Association between homocysteine levels and arterial stiffness in women with systemic sclerosis

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ABSTRACT

Objective. The purpose of this study was to evaluate homocysteine (Hcy) serum levels in women with systemic sclerosis (SSc) compared with healthy controls and to examine possible associations between Hcy and markers of arterial stiffness.

Methods. A cross-sectional study was performed at a single hospital between November 2017 and May 2019: 62 women with SSc and 62 age- and sex-matched healthy controls were enrolled. Pulse wave velocity (PWV) was measured non-invasively along the carotid-femoral arterial segment. Serum Hcy was analysed using immunonephelometric method.

Results. There was a significant difference in Hcy serum levels between SSc female patients and healthy controls (11.9±3.3 vs. 10.3±2.3 μmol/ml, p=0.002). Serum levels of Hcy were positively correlated with PWV (r=0.28, p<0.05), brain natriuretic peptide (BNP) (r=0.36, p<0.05) and disease duration (r=0.38, p<0.05), within the SSc group. In addition, in the linear regression model, higher Hcy concentrations were associated with higher PWV [β=0.74 95% CI (0.085, 1.394); p=0.027], BNP [β=0.04 95% CI (0.014, 0.072); p=0.004] and disease duration [β=0.18 95% CI (0.070, 0.300); p=0.002]. In multiple linear regression model adjusting for covariants, Hcy remained positively related to the PWV [β=0.033 95% CI (0.003, 0.062); p=0.031].

Conclusion. Our findings revealed a positive correlation between Hcy serum levels and PWV, which indicates that high levels of Hcy may predispose to the development of vascular stiffness in patients with SSc.

Introduction

Systemic sclerosis (SSc) is a multisystemic disease featured by microvasculard and immunological disorders along with an excessive accumulation of the components of the connective tissue (collagen, fibronectin, glycosaminoglycans, proteoglycans) that cause cutaneous sclerosis and fibrosis of different organs (1). Vascular involvement in SSc is thought to be the result of immune inflammatory processes that activate and injure the vascular endothelium (2). Such immune vascular injury could increase the risk of arteriopathy.

Homocysteine (Hcy), a nonessential sulfur-containing amino acid, is derived from methionine (3). It is formed during the conversion of methionine to cysteine. Several studies have reported that serum Hcy levels are elevated in SSc patients compared to healthy controls (4-7). Elevated Hcy has been shown to be a moderately strong and independent cardiovascular risk factor in healthy populations (8). This association appears to be particularly strong in older people, in which the level of Hcy has even been shown to be a better predictor of cardiovascular mortality than models based on classic Framingham risk factors (age, gender, total cholesterol, high density lipoprotein cholesterol, smoking habits, and systolic blood pressure) (9). Hcy also has been associated with arterial stiffness (10). Arterial stiffness can reflect arterial elasticity and the burden of arteriosclerosis (11). Pulse wave velocity (PWV) is regarded as the gold standard measurement of large artery stiffness and is one of the markers of hypertension-mediated organ damage, and should be assessed among patients with hypertension according to the guidelines of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) (12). Previous meta-analyses have revealed that PWV is an independent predictor of the development of cardiovascular
disease (CVD), adverse cardiovascular events and all-cause mortality (13-15). At present, PWV is widely used in both clinical practice and epidemiological studies due to its feasibility and clinical significance.

The purpose of this study was to analyse Hcy in a cohort of women with SSc and to investigate possible associations between Hcy serum levels with markers of arterial stiffness.

**Materials and methods**

**Study subjects**

This cross-sectional study was performed at San Cecilio Hospital, Granada, Spain, from November 2017 to May 2019. We prospectively enrolled 62 consecutive female patients affected by SSc ≥18 years old attending our Systemic Autoimmune Diseases Unit. In addition, a control group of 62 healthy women age-matched, recruited mainly among non-medical staff of our hospital that attended their annual medical health examination and who were invited to participate were included. All patients included in this study had normal serum creatinine (Cr) levels, and met the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for SSc (16). Individuals with prevalent cardiovascular disease (acute myocardial infarction, angina pectoris, stroke, or peripheral arterial disease) were excluded.

