Insulin resistance is associated with digital ulcer in patients with systemic sclerosis

E.-K. Park[1], S.-G. Lee[1], B.-H. Kim[2], J.-H. Park[1], S. Lee[3], G.-T. Kim[4]

[1]Division of Rheumatology, Department of Internal Medicine, [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Pusan National University School of Medicine, Pusan National University Hospital, Busan, South Korea; [3]Department of Internal Medicine, Pusan National University School of Medicine, Medical Research Institute, Pusan National University Hospital, Busan, South Korea; [4]Division of Rheumatology, Department of Internal Medicine, Kosin University College of Medicine, Busan, South Korea.

Eun-Kyoung Park, MD
Seung-Geun Lee, MD, PhD
Bo-Hyun Kim, MD, PhD
Ji-Heh Park, MD
Shinwon Lee, MD, PhD
Geun-Tae Kim, MD, PhD

Please address correspondence to:
Seung-Geun Lee,
Division of Rheumatology,
Department of Internal Medicine,
Pusan National University
School of Medicine,
Pusan National University Hospital,
179 Gudeok-Ro, Seo-Gu,
602-739, Busan, South Korea.
E-mail: sglee@pusan.ac.kr

Received on January 30, 2016; accepted in revised form on May 16, 2016.

Clin Exp Rheumatol 2016; 34 (Suppl. 100): S85-S91.

Key words: systemic sclerosis, insulin resistance, ulcers, fibrosis, endothelial cells

Funding: this work was supported by clinical research grant from Pusan National University Hospital 2016.

Competing interests: none declared.

ABSTRACT

Objective. *To investigate the relationship between insulin resistance and digital ulcers (DUs) in patients with systemic sclerosis (SSc).*

Methods. *Using a cross-sectional design, we recruited 73 consecutive female patients with SSc and 109 sex- and age-matched healthy controls in South Korea from July 2014 to June 2015. The magnitude of insulin resistance was measured using the homeostatic model assessment of insulin resistance (HOMA-IR). DUs ever included active and healed DUs and the extent of skin fibrosis was evaluated using the modified Rodnan skin score (MRSS).*

Results. *The HOMA-IR in patients with SSc was significantly higher than that in healthy controls (median 1.18 vs. 0.71, p<0.001). In SSc patients, 7 (9.6%) had active DUs and 14 subjects (19.2%) had healed DUs; thus, DUs ever were observed in 21 cases (28.8%). SSc patients with DUs ever had significantly higher HOMA-IR and MRSS compared with those without this feature (median, 2.05 vs. 0.99, p=0.001 and 14 vs. 9.5, p=0.011, respectively). After adjustment for confounding factors using multivariable logistic regression analyses, the HOMA-IR showed a significant positive association with the presence of DUs ever in patients with SSc (OR=1.43, 95% CI=1.01-2.05, p=0.048). In addition, higher MRSS was significantly correlated with DUs ever (OR=1.11, 95% CI=1.02-1.21, p=0.015).*

Conclusion. *Insulin resistance was independently associated with the presence of DUs in patients with SSc and may be a potential biomarker for SSc micro-vasculopathy. Moreover, our data also suggest a potential contribution of insulin resistance to the pathogenesis of DUs.*

Introduction

Systemic sclerosis (SSc) is a chronic connective tissue disease of unknown aetiology characterised by vascular injury, immune dysregulation and progressive fibrosis of the skin and internal organs. Recently, growing clinical and biologic evidence has supported the concept that SSc is a primarily vascular disease mediated by activation of the immune system and evolving into tissue fibrosis and organ damage (1, 2). Vasculopathy of SSc mainly affects the microvasculature and the underlying pathologic process includes endothelial cell injury and dysfunction contributing to structural alterations, remodelling of the vessel walls and progressive luminal obliterations (1, 3). Vasculopathy is known to cause clinical manifestations such as Raynaud's phenomenon, digital ulcers (DUs) and life threatening pulmonary artery hypertension (PAH) in patients with SSc. In particular, DUs are one of the most frequent complications of SSc and can cause significant pain and impairment of hand function, leading to a considerable impact on quality of life (2). Additionally, DUs have been considered as a clinical indicator of severe vasculopathy which may be associated with other vascular lesions or organ involvement (4-6); however, there are still unmet clinical needs for the identification of reliable predictors and effective treatment for DUs in SSc patients.

Insulin resistance is defined as a metabolic derangement resulting from an impaired physiologic response to insulin (7) and predisposes endothelial dysfunction and arterial stiffness eventually leading to arterial atherosclerosis (8). Insulin resistance is recognised as a major pathogenetic factor for metabolic syndrome (MS) which is associated with increased cardiovascular risk (9, 10). Recently, a growing body of evidence has suggested that the inflammatory process can promote insulin resistance (11) and extensive epidemiologic studies have demonstrated increased risks of insulin resistance and MS in

patients with inflammatory rheumatic diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (12-14). In turn, insulin resistance was reported to be correlated with disease activity in patients with RA and SLE (15, 16). However, little attention has been given to the risk of insulin resistance and its association with the pathogenesis of SSc.

In this study, considering the effects of insulin resistance on the vasculature such as endothelial dysfunction, we hypothesised that insulin resistance may contribute to the process of microvasculopathy, especially DUs, of SSc. Therefore, the aims of this study were to compare the magnitude of insulin resistance between patients with SSc and healthy controls and to investigate the relationship between insulin resistance and DUs.

