)			
Anti-topoisomerase I antibodies (positive)	14(29.8%)			
mRSS	11.5 ± 7.2			
EUSTAR activity index	1.71 ± 1.11			
Medsger severity scale	Normal	Mild	Moderate	Severe
General	28 (59.6%)	12 (25.5%)	6 (12.8%)	1 (2.1%)
Peripheral vascular	1 (2.1%)	25 (53.2%)	9 (19.1%)	12 (25.5%
Skin	–	37 (78.7%)	9 (19.1%)	1 (2.1%)
Joint/tendon	41 (87.2%)	4 (8.5%)	2 (4.3%)	–
Muscle	47 (100%)	–	–	–
GI tract	42 (89.4%)	5 (10.6%)	–	–
Lung	12 (25.5%)	24 (51.1%)	11 (23.4%)	–
Heart	47 (100%)	–	–	–
Kidney	47 (100%)	–	–	–

Data are expressed as mean ± SD or percentile as appropriate

LcSSc limited cutaneous systemic sclerosis, *FVC* forced vital capacity, *DLCO* diffusing capacity for carbon monoxide, *mRSS* modified Rodnan skin score

concentration were significantly higher in patients with SSc compared to the control group (18 [31–10] vs 8.5 [18–4], $p<0.001$; 0.4 [0.67–0.18] vs 0.21 [0.48–0.09], $p=0.012$; 7510 [8731–5990] vs 6435 [7360–5195], $p=0.002$; 4350 [5440–3570] vs 3390 [4168–2903], $p<0.001$; 111 [185–74] vs 70 [127–70], $p=0.010$, respectively). The fasting plasma insulin and HOMA-IR were significantly higher (6.7 [10.5–4.7] vs 4.7 [6.8–4.1], $p=0.008$; 1.7 [2.6–1] vs 1.1 [1.7–0.9], $p=0.015$, respectively); and total cholesterol and low-density lipoprotein cholesterol (LDL-C) were significantly lower in SSc patients than healthy controls (197 ± 45 vs 284 ± 36, $p=0.005$; 118 [148–84] vs 140 [180–115], $p=0.003$, respectively).

At our laboratory, the normal adult range for leukocyte, neutrophil, and CRP are $3.6–10.2\times10^3$/μl, $1.7–7.6\times10^3$/μl and < 0.5 mg/dl, respectively. Among the patients with SSc ($n=47$), 2 (4.3%) had leukocyte count > 10.2×10^3/μl and 2 (4.3%) had neutrophil count > 7.6×10^3/μl. The comparison of the numbers of the subjects with leukocyte and neutrophil counts over the normal range were not statistically significant between SSc and healthy controls. Among 47 patients with SSc, 17 (36.2%) had CRP > 0.5 mg/dl and among 36 healthy controls 7 (19.4%) had CRP > 0.5 mg/dl. The patients with SSc were not different from controls in terms of having CRP > 0.5 mg/dl ($p=0.077$). The markers of inflammation (leukocyte, neutrophil, and CRP) were