At the clinic visit, participants completed questionnaires about their lifestyle characteristics, medical history, and current medication used. Informed consent was obtained for all subjects, and the study was approved by the Research Ethics Committee of Hospital Clinico Universitario San Cecilio in Granada, Spain, and conducted in accordance with the guidelines in the Declaration of Helsinki.

**Cardiovascular assessment**

Current smokers were defined as those who reported having smoked ≥1 cigarette per day regularly during the year preceding the examination. Waist circumference, weight, and height were measured; and body mass index (BMI) was calculated as weight (kg)/height (m²). Two recordings of blood pressure were obtained from the right arm of the seated subjects; measurements were taken in 5-min intervals, then mean values were calculated. Hypertension was defined as a mean of 3 independent measures of blood pressure ≥140/90 mmHg or current use of antihypertensive drugs. Type 2 diabetes mellitus (T2DM) was defined by self-reported use of insulin, or oral hypoglycaemic medications, or a fasting glucose level ≥126 mg/dl. Kidney function was assessed using the estimated glomerular filtration rate (eGFR) calculated by the CKD-Epi study equation (17).

In our study, we assessed arterial stiffness by determining carotid–femoral pulse wave velocity (CFPWV), as previously described (18). Briefly, after 15–20 min in a supine posture, we measured brachial blood pressure and arterial tonometry with an electrocardiogram obtained from the carotid and femoral arteries using a custom transducer (Cardiovascular Engineering, Inc.) (18). Transit distance from the carotid to femoral arteries was assessed with body surface measurements from the suprasternal notch to the carotid and femoral pulse recording sites. We computed CFPWV as the pulse wave transit distance divided by the transit time of the pulse wave from the carotid to femoral arteries, with adjustment for parallel transmission of the arterial pulse wave in the brachiocephalic artery and aortic arch (18). Higher CFPWV indicates greater arterial stiffness.

**Laboratory measurements**

In all the cases, a fasting blood sample was taken in the morning, and was stored at -70°C until the assays were performed.

The sera were tested for creatinine, CRP, BNP and Hcy. Creatinine was determined by Jaffe method (Siemens Healthcare Diagnostic Inc. NY, USA). CRP was measured by turbidimetric immunoassay (Siemens Healthcare Diagnostic Inc. NY, USA). BNP was quantified in heparinised plasma using a solid-phase two-site chemiluminescent immunometric assay (Biomeérieux, France). Serum Hcy (Siemens Healthcare Diagnostic Inc. NY, USA) were measured by immunonephelometric method according to the manufacturer’s recommendations. Antinuclear antibodies were assessed using ELISA kits produced by Generic Assay Dahlwewitz Germany. Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (Siemens Healthcare Diagnostic Inc. NY, USA). Total cholesterol and triglyceride levels were determined by fully enzymatic techniques. High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein B (apoB)–containing lipoproteins with magnesium sulphate and dextran sulphate. Low-density lipoprotein (LDL) was calculated using the Friedewald formula. All other routine serum biochemical parameters were measured at the Department of Clinical Chemistry, San Cecilio Hospital.

**Statistical analysis**

Data were analysed by statistical software SPSS 21 (Chicago, IL, USA), using independent samples t-test, Mann-Whitney U-test, and Chi-square test when appropriate. Spearman’s coefficient and Pearson’s correlation were calculated as suitable to determine the correlation between the bio-chemical parameters, these correlation coefficients are statistical measures of the strength of a monotonic relationship between paired data and that correlation does not imply causation. The correlation between the two variables is denoted by the letter r and quantified with a number, which varies between −1 and +1. Zero means there is no correlation, where 1 means a complete or perfect correlation. The sign of the r shows the direction of the correlation. A negative r means that the variables are inversely related. The strength of the correlation increases both from 0 to +1, and 0 to -1, using the following interpretation: 0.00–0.19 is “very weak”, 0.20–0.39 is “weak”, 0.40–0.59 is “moderate”, 0.60–0.79 is “strong” and 0.80–1 is “very strong”. P-values of less than 0.05 were considered statistically significant. Sample size was estimated for a 0.05% alpha risk. The quantitative data were shown as mean ± standard deviation (SD) and median.