Materials and methods

Study design and population

Using a cross-sectional design, we recruited 73 consecutive female patients with SSc and 109 sex- and age-matched healthy controls (± 2 years), aged between 20 and 75 years, from a university-affiliated rheumatology centre in South Korea from July 2014 to June 2015. Due to the limited number of male subjects with SSc in our centre, we recruited female SSc patients only. All patients with SSc fulfilled the preliminary classification criteria of the American College of Rheumatology for SSc (17). SSc patients were classified as limited or diffuse subset based on the extent of skin involvement according to the classification of LeRoy *et al*. (18) We excluded patients with rheumatic diseases other than SSc or who refused to participate in the present study. Healthy subjects with no history of current or previous rheumatic diseases and not taking any medicine such as oral contraceptives, that can affect insulin resistance were selected randomly from applicants undergoing annual health check-ups in the same centre. For the age matching, patients with SSc and healthy controls were matched by year of birth. If no appropriately matched healthy subject was found, this age-matching criterion was expanded stepwise in age increments or decrements of one year to a maximum of two years (19). All participants provided written informed consent based on the Helsinki Declaration before any study-related procedures. The present study was approved by the Research and Ethical Review Board of Pusan National University Hospital, Busan, South Korea (IRB no. H-1402-005-014).

Clinical and laboratory assessments

Demographics and anthropometric parameters such as height, weight, body mass index (BMI), waist circumference, and blood pressure were collected and measured by interview and review of the participants' medical records. BMI was calculated by dividing body weight in kilograms by the square of height in meters (kg/m^2). Waist circumference was measured using constant tension tape at the midpoint between the lower part of the lowest rib and the highest point of the superior iliac crest on the mid-axillary line. Blood pressure was assessed using a TM-2655P apparatus (A&D Company Ltd, Tokyo, Japan) as the mean of two measurements taken at an interval of 5 minutes and hypertension was defined as blood pressure ≥140/90 mmHg or requiring antihypertensive drugs. Current or previous smoking history was also obtained.

Laboratory assessments including fasting serum glucose and insulin, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglyceride (TG) and C-reactive protein (CRP) were conducted using 12-hour overnight blood samples. Fasting glucose and insulin levels were determined using the glucose oxidase method (Synchron LX-20, Beckman Coulter Inc., Fullerton, CA, USA) and radioimmunoassay (Diagnostic Product Co., Los Angeles, CA, USA), respectively. TC, TG and HDL-C concentrations were assessed with an enzymatic colorimetric reagent (Roche Diagnostics, Zurich, Switzerland) and a P800 Module (Roche Diagnostics) and the concentration of LDL-C was calculated by the Friedewald formula. CRP was analysed by particle-enhanced immunoturbidimetry (Tina-quant C-reactive protein assay, Roche Diagnostics) with a P800 Module (Roche Diagnostics). Insulin resistance was measured using the homeostasis model assessment for insulin resistance (HOMA-IR), calculated with the formula developed by Matthews *et al*. (20) as follows: HOMA-IR = [fasting serum insulin (μIU/mL)] x [fasting serum glucose (mg/dL)]/405.

Regarding patients with SSc, the following data were also obtained; disease duration, the presence of organ involvement including DUs, autoantibody profile and current medications. Active DUs were defined as loss of epithelialisation and tissues involving the epidermis, dermis, subcutaneous tissue and sometimes also involving the bone according to the criteria by Amanzi *et al*. (21) DUs ever included both active and healed DUs (22). The extent of skin involvement was evaluated using the modified Rodnan skin score (MRSS) by a rheumatologist who was blinded to all clinical data. Interstitial lung disease (ILD) was diagnosed on the basis of high-resolution computed tomography findings such as diffuse ground glass opacity, consolidation, or infiltrate and pulmonary artery hypertension was defined as pulmonary arterial pressure >35 mmHg using colour Doppler echocardiography at least 2 occasions by a cardiologist. Gastrointestinal (GI) tract involvement was determined by clinical symptoms, including dysphagia, heartburn or reflux oesophagitis requiring proton pump inhibitors, or by small bowel bacterial overgrowth. The antinuclear antibody expression was evaluated by indirect immunofluorescence on HEp-2 cells and the anti-topoisomerase I antibody expression was assessed by immunoblot assay (EUROLINE anti-ENA profile 1). The presence of anti-centromere antibody was determined by the typical pattern of indirect immunofluorescence assay using HEp-2 cells.

Statistical analysis

Data with normal and non-normal distribution were summarised as the means and standard deviations (SD) and medians and interquartile range (IQR), re-

Table I. Baseline clinical and metabolic characteristics of study subjects.