Table 2 Demographics and clinical features of SSc patients and controls

	SSc ($n=47$)	Healthy controls ($n=36$)	p
Age, years (mean ± SD)	52.1 ± 12.4	49.4 ± 8.4	0.256
Female, *n* (%)	42 (89.4%)	33 (91.7%)	1.000
Systolic BP, mmHg (mean ± SD)	120 [120–110]	123 [124–110]	0.902
Diastolic BP, mmHg (mean ± SD)	73 [80–70]	75 [84–70]	0.934
Height, cm (mean ± SD)	164 [165–159]	163 [165–160]	0.934
Weight, kg (mean ± SD)	72 [83–61]	68 [78–66]	0.360
Waist circumference, cm (mean ± SD)	86 ± 12	89 ± 8.5	0.174
Body mass index, kg/m^2 (mean ± SD)	27.4 ± 4.8	26 ± 2.2	0.148
Smoking (current) [*n* (%)]	11 (23.4)	12 (33.3)	0.335
ESR, mm/h	18 [31–10]	8.5 [18–4]	< 0.001
CRP, ng/ml	0.4 [0.67–0.18]	0.21 [0.48–0.09]	0.012
Leukocyte, 10^3/µl	7510 [8731–5990]	6435 [7360–5195]	0.002
Neutrophil, 10^3/µl	4350 [5440–3570]	3390 [4168–2903]	< 0.001
Hemoglobin, g/dl	12.8 ± 1.7	12.8 ± 1.2	0.916
Homocysteine, mg/dl	12.7 [5.3–9.7]	11.3 [13.2–10]	0.220
Brain natriuretic peptide, mg/dl	111 [185–74]	70 [127–70]	0.010
Uric acid, mg/dl	4.1 [4.8–3.4]	3.9 [4.4–3.2]	0.111
Galectin-3, mg/dl	6.7 [8.3–5.5]	7.6 [9.1–6.5]	0.096
Fasting plasma glucose, mg/dl	95.4 ± 12.7	92.5 ± 8.9	0.247
Insulin, mg/dl	6.7 [10.5–4.7]	4.7 [6.8–4.1]	0.008
HOMA	1.7 [2.6–1]	1.1 [1.7–0.9]	0.015
HbA1C, %	5.5 [5.9–5.3]	5.3 [5.7–5.1]	0.100
Total cholesterol, mg/dl	197 ± 45	284 ± 36	0.005
LDL-cholesterol, mg/dl	118 [148–84]	140 [180–115]	0.003
HDL-cholesterol, mg/dl	52.4 ± 15.2	52.7 ± 13	0.936
Triglyceride, mg/dl	104 [143–81]	120 [139–81]	0.578

Data are expressed as mean ± SD, median [IQR] or percentile as appropriate

BP blood pressure, *BMI* body mass index, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *HOMA-IR* homeostatic model assessment of insulin resistance

higher in patients with SSc compared to controls. However, there were no differences between the two groups in terms of having any parameters over the normal range.

When we tested the number of the participants with a BMI ≥ 30, among 47 SSc patients 20 (42.6%) were obese and among 36 healthy controls, 1 (2.8%) was obese. The obesity was significantly higher in SSc compared to controls ($p < 0.001$). EAT did not differ between limited and diffuse disease subsets (6 [7–5] vs 6 [7.25–4.75], $p = 0.499$).

Comparison of conventional echocardiography data of the study subjects

The conventional echocardiographic measurements displayed no difference between the two groups (Table 3). EAT thickness was significantly higher in patients with SSc compared to the control group (6 [7–5] vs 5 [6.75–3.25], $p = 0.041$) (Fig. 1). There was no other significantly different echocardiographic parameter between the patients and controls.

Associations between EAT thickness and cardio-metabolic risk factors, anthropomorphic and inflammatory parameters in SSc patients

There was a positive correlation between the EAT thickness and cardiovascular risk factors including age, ESR, CRP, insulin, HbA1c, total cholesterol and LDL ($r = 0.574$, $p < 0.001$; $r = 0.352$, $p = 0.015$; $r = 0.334$, $p = 0.022$; $r = 0.290$, $p = 0.048$; $r = 0.317$, $p = 0.030$; $r = 0.396$, $p = 0.006$ and $r = 0.349$, $p = 0.016$, respectively) in patients with SSc. In healthy subjects, no relationship between the EAT thickness and these parameters, except a positive correlation with CRP ($r = 0.463$, $p = 0.004$), was demonstrated. There was a negative correlation between the EAT thickness and the lung domain of the Medsger severity scale ($r = -0.575$, $p > 0.001$). After adjustment for age and sex, significant associations with EAT thickness remained for insulin ($p = 0.009$) and the lung domain of the Medsger severity scale ($p = 0.004$), and a previously hidden association with HOMA-IR was detected ($p = 0.016$). The correlations