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(Q1–Q3) as suitable. To test if we can admit that the distribution is normal, we use the Shapiro-Wilk test. Linear regression was used to examine the cross-sectional associations of serum Hcy concentrations with PWV, BNP and disease duration. Multiple regression analysis was performed to determine the relationship between PWV, age, smoking, BMI, blood pressure, as well as serum levels of Hcy, HDL-c and glucose.

Results

Characteristics of the study subjects
The main features of the 62 women with SSc and 62 controls included in this study are shown in Table I. The mean age (SD) of the patients was 53.2±10.1 years. The majority were Caucasian (90.5%). The mean disease duration was 8.8±6.9 years. Forty-four (70.9%) patients had a limited form of the disease, 11 (17.7%) had a diffuse form, and 7 (11.3%) had SSc without skin involvement. Twelve (19%) patients had calcinosis and all patients had Raynaud Phenomenon. Three patients were treated with methotrexate. In addition, a total of 62 healthy women were included in our study as controls; mean age (SD) 52.7±9.7 years. Most of them were also Caucasian (98.3%).

Laboratory results
Laboratory tests of the patients and healthy controls included in the present study are shown in Table II. The mean CRP, ESR and BNP in SSc patients were 0.4±0.4 mg/dl, 21.1±16.0 mm/1h, and 41.2±27.5 pg/ml, respectively. Antinuclear antibodies (ANAs) were detected in 54 patients (87.1%), anticientromere antibodies in 34 patients (54.8%), anti-Scl-70 antibodies in 6 patients (9.6%), and anti-RNA-polymerase III antibodies in 4 patients (6.4%). Serum Hcy concentrations were significantly higher in the SSc patients than those in the control group: [11.9±3.3 vs. 10.3±2.3, μmol/l; p=0.002] (Fig. 1).

Cardiovascular disease risk factors
As shown in Table I, patients had a mean BMI of 26.3±4.9 kg/m², waist circumference of 83.5±11.0 cm and PWV of 7.5±1.2 m/s. Eleven (17.7%) of them are current smokers. Two patients (3.2%) had diabetes mellitus.

Healthy controls had a mean BMI of 25.9±4.3 kg/m², waist circumference of 83.1±13.4 cm and PWV of 7.3±1.4 m/s. Fifteen (24.2%) of them are current smokers. Two controls (3.2%) had diabetes mellitus.

Association between Hcy levels and cardiovascular risk factors or disease features in patients with SSc
Table III shows the correlation coefficients between Hcy and other markers in patients with SSc. Hcy serum levels showed a statistically significant positive weak correlation with PWV (r=0.28, p=0.02), BNP (r=0.36, p=0.004) and associations with other cardiovascular risk factors.
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However, there was no correlation of Hcy levels with age, BMI, waist circumference, or smoking status. In the linear regression model, higher Hcy concentrations were associated with higher PWV \(\beta=0.74\), 95% CI (0.085, 1.394); \(p=0.027\), BNP \(\beta=0.04\), 95% CI (0.014, 0.072); \(p=0.004\) and disease duration \(\beta=0.18\), 95% CI (0.070, 0.300); \(p=0.002\) (Fig. 2).

Multiple linear regression analysis (Table IV) shows the possible influence of age, BMI, current smoking, blood pressure, serum Hcy, HDL-c and glucose levels in predicting PWV.

**Discussion**

Systemic sclerosis (SSc) is a multisystem disease characterised by vasculopathy and organ fibrosis (19). Cardiovascular complications in SSc include peripheral vascular disease, cerebrovascular disease, coronary disease and primary myocardial disease (20). Hyperhomocysteinemia (HHcy) was recognised as a modifiable independent risk factor for coronary, cerebral and peripheral vascular diseases (21-23), and experimental studies have also suggested that Hcy induces vascular endothelium injury and leads to vascular damage (24). In our study, we found significantly elevated Hcy serum levels in patients with SSc compared with healthy controls, in accordance with several reports (4-7). We also have found higher BNP serum levels in SSc female patients compared to healthy controls. Brain natriuretic peptide (BNP) level assessment has become a strong and well-recognised indicator of the cardiovascular risk in SSc (25).