Variables	SSc (n=73)	Healthy subjects (n=109)	*p*-value
Age, years, mean ± SD	53.1 ± 10.8	51.5 ± 10.9	0.316
BMI, kg/m^2, mean ± SD	21.8 ± 2.8	23.2 ± 3.5	0.006
Waist circumference, cm, mean ± SD	75.8 ± 8.9	77.7 ± 8.3	0.136
Fasting serum glucose, mg/dL, mean ± SD	88.3 ± 14.7	88.8 ± 15.6	0.771
Fasting serum insulin, μIU/mL, median (IQR)	5.52 (4.07-10.29)	3.35 (2.3-4.54)	<0.001
HOMA-IR, median (IQR)	1.18 (0.83-2.38)	0.71 (0.47-0.99)	<0.001
Type 2 diabetes mellitus, n (%)	2 (2.7)	5 (4.6)	0.704
SBP, mmHg, mean ± SD	112.2 ± 15.6	119.2 ± 16.4	0.004
DBP, mmHg, mean ± SD	69.5 ± 11.7	72.9 ± 10.7	0.038
Hypertension, n (%)	6 (8.2)	8 (7.3)	1.000
Fasting serum LDL-C, mg/dL, mean ± SD	110.6 ± 31.3	118.1 ± 36.7	0.155
Fasting serum HDL-C, mg/dL, mean ± SD	53.5 ± 14.8	57.5 ± 12.8	0.053
Fasting serum TG, mg/dL, median (IQR)	107 (74.3 -163.8)	78 (63.5 - 109.5)	0.001
CRP, mg/dL, median (IQR)	0.09 (0.03-0.34)	0.04 (0.02-0.1)	0.001
Current or previous smoker	1 (1.3)	7 (6.4)	0.147
Disease duration, months, median (IQR)	84 (36 - 127)		
MRSS, median (IQR)	10 (7 - 16)		
Limited SSc, n (%)	39 (53.4)		
DUs ever, n (%)	21 (28.8)		
Active DUs, n (%)	7 (9.6)		
Healed DUs, n (%)	14 (19.2)		
ILD, n (%)	40 (54.8)		
PAH, n (%)	11 (15.1)		
GI tract involvement, n (%)	37 (50.7)		
Anti-nuclear antibody, n (%)	72 (98.6)		
Anti-centromere antibody, n (%)	17 (23.3)		
Anti-topoisomerase I antibody, n (%)	26 (35.6)		
Current medication			
Glucocorticoids, n (%)	32 (43.8)		
Penicillamine, n (%)	27 (37)		
Methotrexate, n (%)	7 (9.6)		
Vasodilators, n (%)	35 (47.9)		
Anti-platelet agents, n (%)	55 (75.3)		

SSc: systemic sclerosis; BMI: body mass index; HOMA-IR: homeostasis model assessment for insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride; CRP: C-reactive protein; MRSS: modified Rodnan skin score; DUs: digital ulcers; ILD: interstitial lung disease; PAH: pulmonary artery hypertension; GI: gastrointestinal.

spectively, for continuous variables and as the number of cases with percentages for categorical variables. The distribution of continuous variables was evaluated by the Kolmogorov-Smirnov test. For group comparisons, Student's *t* test or the Mann-Whitney U test was performed for continuous variables and the χ^2 test or Fisher's exact test was conducted for categorical variables, as appropriate. Spearman's correlation analysis was carried out to estimate the relationship between HOMA-IR and other variables in patients with SSc. Using multivariable backward logistic regression analysis including variables with $p<0.2$ in the univariable logistic analyses, the odds ratios (OR) with 95% confidence intervals (CI) were calculated to determine the association between the magnitude of insulin resistance and DUs ever. All statistical tests were two-sided and values of $p<0.05$ were considered to be statistically significant. All statistical analyses were carried out using STATA v. 11.1 for Windows software (StataCorp LP, College Station, TX, USA).

Results

Table I summarises the baseline clinical and metabolic characteristics of patients with SSc and healthy subjects. The mean (± SD) age of SSc patients was 53.1 (± 10.8) years and the median (IQR) disease duration was 84 (36-127) months. Thirty-nine patients (53.4%) were classified as limited subset and 34 (46.6%) had diffuse SSc, while the median (IQR) MRSS was 10 (7-16). Seven (9.6%) and 14 subjects (19.2%) had active and healed DUs, respectively; thus, DUs ever were observed in 21 cases (28.8%). ILD, PAH, and GI tract involvement were found in 40 (54.8%), 11 (15.1%), and 37 (50.7%) cases, respectively. The anti-centromere antibody was presented in 17 patients (23.3%) and anti-topoisomerase I antibody in 26 (35.6%). Of interest, as compared with healthy subjects, we detected a significant increase of the HOMA-IR in patients with SSc, despite of a significantly lower BMI. No significant difference in waist circumference between SSc patients and healthy controls was observed. The TG and CRP levels in patients with SSc were significantly higher than those in healthy subjects whereas SSc patients had significantly lower both systolic and diastolic blood pressure.

Comparisons of clinical characteristics in patients with SSc according to the presence of DUs ever are shown in Table II. The HOMA-IR in SSc patients with DUs ever was significantly higher than that in SSc patients without DUs ever (median, 2.05 vs. 0.99; $p=0.001$). In addition, SSc patients with DUs ever had significantly higher MRSS than those without DUs ever (median, 14 vs. 9.5, $p=0.011$). Further, the fasting serum TG concentrations were higher and the disease duration was longer in SS patients with DUs ever than in those without this feature, although these factors did not reach statistical significance. There were no significant differences between the two groups according to age, CRP, BMI, waist circumferences, systolic blood pressure, diastolic blood pressure, lipid profile, and the proportions of limited SSc, ILD, PAH and GI tract involvement. The frequency of the autoantibody profiles also did not significantly differ between the two groups. In addition, no significant differences in the clinical characteristics were observed between SSc patients with active and healed DU (data not shown).