Table 3 Conventional echocardiography data of SSc patients and healthy controls

	SSc ($n=47$)	Controls ($n=36$)	p
EAT, mm	6 [7–5]	5 [6.75–3.25]	0.041*
LVEDV, ml	89 [103–79]	93 [108–79]	0.343
LVESV, ml	21 [29–18.6]	22 [28–19]	0.897
LV mass index, g/m^2	86 ± 19	82 ± 18	0.330
LVEDD, cm	4.4 [4.7–4.2]	4.5 [4.8–4.2]	0.292
LVESD, cm	2.6 ± 0.33	2.5 ± 0.34	0.698
LVEF (%)	74 [77–71]	76 [78–73]	0.058
E, m/s	0.78 [0.87–0.70]	0.85 [0.95–0.70]	0.186
A, m/s	0.80 [0.93–0.63]	0.71 [0.83–0.64]	0.106
Deceleration time, ms	207 [226–191]	196 [220–182]	0.081
E/*A*, ratio	0.88 [1.35–0.72]	1.16 [1.36–0.87]	0.149
E′, cm/s	0.08 [0.11–0.07]	0.10 [0.11–0.08]	0.088
E/*E′*, ratio	9 [11–7.1]	8.9 [9.6–7.1]	0.361
PASP, mmHg	0 [25–0]	0 [25–0]	0.525
TAPSE	21 ± 3.9	21 ± 4.6	0.704

Data are expressed as mean ± SD except where indicated otherwise

EAT epicardial adipose tissue, *LV* left ventricular, *LVEDV* left ventricular end diastolic volume, *LVESV* left ventricular end systolic volume, *LVEDD* left ventricular end diastolic diameter, *LVESD* left ventricular end systolic diameter, *LVEF* left ventricular ejection fraction, *A* late peak mitral inflow velocity, *E′* early diastolic velocity at basal mitral annulus, *E*/*E′* ratio of peak early diastolic velocity to early diastolic velocity at basal mitral annulus, *PASP* pulmonary arterial systolic pressure, *TAPSE* tricuspid annular plane systolic excursion

* $p < 0.05$

between EAT and anthropomorphic, inflammatory and metabolic parameters are shown in Table 4. Disease duration correlated positively with EAT thickness, but it was not observed after adjustment for age and sex. To create a multivariable regression model predictive for the EAT thickness, we decided to include ESR, CRP, insulin, HbA1c, LDL-cholesterol among the variables which were found significantly different between patients and controls on univariate analysis. We dropped the lung domain of Medsger severity scale because of our concern about the accuracy of the result in our study population. Also, we excluded total cholesterol from the analysis because of the collinearity with LDL-cholesterol. The results of this analysis revealed that about 30% of the total variability in logEAT is explained by the variables (adjusted R square = 0.322). The model had explanatory power (ANOVA table, $F=4.646$, $p=0.001$). The age and insulin had predictive ability for logEAT ($p=0.001$; $p=0.015$, respectively). When we ran the regression model in healthy subjects we did not detect significant results (ANOVA, $F=2.289$, $p=0.063$).

Discussion

Patients with SSc were shown to be at increased risk for cardiovascular disease on which the impact of the disease itself may be greater even than that of hypertension or diabetes [28]. In a study, the contribution of traditional cardiovascular risk factors was small, and the other disease-related factors such as disease duration, renal involvement, and pulmonary arterial hypertension were more involved in cardiac disease [29].

The main finding in our study was the increased burden of EAT in patients with SSc, compared to healthy subjects. To the best of our knowledge, this is the first study to investigate EAT thickness in SSc patients by using echocardiography. In the literature, there is only one study which evaluated the epicardial fat volume (EFV) with coronary computed tomography angiography in SSc. In their study, the EFV was reported to be greater in SSc than healthy subjects and found to be associated with the presence and severity of SSc, independent of the cardiovascular risk factors [30]. The severity of SSc was defined by the presence of pulmonary arterial hypertension (PAH) and it was found to be associated with higher EFV. Unlike their study, we used an echocardiography technique and excluded patients with PAH to avoid its possible confounding effect.