Elshamy et al. (26), reported a significant increase in the mean values of serum levels of N-terminal pro-brain natriuretic peptide in SSc patients compared to controls. Interestingly, we disclosed a significant positive correlation between Hcy serum levels and BNP in our patients with SSc, independent of age. Washio et al. (27) found that BNP levels were increased in the high Hcy group in patients with acute myocardial infarction.

Pulse wave velocity (PWV) is a well-validated index of arterial distensibility, large artery stiffness is a powerful and independent predictor of cardiovascular risk (28, 29). In our study, there was no significant difference in PWV between SSc patients and healthy controls that were in agreement with the study of Bartoloni et al. (30), this result may be biased by the higher proportion of smokers in the control group or by the higher proportion of patients under calcium-channel blockers treatment (31, 32).

Smooth muscle tone influences the stiffness of the elastic and muscular arteries, and removal of the vascular endothelium modifies large artery mechanics in vivo (33, 34), suggesting a degree of functional regulation of large artery stiffness by endothelium-derived vasoactive mediators. In the present study, we report for first time that high Hcy serum levels were positively as-

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**Table III.** Correlations between Hcy and study parameters in SSc patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.24</td>
<td>0.054</td>
</tr>
<tr>
<td>Height</td>
<td>-0.07</td>
<td>0.614</td>
</tr>
<tr>
<td>Body weight</td>
<td>0.04</td>
<td>0.745</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.08</td>
<td>0.559</td>
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<tr>
<td>Waist circumference</td>
<td>0.04</td>
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</tr>
<tr>
<td>Hypertension</td>
<td>0.08</td>
<td>0.543</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.17</td>
<td>0.189</td>
</tr>
<tr>
<td>Disease duration</td>
<td>0.38</td>
<td>0.002</td>
</tr>
<tr>
<td>PWV</td>
<td>0.28</td>
<td>0.027</td>
</tr>
<tr>
<td>Prednisone dose</td>
<td>-0.03</td>
<td>0.690</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>-0.08</td>
<td>0.374</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.18</td>
<td>0.155</td>
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<tr>
<td>Erythrocyte sedimentation rate</td>
<td>0.05</td>
<td>0.721</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>0.38</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum urea</td>
<td>0.35</td>
<td>0.005</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.3</td>
<td>0.007</td>
</tr>
<tr>
<td>Serum phosphate</td>
<td>0.18</td>
<td>0.165</td>
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<tr>
<td>Serum calcium</td>
<td>0.09</td>
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<tr>
<td>Glucose</td>
<td>-0.20</td>
<td>0.127</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.14</td>
<td>0.283</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.11</td>
<td>0.414</td>
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<tr>
<td>HDL-C</td>
<td>-0.06</td>
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<tr>
<td>Triglycerides</td>
<td>0.20</td>
<td>0.116</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.19</td>
<td>0.146</td>
</tr>
<tr>
<td>BNP</td>
<td>0.36</td>
<td>0.004</td>
</tr>
</tbody>
</table>

BNP: brain natriuretic peptide; eGFR: estimated glomerular filtration rate; HDL: high density lipoprotein; Hcy: homocysteine; LDL: low density lipoprotein; PWV: pulse wave velocity; SSc: systemic sclerosis.
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We also found a positive correlation between Hcy serum levels and disease duration in women with SSc. In this regard, Sabio et al. (41) described a positive correlation between Hcy serum levels and disease duration in women with Systemic Lupus Erythematosus.

There are several limitations in our study that should be considered. First, this study was a cross-sectional analysis that reflected the status of a population in a particular period. The cross-sectional design of this study does not allow drawing causal inferences. This study focused only on SSc women; therefore, the findings of this study cannot be generalised to men with SSc. The lack of data on folate and vitamin B12 status in these subjects made it impossible to analyse their potential influence on Hcy serum levels.

In conclusion, our findings revealed a positive correlation between Hcy serum levels and PWV, which indicates that high levels of Hcy may be involved in the development of vascular stiffness in patients with SSc.

Competing interests
M.A. González-Gay received grants/research support from Abbvie, MSD, Jannsen and Roche, and had consultation fees/participation in company sponsored speakers bureau from Abbvie, Pfizer, Roche, Sanofi, Celgene and MSD. The other co-authors have declared no competing interests.

References


