Elevated Serum Homocysteine Levels Were Not Correlated with Serum Uric Acid Levels, but with Decreased Renal Function in Gouty Patients

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INTRODUCTION

Gout is a chronic, progressive illness, which is characterized by hyperuricemia, acute and chronic arthropathies, tophi, and urate urolithiasis (1). Metabolic syndrome with enhanced cardiovascular disease (CVD), chronic kidney disease (CKD), and obesity are associated with the course of gout (2, 3). Patients with chronic gout have a higher mortality rate due to cardiovascular comorbidities (4-6). Several mechanisms underlying links between hyperuricemia and CVD in gout have been suggested (7), but there are still many uncertainties.

Homocysteine (Hcy) is a non-essential amino acid formed by the conversion of methionine to cysteine (8). Clinical manifestations of homocystinuria include developmental delays, osteoporosis, ocular abnormalities, thromboembolic disease, and severe premature atherosclerosis. In animal studies, a metabolic pathway of cellular growth and intercellular matrix synthesis by Hcy derivatives is viewed in the context of the pathogenesis of arteriosclerosis (9). Likewise, hyperhomocysteinemia is believed to be a risk factor of CVD in humans, possibly due to the endothelial cell damage (8, 10, 11).

Both hyperhomocysteinemia and gout are associated with CVD. However, the exact relationships between hyperhomocysteinemia and gout remain obscure. There are a few reports on the serum Hcy levels in patients with gout, however, the results showed discrepancies. Some studies revealed an elevation of serum Hcy in gout (11-13), which was not confirmed elsewhere (14). Factors confounding the levels of Hcy in gout have not been explored. In this study, we therefore aimed to examine the levels of Hcy and its potential confounders in patients with gout.

MATERIALS AND METHODS

Study design and patient population

This cross-sectional, retrospective study included 91 male patients with gout and 97 age-matched healthy male controls. Patients with gout, diagnosed in accordance with the revised American College of Rheumatology criteria (15), were randomly enrolled from inpatient and outpatient clinics at Chung-Ang University Hospital in Seoul, Korea from July 1, 2011 to June 30, 2012. Age-matched healthy controls entered the study after medical examination.

Clinical and laboratory assessment

Age, sex, medication history, and duration of gout were recorded. Clinical and laboratory data were collected at the blood sampling. Serum Hcy, glucose, uric acid (UA), blood urea nitrogen

Hyperhomocysteinemia is one of the important factors of the cardiovascular disease, and gout is well known to be associated with cardiovascular disease. There are a few reports on the serum homocysteine (Hcy) levels in patients with gout, however, the results showed discrepancies. In this study, we measured Hcy levels in patients with gout and examined factors associated with the levels of serum Hcy. Ninety-one male patients with gout and 97 age-matched healthy male controls were enrolled in the study. Serum uric acid levels were not significantly different between gout and healthy control groups. However, serum Hcy levels were significantly higher in patients with gout compared to controls (13.96 ± 4.05 μM/L vs 12.67 ± 3.52 μM/L, P = 0.035). In gout group, patients with 1-2 stages of chronic kidney disease (CKD) had significantly lower serum Hcy than those with 3-5 stages of CKD (13.15 ± 3.46 μM/L vs 17.45 ± 4.68 μM/L, P < 0.001). Multivariate linear analysis revealed an inverse association between serum Hcy and estimated glomerular filtration rate (eGFR) (β = -0.107, P < 0.001). In conclusion, serum Hcy was elevated in male patients with gout. Hyperhomocysteinemia was not correlated with serum uric acid, but it was inversely associated with impaired renal function.