EAT is associated with the release of many pro-inflammatory chemokines that suggest a particular association with inflammation [31, 32]. Additionally, epicardial adipose tissue is known to secrete inflammatory cytokines sufficient

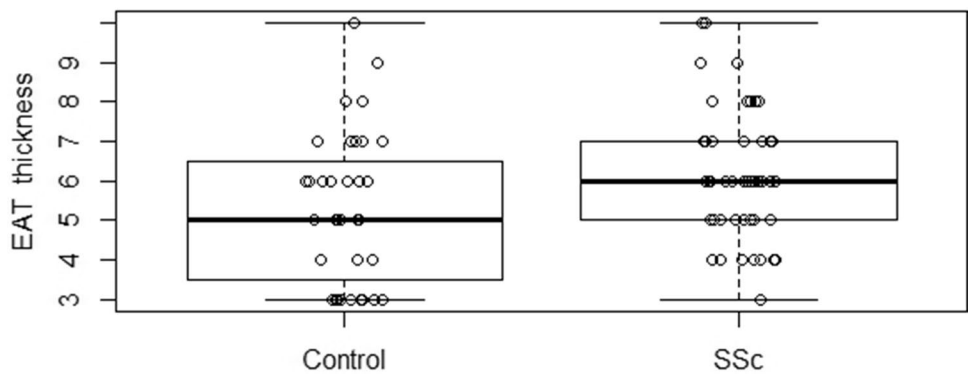

Fig. 1 Epicardial adipose tissue (EAT) thickness in SSc and controls

Table 4 Correlations of EAT and cardio-metabolic risk factors, anthropomorphic and inflammatory parameters assumed to contribute in the cardiovascular risk in SSc patients

	Spearman (rho)	*p** value	Adjusted *p*** value
Age, years (mean ± SD)	0.574	< 0.001*	–
Disease duration, years	0.307	0.036	0.881
mRSS	− 0.142	0.351	0.295
Weight, kg (mean ± SD)	0.160	0.282	0.161
Waist circumference, cm (mean ± SD)	0.162	0.275	0.159
Body mass index, kg/m^2 (mean ± SD)	0.200	0.177	0.091
ESR, mm/h	0.352	0.015*	0.328
CRP, mg/dl	0.334	0.022*	0.512
Leukocyte, 10^3/µl	0.013	0.930	0.772
Neutrophil, 10^3/µl	0.056	0.710	0.758
Fasting plasma glucose, mg/dl	0.123	0.440	0.576
Insulin, mg/dl	0.290	0.048*	0.009*
HOMA	0.274	0.062	0.016*
HbA1C, %	0.317	0.030*	0.192
Total cholesterol, mg/dl	0.396	0.006*	0.465
LDL-cholesterol, mg/dl	0.349	0.016*	0.777
HDL-cholesterol, mg/dl	− 0.145	0.335	0.811
Triglyceride, mg/dl	0.193	0.194	0.738
Homocysteine, mg/dl	0.233	0.133	0.708
Brain natriuretic peptide, mg/dl	0.250	0.114	0.699
EUSTAR activity index	− 0.213	0.160	0.214
Medsger-general	− 0.237	0.109	0.228
Medsger-peripheral vascular	− 0.151	0.312	0.590
Medsger-skin	− 0.052	0.729	0.169
Medsger-joint–tendon	− 0.137	0.371	0.347
Medsger-GI tract	− 0.244	0.098	0.410
Medsger-lung	− 0.575	< 0.001*	0.004*

mRSS modified Rodnan skin score, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *HOMA-IR* homeostatic model assessment of insulin resistance

*p** Spearman correlation coefficient *p* values

*p*** Multiple variable linear regression was used for adjustment of age and sex

to cause a systemic inflammatory effect [33]. Our study revealed that the inflammatory markers such as ESR, CRP, and leukocyte and neutrophil counts were higher in SSc than healthy controls. Within the SSc patients, we showed a correlation between EAT thickness and CRP and ESR, thus, suggesting that amount of EAT may be correlated to the degree of inflammation, which is a key component in the progression of atherosclerosis. Although it is not possible in our study to discriminate whether this finding is associated with the inflammation due to the disease itself or related to the atherosclerotic process, this relation cannot be ignored. However, we tried to eliminate the contribution of the disease as much as possible by excluding the patients with severe organ involvements, short disease duration, and high disease activity. The patients in our study seem to have had moderate disease-related inflammation since disease duration was longer than expected in the early inflammatory-cellular phase [34]. Moreover, they did not have severe organ manifestation and disease activity scores were low. Our results seem to represent a possible link between EAT thickness and the lung domain of the Medsger severity scale. As we tried to exclude the patients with severe organ involvement, many items of the Medsger severity scale were not fulfilled as a result. Although most of our patients were assessed to have mild pulmonary involvement, based on their physical examination or other laboratory investigations, the lung domain of the Medsger severity scale was worse than expected. The main contributor to the lung domain in our patients was the pulmonary function test results. We have linked this result to patients' lack of co-operation in performing the pulmonary function tests correctly. Therefore, we had a concern about the misinterpretation of the severity scale in assessing disease status for our study population. Moreover, we demonstrated no difference

in EAT thickness between the disease-related involvements and disease subtypes.