Subsequently, whether any specific manifestations or clinical parameters of SSc correlated with insulin resistance was examined. Except for DUs ever, the HOMA-IR did not differ signifi-

Table II. Comparisons of clinical features in patients with systemic sclerosis according to the presence of digital ulcer.

Variables	No DUs (n=52)	DUs ever (n=21)	*p*-value
Age, years, mean ± SD	54.2 ± 11.2	50.6 ± 9.5	0.202
CRP, mg/dL, median (IQR)	0.07 (0.03 - 0.34)	0.14 (0.07 - 0.35)	0.335
HOMA-IR, median (IQR)	0.99 (0.72 - 1.76)	2.05 (1.29 - 3.3)	0.001
BMI, kg/m^2, mean ± SD	21.8 ± 2.8	21.8 ± 2.9	0.951
Waist circumference, cm, mean ± SD	75.6 ± 9.3	76.1 ± 7.9	0.841
SBP, mmHg, mean ± SD	111.7 ± 14.1	113.6 ± 19.3	0.638
DBP, mmHg, mean ± SD	69.1 ± 11.2	70.5 ± 13.1	0.623
Fasting serum LDL-C, mg/dL, mean ± SD	110.5 ± 32.1	110.9 ± 30.1	0.961
Fasting serum HDL-C, mg/dL, mean ± SD	55 ± 14.8	50 ± 14.4	0.174
Fasting serum TG, mg/dL, median (IQR)	101 (73 - 165)	131 (88 - 165)	0.092
Disease duration, months, median (IQR)	72 (33.8 - 123)	101 (72 - 143.5)	0.094
MRSS, median (IQR)	9.5 (5 - 13.8)	14 (9 - 20.5)	0.011
Limited SSc, n (%)	29 (55.8)	10 (47.6)	0.608
ILD, n (%)	25 (48.1)	15 (71.4)	0.118
PAH, n (%)	9 (17.3)	2 (9.5)	0.494
GI involvement, n (%)	24 (46.2)	13 (61.9)	0.302
Anti-nuclear antibody, n (%)	51 (98.1)	21 (100)	1.000
Anti-centromere antibody, n (%)	14 (28.6)	3 (15)	0.358
Anti-topoisomerase I antibody, n (%)	20 (38.5)	6 (28.6)	0.591
Current medication			
Glucocorticoids, n (%)	23 (44.2)	9 (42.9)	1.000
Penicillamine, n (%)	19 (36.5)	8 (38.1)	1.000
Methotrexate, n (%)	5 (9.6)	2 (9.6)	1.000
Vasodilators, n (%)	23 (44.2)	12 (57.1)	0.438
Anti-platelet agents, n (%)	38 (73.1)	17 (81)	0.561

DUs: digital ulcers; CRP: C-reactive protein; HOMA-IR: homeostasis model assessment for insulin resistance; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride; MRSS: modified Rodnan skin score; SSc: systemic sclerosis; ILD: interstitial lung disease; PAH: pulmonary artery hypertension; GI: gastrointestinal.

cantly according to the subset of SSc or the presence of ILD or PAH (Fig. 1). GI tract involvement was not associated with HOMA-IR, either (data not shown). Correlation analysis showed a significant positive relationship between HOMA-IR and MRSS (Spearman's ϱ=0.253, p=0.031), as shown in Fig. 2. Waist circumferences were also positively related to increased HOMA-IR, whereas the disease duration and the CRP levels did not show significant correlations with insulin resistance (Fig. 2).

Table III shows the logistic regression models for the presence of DUs ever in patients with SSc. In the univariate analyses, increased HOMA-IR and MRSS were significantly associated with the presence of DUs ever. ILD also tended to relate with DUs ever, but this association was not statistically significant (p=0.075). No clear associations with DUs ever were observed for disease duration, age, CRP, BMI, waist circumferences and serum TG levels. After adjustment for confounding factors using a multivariable logistic regression model with backward selection, HOMA-IR showed a significant positive association with the presence of DUs ever in patients with SSc (OR=1.43, 95% CI=1.01-2.05, p=0.048). In addition, the relationship between higher MRSS and DUs ever was also statistically significant in the multivariable logistic regression model (OR=1.11, 95% CI=1.02-1.21, p=0.015).

Discussion

In the present study, the magnitude of insulin resistance was found to be significantly higher in SSc patients despite of lower BMI, as compared with in healthy controls. Increased HOMA-IR was an independent risk factor for DUs ever in patients with SSc after adjusting for confounding factors, whereas insulin resistance was not associated with other clinical manifestations such as ILD, PAH, and GI involvement. These results suggest the possible contribution of insulin resistance to the pathologic process of vasculopathy, especially DUs, in patients with SSc. Furthermore, MRSS was also found to be significantly related to the presence of DUs ever, and a significant positive correlation between insulin resistance and MRSS was observed, indicating that insulin resistance may be involved in the pathogenesis of skin fibrosis in SSc patients.