We found a significant association between the EAT thickness and LDL and total cholesterol levels in SSc patients compared to healthy controls. Serum cholesterol levels are reported to be associated with EAT thickness in autoimmune diseases [35]. There are controversial results suggesting cholesterol levels as a risk factor for cardiovascular disease in SSc. Based on the current data, the role of hypercholesterolemia in SSc-associated cardiovascular disease is unclear, with some studies reporting increased, decreased or similar levels of LDL and triglycerides in SSc compared to controls [28]. Our study revealed the insulin and HOMA-IR were significantly higher in patients with SSc, and the EAT thickness correlated positively with insulin and HbA1c. Based on the multivariable regression analysis, insulin and age have predictive ability for EAT thickness. As we excluded the major risk factors for cardiovascular diseases in the study, only two patients were able to fulfill metabolic syndrome (MS) criteria. Since insulin and its derivative HOMA are closely related to insulin resistance and metabolic syndrome, we suggest that our patients may be at risk even though they do not fulfill the metabolic syndrome criteria [36]. Although the number of patients in our study is too small to demonstrate this relationship definitely, our results support the evidence for an association between EAT and metabolic syndrome [37]. In connection with this result, we found that the obesity was more common in the patients with SSc which may be linked to the high frequency of patients with limited cutaneous SSc in our study that is known to be less severe than diffuse subtype in terms of malnutrition and its relevant measure BMI. We preferred to interpret this finding related to the results of univariable analysis and multivariable regression analysis which revealed an association with EAT and metabolic risk factors. Based on the results of our study, we speculate that there is a close link between EAT thickness and traditional cardiovascular risk factors in our patients.

The conventional echocardiography data had no statistically significant differences, other than EAT thickness, between SSc patients and healthy controls. In the early phases of cardiac involvement in SSc, diastolic dysfunction is known to precede the other findings. The parameters related to myocardial diastolic function were not statistically different between our SSc patients and healthy subjects.

We excluded the patients receiving steroids while designing the study to avoid drug effects because the utilization of glucocorticoids in SSc is still controversial, and the data about adding glucocorticoid to other immunosuppressive drugs could lead to better control of the disease is scarce. Glucocorticoids are mainly part of the therapeutic strategy in the management of interstitial lung disease, diffuse cutaneous disease, arthritis or myositis in SSc [38]. The patients enrolled in our study did not exhibit severe clinical involvements which require steroid treatment and were steroid-free for at least 1 year prior to enrollment.

As a limitation of our study, we did not directly evaluate atherosclerosis with arterial angiography in the patients. Nonetheless, all patients were asymptomatic in this regard and overt clinical atherosclerotic risk factors were eliminated as much as possible. However, carotid intima–media thickness (IMT) might be an option to evaluate the prediction of subclinical atherosclerosis and oxidized LDL can be considered fort he association with atherosclerosis. Using a noninvasive technique which is a surrogate of atherosclerosis appeared more appropriate in asymptomatic patients. Another issue is the limitations of conventional echocardiography which may be less sensitive and reliable for measuring fat thickness especially in obese patients [39, 40]. However, echocardiographic measurement of EAT thickness is comparable to MRI measurements of visceral fat [15], and echocardiography is still easier and more accessible than MRI and computed tomography. The other point which may be considered as a limitation is that 13 of our patients were under immunosuppressive treatment. Although immunosuppressive drugs have been mentioned as potentially cardiotoxic agents in previous studies, their effects on the cardiovascular system are still unclear [41].