An inflammatory process driven by proinflammatory cytokines such as tumour necrosis factor-α and interleukin-6 has been recognised to promote insulin resistance (23). Thus, recently great attention has been paid to the relationship between insulin resistance and inflammatory rheumatic diseases such RA and SLE. Most previous studies have reported that patients with RA or SLE had higher insulin resistance or frequency of MS compared with the general population (13, 14), which collectively could contribute to accelerated atherosclerosis and increased burden of cardiovascular diseases (CVDs). While previous reports have shown that SSc is associated with increased risk of CVDs (24, 25), studies regarding insulin resistance in patients with SSc are lacking. In this regard, our result showing significantly increased insulin resistance in SSc patients seems noteworthy. Peralta-Amaro *et al*. reported that the prevalence of MS in SSc patients was 36.4%, although their sample size was small (n=55) and they did not compare the frequency of MS between SSs patients and healthy controls or analyse the magnitude of insulin resistance such as the HOMA-IR (26). Therefore, the present study provides more comprehensive information on the metabolic status of SSc patients. Notably, the present study showed that SSc patients had a significantly lower BMI as compared with controls, in accordance with previous studies (24, 25, 27-30). Thus, our study showed a paradoxical association between insulin resistance and obesity represented by BMI in patients with SSc. Malnutrition due to intestinal malabsorption, oesophageal hypomotility, reduced physical activity, muscle wasting, and

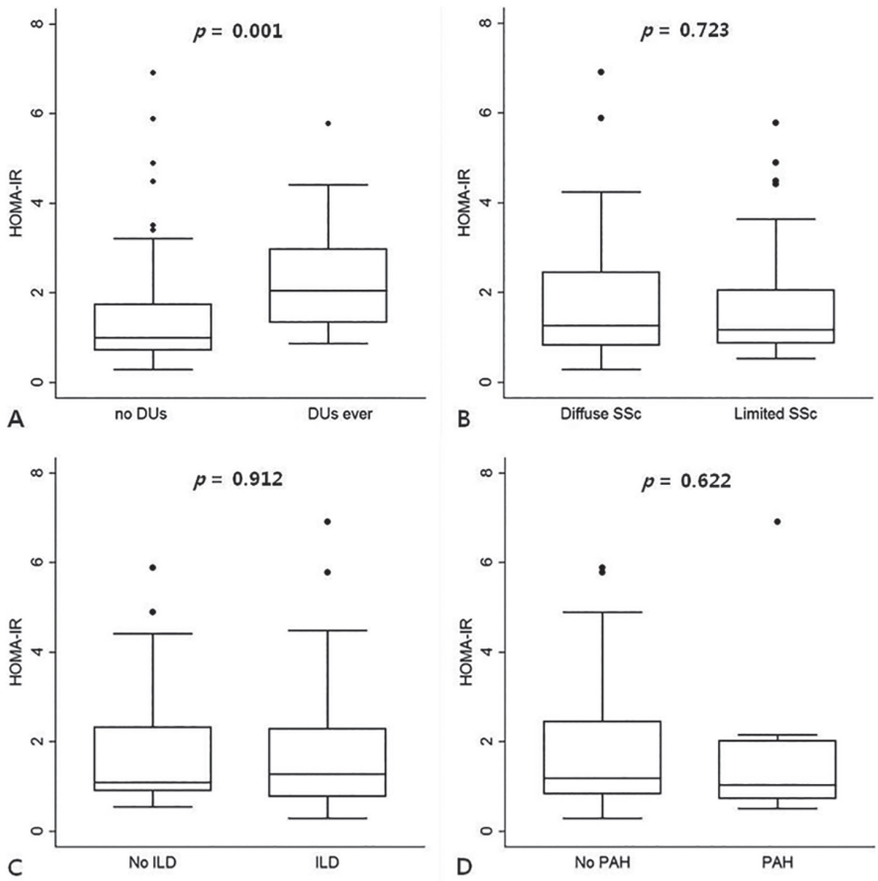

Fig. 1. Comparison of the homeostasis model assessment for insulin resistance according to clinical features.

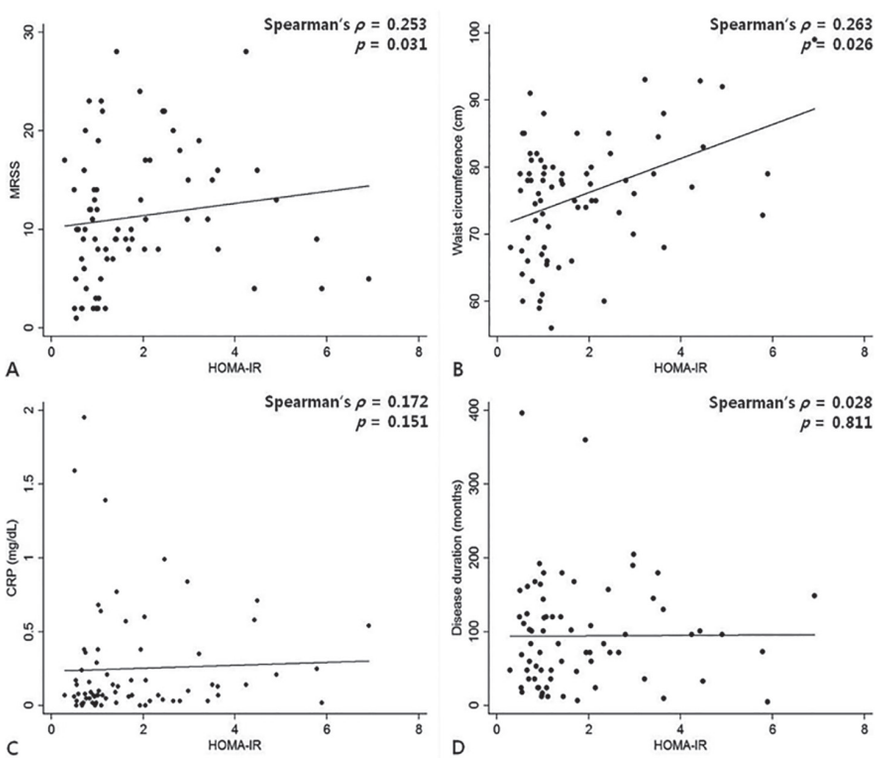

Fig. 2. Correlation analyses between the homeostasis model assessment for insulin resistance and clinical features.