As our study aimed to investigate the EAT thickness in SSc patients without overt cardiovascular disease, the design of our study does not allow us to evaluate atherosclerosis in the disease group. This prevents us from detecting a cut-off for EAT thickness associated with the presence of significant coronary artery disease in SSc. There is a need for future studies investigating the relationship between EAT thickness and overt atherosclerotic cardiac or cerebral diseases in a more heterogeneous group in terms of cardiovascular disease.

In conclusion, in SSc patients without overt cardiac disease, the EAT thickness is greater than matched healthy subjects and associated with both inflammatory markers and metabolic risk factors. Based on the results of our study, we speculate that EAT thickness is related to traditional cardiovascular risk factors in our patients rather than the factors related to disease or disease activity. Although this is one of the first reports and there is no comparable data, we suggest EAT thicknesses measurement as a candidate for assessing the atherosclerotic risk in SSc patients. The follow-up studies in large cohorts may offer insight into the association of EAT with cardiovascular events in SSc patients.

Acknowledgements The authors are grateful to Mr. Jeremy Jones of the Academic Writing Department of Kocaeli University, Izmit, Turkey, for his assistance in editing the English used and for his help and advice concerning the contents of this manuscript.

Author contributions The authors certify that they take responsibility for the entire work, and they agree that any questions related to the work in the future are appropriately and fully investigated. DTK acquired the clinical data, contributed to the design of the work, performed all the statistical analysis and drafted the manuscript. TS acquired the echocardiography data, data interpretation and revised the work for important intellectual content. ST, OOI, and AY contributed to data interpretation and critical revision of the data and the manuscript AC coordinated the study, data interpretation and contributed to the revision of the data and the drafting of the manuscript.

Funding This study had no financial support.

Compliance with ethical standards

Conflict of interest None of the authors has financial or non-financial conflicts of interest to disclose.

Ethical approval This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by Kocaeli University School of Medicine Ethics Committee for noninvasive clinical trials with protocol number 178 in 16th June 2015 (KOU KAEK 2015/178). The data of this study were derived during a previous study entitled "Evaluation of the ventricular dysfunction by two-dimensional speckle tracking echocardiography in SSc patients without pulmonary hypertension".