glucocorticoid treatment may reduce the BMI in SSc patients (27). Besides obesity, which is directly linked to insulin resistance through the action of adipokines, a growing body of evidence suggests that inflammation cause insulin resistance, as mentioned above. We presumed that inflammation may be a more important contributor of insulin resistance in SSc patients than obesity, which may explain a paradoxical association between insulin resistance and obesity in our data. Significantly higher CRP levels in SSc patients, as shown in Table I, may support this notion. Similar to our data regarding SSc patients, RA patients with low BMI (<20 kg/m^2) were also paradoxically found to be associated with an increased risk of CVDs (31). The inflammatory status of RA can lead to low muscle mass with a high percentage of fat mass, termed "rheumatoid cachexia", which correlates with CVDs mortality (31, 32). However, regarding patients with SSc, both the lean body and fat mass were found to be reduced in previous studies (29, 30). Hence, further investigations are needed to elucidate the complex interplay between obesity and cardiovascular risks including insulin resistance in patients with SSc.

A major finding of our study was a significant relationship between increased insulin resistance and the presence of DUs in patients with SSc. DUs are a significant clinical burden for patients with SSc and are associated with poorer prognosis and quality of life. For proper treatment, early and prompt identification of SSc patients at high risk of DUs is crucial. Accordingly, vascular biomarkers to assess and predict SSc vasculopathy including pro- and anti-angiogenic factors, autoantibodies, chemokines and endothelial cell adhesion molecules have been extensively investigated (1, 2, 33). Among them, certain vascular biomarkers such as endothelin-1 (ET-1), and their pathways have been suggested to become target for novel therapies of SSc vasculopathy (2). Thus, considering our data, it is presumed that insulin resistance may have a potential prognostic implication in DUs of SSc patients. In addition, future clinical studies focusing on

Table III. Logistic regression models for the presence of digital ulcer ever in patients with systemic sclerosis.

Variables	Crude OR (95% CI)	*p*-value	Adjusted OR* (95% CI)	*p*-value
HOMA-IR	1.46 (1.02 - 2.07)	0.037	1.43 (1.01 - 2.05)	0.048
MRSS	1.11 (1.02 - 1.21)	0.01	1.11 (1.02 - 1.21)	0.015
ILD	2.7 (0.91 - 8.05)	0.075		
Disease duration, months	1.01 (0.99 - 1.01)	0.149		
Age, years	0.97 (0.92 - 1.01)	0.202		
CRP, mg/dL	0.85 (0.21 - 3.59)	0.829		
BMI, kg/m^2	1.01 (0.84 - 1.21)	0.95		
Waist circumference, cm	1.01 (0.95 - 1.07)	0.838		
TG, mg/dL	1.01 (0.99 - 1.01)	0.273		

*Estimated using multivariable logistic regression model with backward selection including HOMA-IR, MRSS, ILD and disease duration.
OR: odds ratios; CI: confidence intervals; HOMA-IR: homeostatic model assessment of insulin resistance; MRSS: modified Rodnan skin thickness score; ILD: interstitial lung disease; CRP: C-reactive protein; BMI: body mass index; TG: triglyceride.

the effect of insulin-sensitising agents on DUs will be necessary to assess the role of insulin resistance as a therapeutic target in SSc vasculopathy.

Endothelial injury and dysfunction, which can be initiated by ischaemia reperfusion, free radical injury, immune activations, and environmental factors, have been postulated as an early pathologic event in vasculopathy of SSc (1, 34, 35). An injured endothelium can disturb the delicate balance between vasodilatation and vasoconstriction leading to decreased efficacy of vasodilators such as nitric oxide and overproduction of vasoconstrictors such as ET-1 in SSc (1). A strikingly similar pathway is also observed for insulin resistance in endothelial dysfunction. Vascular insulin resistance reduces the activity of the downstream insulin signalling pathways such as the PI3K-Akt pathway, which in turn can decrease endothelial nitric oxide synthetase enzymatic activity leading to reduced vasodilatation (36). In addition, systemic insulin resistance promotes overproduction of the vasoconstrictor ET-1 via the mitogen-activated protein kinase pathway (37). Thus, endothelial dysfunction may be a common pathologic feature to both vasculopathy of SSc and insulin resistance, a notion that would support the significant relationship between HOMA-IR and DUs observed in the present study. However, it is difficult to delineate whether the effect of insulin resistance on DUs of SSc is contributory or causal based on our study and further experimental researches are obviously needed in this area.