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Chizzolini C, Brembilla NC, Montanari E, Truchetet ME (2011) Fibrosis and immune dysregulation in systemic sclerosis. Autoimmun Rev 10:276–281. https://doi.org/10.1002/art.30380
2. Kahan A, Allanore Y (2006) Primary myocardial involvement in systemic sclerosis. Rheumatol (Oxf) 45(Suppl. 4):7
3. Mavrogeni S, Koutsogeorgopoulou L, Karabela G, Stavropoulos E, Katsifis G, Raftakis J, Plastiras S, Noutsias M, Markousis-Mavrogenis G, Kolovou G (2017) Silent myocarditis in systemic sclerosis detected by cardiovascular magnetic resonance using Lake Louise criteria. BMC Cardiovasc Disord 17(1):187. https://doi.org/10.1186/s12872-017-0619-x
4. Au K, Singh MK, Bodukam V, Bae S, Maranian P, Ogawa R, Spiegel B, McMahon M, Hahn B, Khanna D (2011) Atherosclerosis in systemic sclerosis: a systematic review and meta-analysis. Arthritis Rheum 63:2078–2090. https://doi.org/10.1002/art.30380
5. Ungprasert P, Charoenpong P, Ratanasrimetha P, Thongprayoon C, Cheungpasitporn W, Suksaranjit P (2014) Risk of coronary artery disease in patients with systemic sclerosis: a systematic review and meta-analysis. Clin Rheumatol 33:1099–1104. https://doi.org/10.1007/s10067-014-2681-4
6. Szucs G, Tímár O, Szekanecz Z, Dér H, Kerekes G, Szamosi S, Shoenfeld Y, Szegedi G, Soltész P (2007) Endothelial dysfunction precedes atherosclerosis in systemic sclerosis—relevance for prevention of vascular complications. Rheumatol (Oxf) 46:759–762
7. Blagojevic J, Matucci Cerinic M (2007) Macrovascular involvement in systemic sclerosis: comorbidity or accelerated atherosclerosis? Curr Rheumatol 9:181–182
8. Montecucco F, Mach F (2009) Common inflammatory mediators orchestrate pathophysiological processes in rheumatoid arthritis and atherosclerosis. Rheumatol (Oxf) 48:11–22. https://doi.org/10.1093/rheumatology/ken395
9. Avina-Zubieta JA, Thomas J, Sadatsafavi M, Lehman AJ, Lacaille D (2012) Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis 71:1524–1529. https://doi.org/10.1136/annrheumdis-2011-200726
10. Szentpetery A, Healy GM, Brady D, Haroon M, Gallagher P, Redmond CE, Fleming H, Duignan J, Dodd JD, FitzGerald O (2018) Higher coronary plaque burden in psoriatic arthritis is independent of metabolic syndrome and associated with underlying disease severity. Arthritis Rheumatol 70:396–407. https://doi.org/10.1002/art.40389
11. Matloch Z, Kotulak T, Haluzık M (2016) The role of epicardial adipose tissue in heart disease. Physiol Res 65:23–32
12. Parisi V, Rengo G, Pagano G, D'Esposito V, Passaretti F, Caruso A, Grimaldi MG, Lonobile T, Baldascino F, De Bellis A, Formisano P, Ferrara N, Leosco D (2015) Epicardial adipose tissue has an increased thickness and is a source of inflammatory mediators in patients with calcific aortic stenosis. Int J Cardiol 186:167–169. https://doi.org/10.1016/j.ijcard.2015.03.201
13. Lima-Martínez MM, Colmenares L, Campanelli Y, Paoli M, Rodney M, Santos RD, Iacobellis G (2018) Epicardial adipose tissue thickness and type 2 diabetes risk according to the FINDRISC modified for Latin America. Clin Investig Arterioscler 18:30077–30079. https://doi.org/10.1016/j.arteri.2018.06.002
14. Dey D, Wong ND, Tamarappoo B, Nakazato R, Gransar H, Cheng VY, Ramesh A, Kakadiaris I, Germano G, Slomka PJ, Berman DS (2010) Computer-aided non-contrast CT-based quantification of pericardial and thoracic fat and their associations with coronary calcium and metabolic syndrome. Atherosclerosis 209:136–141. https://doi.org/10.1016/j.atherosclerosis.2009.08.032
15. Iacobellis G, Assael F, Ribaudo MC, Zappaterreno A, Alessi G, Di Mario U, Leonetti F (2003) Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction. Obes Res 11:304–310
16. Van den Hoogen F, Khanna D, Fransen J et al (2013) Classification criteria for systemic sclerosis: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 72:1747–1755. https://doi.org/10.1136/annrheumdis-2013-204424
17. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, Rowell N, Wollheim F (1988) Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 15:202–205
18. Clements P, Lachenbruch P, Siebold J et al (1995) Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol. 22:1281–1285
19. Medsger TA Jr, Bombardieri S, Czirjak L, Scorza R, Della Rossa A, Bencivelli W (2003) Assessment of disease severity and prognosis. Clin Exp Rheumatol 21:42–46
20. Valentini G, Iudici M, Walker UA, Jaeger VK, Baron M, Carreira P (2017) The European Scleroderma Trials and Research group (EUSTAR) task force for the development of revised activity criteria for systemic sclerosis: derivation and validation of a preliminarily revised EUSTAR activity index. Ann Rheum Dis. 76:270–276
21. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ, National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment

of High Blood Pressure, National High Blood Pressure Education Program Coordinating Committee (2003) The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 289:2560–272
22. Hsu VM, Moreyra AE, Wilson AC, Shinnar M, Shindler DM, Wilson JE, Desai A, Seibold JR (2008) Assessment of pulmonary arterial hypertension in patients with systemic sclerosis: comparison of noninvasive tests with results of right-heart catheterization. J Rheumatol 35:458–465
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
24. WHO (1995) Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 854:1–452
25. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 285:2486–2497. https://doi.org/10.1001/jama.285.19.2486
26. Lang RM, Bierig M, Devereux RB et al (2005) Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 18:1440–1463
27. Porter TR, Shillcutt SK, Adams MS, Desjardins G, Glas KE, Olson JJ, Troughton RW (2015) Guidelines for the use of echocardiography as a monitor for therapeutic intervention in adults: a report from the American Society of echocardiography. J Am Soc Echocardiogr 28:40–56. https://doi.org/10.1016/j.echo.2014.09.009
28. Chu SY, Chen YJ, Liu CJ, Tseng WC, Lin MW, Hwang CY, Chen CC, Lee DD, Chen TJ, Chang YT, Wang WJ, Liu HN (2013) Increased risk of acute myocardial infarction in systemic sclerosis: a nationwide population-based study. Am J Med 126:982–988. https://doi.org/10.1016/j.amjmed.2013.06.025
29. Ali H, Ng KR, Low AH (2015) A qualitative systematic review of the prevalence of coronary artery disease in systemic sclerosis. Int J Rheum Dis 18:276–286. https://doi.org/10.1111/1756-185X.12566
30. Long BD, Stojanovska J, Brown RKJ, Attili AK, Jackson EA, Ognenovski V (2017) Increased epicardial fat volume is independently associated with the presence and severity of systemic sclerosis. Acad Radiol 24:1473–1481. https://doi.org/10.1016/j.acra.2017.07.003
31. Rabkin SW (2014) The relationship between epicardial fat and indices of obesity and the metabolic syndrome: a systematic review and meta-analysis. Metab Syndr Relat Disord 12:31–42. https://doi.org/10.1089/met.2013.0107
32. Singh N, Singh H, Khanijoun HK, Iacobellis G (2007) Echocardiographic assessment of epicardial adipose tissue–a marker of visceral adiposity. McGill Med J 10:26–30
33. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, Sarov-Blat L, O'Brien S, Keiper EA, Johnson AG, Martin J, Goldstein BJ, Shi Y (2003) Human epicardial adipose tissue is a source of inflammatory mediators. Circulation 108(20):2460–2466
34. Medsger TA Jr, Silman AJ, Steen VD et al (1999) A disease severity scale for systemic sclerosis: development and testing. J Rheumatol 26:2159–2167
35. Temiz A, Gökmen F, Gazi E, Akbal A, Barutçu A, Bekler A, Altun B, Tan YZ, Güneş F, Şen H (2015) Epicardial adipose tissue thickness, flow-mediated dilatation of the brachial artery, and carotid intima-media thickness, Associations in rheumatoid arthritis patients. Herz 40:217–224. https://doi.org/10.1007/s00059-014-4140-z
36. Iacobellis G, Ribaudo MC, Assael F, Vecci E, Tiberti C, Zappaterreno A, Di Mario U, Leonetti F (2003) Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. J Clin Endocrinol Metab 88:5163–5168
37. Kim BJ, Kim HS, Kang JG, Kim BS, Kang JH (2016) Association of epicardial fat volume and nonalcoholic fatty liver disease with metabolic syndrome: From the CAESAR study. J Clin Lipidol 10:1423–1430. https://doi.org/10.1016/j.jacl.2016.09.007
38. Iudici M, Fasano S, Iacono D, Russo B, Cuomo G, Valentini G (2014) Prevalence and factors associated with glucocorticoids (GC) use in systemic sclerosis (SSc): a systematic review and metaanalysis of cohort studies and registries. Clin Rheumatol 33:153–164. https://doi.org/10.1007/s10067-013-2422-0
39. Flüchter S, Haghi D, Dinter D, Heberlein W, Kühl HP, Neff W, Sueselbeck T, Borggrefe M, Papavassiliu T (2007) Volumetric assessment of epicardial adipose tissue with cardiovascular magnetic resonance imaging. Obesity 15:870–878
40. Abbara S, Desai JC, Cury RC, Butler J, Nieman K, Reddy V (2005) Mapping epicardial fat with multi-detector computed tomography to facilitate percutaneous transepicardial arrhythmia ablation. Eur J Radiol 57:417–422
41. Yiu KH, Schouffoer AA, Marsan NA, Ninaber MK, Stolk J, Vlieland TV, Scherptong RW, Delgado V, Holman ER, Tse HF, Huizinga TW, Bax JJ, Schuerwegh AJ (2011) Left ventricular dysfunction assessed by speckle tracking strain analysis in patients with systemic sclerosis: relationship to functional capacity and ventricular arrhythmias. Arthrit Rheumat 63:3969–3978. https://doi.org/10.1002/art.30614