The HOMA-IR was positively correlated with MRSS in our study, suggesting a potential association between insulin resistance and skin fibrosis in patients with SSc. Fibrosis, a pathologic hallmark of SSc, results from a complex series of vascular and immune-mediated injuries in SSc patients. Transforming growth factor-β (TGF-β) is considered the master regulator of fibrosis through initiating fibroblast activation and myofibroblast differentiation. Peroxisome proliferator-activated receptor-γ (PPAR-γ), which plays a key role in adipogenesis, vascular remodelling, and insulin sensitivity is known to possess anti-fibrotic properties by blocking TGF-β signalling (38, 39). Insulin sensitising drugs such as rosiglitazone, a potent PPAR-γ agonist, have been reported to prevent and attenuate the fibrotic process in a bleomycin-induced scleroderma mouse model *in vivo* (40). In addition, the levels of adiponectin, a marker of PPAR-γ activity, have been demonstrated to be inversely correlated with the MRSS in SSc patients (41). The biologic action of PPAR-γ in the pathologic process of SSc suggests a novel link between metabolism and fibrogenesis and given these findings, it is conceivable that insulin resistance was associated with the extent of skin fibrosis in the present study.

There are a number of potential limitations in our study that are worth noting. First, because of the cross-sectional design, any interpretation of causality between insulin resistance and DUs should be made with caution, as mentioned above. Considering the pathologic process of SSc micro-vasculopathy, it can be assumed that insulin resistance may be a contributory or ancillary factor rather than a primary cause of the development of DUs. Further longitudinal or experimental studies are needed to determine the role of insulin resistance in SSc micro-vasculopathy. Second, our study only included subjects from a single centre located in a seacoast region of South Korea. Thus, lifestyle factors such as dietary habits and physical activity related to geographic characteristics could not be fully adjusted for, which may act as confounding factors for our results (12). Lastly, because only female subjects were recruited, any interactions between sex, insulin resistance, and DUs could not be evaluated.

In conclusion, insulin resistance was found to be independently associated with the presence of DUs in patients with SSc and may be a potential biomarker for SSc micro-vasculopathy. Our data also suggest a potential contribution of insulin resistance to the pathologic process of DUs considering its effect on endothelial dysfunction or injury. Further researches are needed to investigate whether the insulin signalling pathway may be a potential therapeutic target for vasculopathy of SSc.

Acknowledgments

We specially thank the late Professor Sung-Il Kim who devoted himself to education, research, and patient care in the Division of Rheumatology, Department of Internal Medicine, Pusan National University School of Medicine (1963~2011).

References

1. MATUCCI-CERINIC M, KAHALEH B, WIGLEY FM: Review: evidence that systemic sclerosis is a vascular disease. *Arthritis Rheum* 2013; 65: 1953-62.
2. CHORA I, GUIDUCCI S, MANETTI M *et al.*: Vascular biomarkers and correlation with peripheral vasculopathy in systemic sclerosis. *Autoimmun Rev* 2015; 14: 314-22.

3. GALLUCCIO F, MATUCCI-CERINIC M: Two faces of the same coin: Raynaud phenomenon and digital ulcers in systemic sclerosis. *Autoimmun Rev* 2011; 10: 241-3.
4. AVOUAC J, MEUNE C, RUIZ B *et al*.: Angiogenic biomarkers predict the occurrence of digital ulcers in systemic sclerosis. *Ann Rheum Dis* 2012; 71: 394-9.
5. BRUNI C, GUIDUCCI S, BELLANDO-RANDONE S *et al*.: Digital ulcers as a sentinel sign for early internal organ involvement in very early systemic sclerosis. *Rheumatology* (Oxford) 2015; 54: 72-6.
6. BARSOTTI S, STAGNARO C, DELLA ROSSA A: Systemic sclerosis: a critical digest of the recent literature. *Clin Exp Rheumatol* 2015; 33: S3-14.
7. BRILLANTE DG, O'SULLIVAN AJ, HOWES LG: Arterial stiffness in insulin resistance: the role of nitric oxide and angiotensin II receptors. *Vasc Health Risk Manag* 2009; 5: 73-8.
8. ZHENG C, LIU Z: Vascular function, insulin action, and exercise: an intricate interplay. *Trends Endocrinol Metab* 2015; 26: 297-304.
9. JEPPESEN J, HANSEN TW, RASMUSSEN S, IBSEN H, TORP-PEDERSEN C, MADSBAD S: Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease: a population-based study. *J Am Coll Cardiol* 2007; 49: 2112-9.
10. MOTTILLO S, FILION KB, GENEST J *et al*.: The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; 56: 1113-32.
11. GREMESE E, FERRACCIOLI G: The metabolic syndrome: the crossroads between rheumatoid arthritis and cardiovascular risk. *Autoimmun Rev* 2011; 10: 582-9.
12. LEE SG, KIM JM, LEE SH *et al*.: Is the frequency of metabolic syndrome higher in South Korean women with rheumatoid arthritis than in healthy subjects? *Korean J Intern Med* 2013; 28: 206-15.
13. ZHANG J, FU L, SHI J *et al*.: The risk of metabolic syndrome in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Plos One* 2013; 8: e78151.
14. PARKER B, BRUCE I: SLE and metabolic syndrome. *Lupus* 2013; 22: 1259-66.
15. STAGAKIS I, BERTSIAS G, KARVOUNARIS S *et al*.: Anti-tumor necrosis factor therapy improves insulin resistance, beta cell function and insulin signaling in active rheumatoid arthritis patients with high insulin resistance. *Arthritis Res Ther* 2012; 14: R141.
16. SMOLEN JS, STEINER G, ARINGER M: Anticytokine therapy in systemic lupus erythematosus. *Lupus* 2005; 14: 189-91.
17. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980; 23: 581-90.
18. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
19. VAN STAA TP, GEUSENS P, BIJLSMA JW, LEUFKENS HG, COOPER C: Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 3104-12.
20. MATTHEWS DR, HOSKER JP, RUDENSKI AS, NAYLOR BA, TREACHER DF, TURNER RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28 :412-9.
21. AMANZI L, BRASCHI F, FIORI G *et al*.: Digital ulcers in scleroderma: staging, characteristics and sub-setting through observation of 1614 digital lesions. *Rheumatology* (Oxford) 2010; 49: 1374-82.
22. KHIMDAS S, HARDING S, BONNER A, ZUMMER B, BARON M, POPE J: Associations with digital ulcers in a large cohort of systemic sclerosis: results from the Canadian Scleroderma Research Group registry. *Arthritis Care Res* 2011; 63: 142-9.
23. OSBORN O, OLEFSKY JM: The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* 2012; 18: 363-74.
24. NGIAN GS, SAHHAR J, PROUDMAN SM, STEVENS W, WICKS IP, VAN DOORNUM S: Prevalence of coronary heart disease and cardiovascular risk factors in a national cross-sectional cohort study of systemic sclerosis. *Ann Rheum Dis* 2012; 71: 1980-3.
25. MAN A, ZHU Y, ZHANG Y *et al*.: The risk of cardiovascular disease in systemic sclerosis: a population-based cohort study. *Ann Rheum Dis* 2013; 72: 1188-93.
26. PERALTA-AMARO AL, CRUZ-DOMINGUEZ MDEL P, OLVERA-ACEVEDO A, VERA-LASTRA OL: Prevalence of metabolic syndrome and insulin resistance in system sclerosis. *Rev Med Ins Mex Seguro Soc* 2015; 53: 476-83.
27. MOK CC, CHAN PT, CHAN KL, MA KM: Prevalence and risk factors of low bone mineral density in Chinese patients with systemic sclerosis: a case-control study. *Rheumatology* (Oxford) 2013; 52: 296-303.
28. MARIGHELA TF, GENARO PDE S, PINHEIRO MM, SZEJNFELD VL, KAYSER C: Risk factors for body composition abnormalities in systemic sclerosis. *Clin Rheumatol* 2013; 32: 1037-44.
29. SOUZA RB, BORGES CT, TAKAYAMA L, ALDRIGHI JM, PEREIRA R: Systemic sclerosis and bone loss: the role of the disease and body composition. *Scand J Rheumatol* 2006; 35: 384-7.
30. CARAMASCHI P, BIASI D, CAIMMI C *et al*.: Relationship between body composition and both cardiovascular risk factors and lung function in systemic sclerosis. *Clin Rheumatol* 2014; 33: 77-82.
31. SUMMERS GD, METSIOS GS, STAVROPOULOS-KALINOGLOU A, KITAS GD: Rheumatoid cachexia and cardiovascular disease. *Nat Rev Rheumatol* 2010; 6: 445-51.
32. NURMOHAMED MT, HESLINGA M, KITAS GD: Cardiovascular comorbidity in rheumatic diseases. *Nat Rev Rheumatol* 2015; 11: 693-704.
33. YALCINKAYA Y, PEHLIVAN O, OMMA A *et al*.: The relationship between nailfold capillaroscopic assessment and telangiectasia score with severity of peripheral vascular involvement in systemic sclerosis. *Clin Exp Rheumatol* 2015; 33: S92-7.
34. SILVA I, TEIXEIRA A, OLIVEIRA J, ALMEIDA I, ALMEIDA R, VASCONCELOS C: Predictive value of vascular disease biomarkers for digital ulcers in systemic sclerosis patients. *Clin Exp Rheumatol* 2015; 33: S127-30.
35. ZHANG SZ, XU D, LI MT *et al*.: Telangiectasia as a potential clinical marker of microvascular lesions in systemic sclerosis patients from EUSTAR data in China. *Clin Exp Rheumatol* 2015; 33: S106-10.
36. JIA G, SOWERS JR: Endothelial dysfunction potentially interacts with impaired glucose metabolism to increase cardiovascular risk. *Hypertension* 2014; 64: 1192-3.
37. KIM JA, JANG HJ, MARTINEZ-LEMUS LA, SOWERS JR: Activation of mTOR/p70S6 kinase by ANG II inhibits insulin-stimulated endothelial nitric oxide synthase and vasodilation. *Am J Physiol Endocrinol Metab* 2012; 302: E201-8.
38. GHOSH AK, BHATTACHARYYA S, LAKOS G, CHEN SJ, MORI Y, VARGA J: Disruption of transforming growth factor beta signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor gamma. *Arthritis Rheum* 2004; 50: 1305-18.
39. TONTONOZ P, SPIEGELMAN BM: Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem* 2008; 77: 289-312.
40. WU M, MELICHIAN DS, CHANG E, WARNER-BLANKENSHIP M, GHOSH AK, VARGA J: Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. *Am J Pathol* 2009; 174: 519-33.
41. LAKOTA K, WEI J, CARNS M *et al*.: Levels of adiponectin, a marker for PPAR-gamma activity, correlate with skin fibrosis in systemic sclerosis: potential utility as biomarker? *Arthritis Res Ther* 2012; 14: R102.