Keywords: Homocysteine; Uric Acid; Gout; Glomerular Filtration Rate
(BUN), creatinine (Cr), cholesterol profile, and other laboratory investigations were performed in all participants. Serum Hcy levels were measured by a competitive immunoassay using direct chemiluminescent technology (Siemens Centaur Immunoassay Systems, Las Vegas, NV, USA). The estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) formula (16): eGFR (mL/min/1.73 m²) = 175 * serum creatinine [exp(-1.154)] * age [exp(-0.203)] * (0.742 if female) * (1.21 if black). Then the stages of CKD were determined according to eGFR levels as follows: stage 1, GFR more than 90 mL/min/1.73 m²; stage 2, GFR between 60-89 mL/min/1.73 m²; stage 3, GFR between 30-59 mL/min/1.73 m²; stage 4, GFR between 15-29 mL/min/1.73 m²; stage 5, GFR less than 15 mL/min/1.73 m² (17).

**Group analysis**

All values, including serum Hcy levels in patients with gout, were compared to those in controls. Patients were divided into groups according to renal function and type of uric acid lowering drug; and thereafter serum Hcy levels in the formed two groups were compared. We performed correlation analyses between serum Hcy and other clinical and laboratory parameters, including renal function, blood pressure, and cholesterol profile in patients with gout.

**Statistical analysis**

Distributions of all parameters were checked by the Kolmogorov-Smirnov test. All measurements with normal distribution were represented as mean ± standard deviation. For the non-normal distribution, median (interquartile range) expression was used. The Mann-Whitney test was employed to compare baseline demographic and clinical data, as well as differences between groups. Associations between serum Hcy levels and other parameters were analyzed by the Spearman’s correlation test. To evaluate relative contribution of hyperhomocysteinemia in gout, a multiple linear regression model was employed. All analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA), and statistical significance was set at P = 0.05 level.

**Ethics statement**

This study was approved by the institutional review board (IRB) of the Chung-Ang University Hospital (IRB No. C2013170[1130]). Informed consent requirement was waived by the IRB.

**RESULTS**

**Patient characteristics**

We collected data from 91 male patients with gout and 97 age- and sex-matched healthy controls. The disease duration in gout patients was 3.2 ± 1.4 yr. Twenty-one patients (23.1%) were on antihypertensive drugs (calcium channel blockers 10/91 [11%]; angiotensin II receptor blockers 10/91 [11%]; beta-blockers 4/91 [4.4%]; diuretics 3/91 [3.3%]; low-dose acetylsalicylic acid 5/91 [5.5%]; statins 10/91 [11%]). Basic characteristics of the study participants are presented in Table 1.

**Homocysteine in patients with gout**

The serum uric acid was not significantly different between patients and controls (6.15 ± 2.23 mg/dL vs 5.82 ± 1.22 mg/dL, P = 0.956). However, patients presented with significantly higher levels of serum Hcy than controls (13.96 ± 4.05 μM/L vs 12.67 ± 3.52 μM/L, P = 0.035) (Fig. 1). The gout group had higher serum BUN level compared to the control group (16.44 ± 5.62 mg/dL vs 14.42 ± 3.63 mg/dL, P = 0.015), however there was no significant difference in serum Cr level and eGFR between these two groups (Table 1).

In gout group, patients with 1-2 stages of CKD (n = 74) had significantly lower serum Hcy levels than those with 3-5 stages of CKD (n = 17) (13.15 ± 3.46 μM/L vs 17.45 ± 4.68 μM/L, P < 0.001) (Table 2). However, there were no significant differences in serum Hcy, uric acid, BUN and Cr levels between 1-2 stages of CKD and healthy control (13.15 ± 3.46 μM/L vs 12.67 ± 3.52 μM/L, P = 0.371; 6.07 ± 2.29 mg/dL vs 5.82 ± 1.22 mg/dL, P = 0.360; 14.97 ± 3.75 mg/dL vs 14.42 ± 3.63 mg/dL, P = 0.336; 1.05 ± 0.15 mg/dL vs 1.06 ± 0.16 mg/dL, P = 0.496, respectively).

Table 1. Basic characteristics of study participants

| Parameters                  | Mean ± SD Gout (n = 91) | Mean ± SD Control (n = 97) | Pvalue |
|-----------------------------|-------------------------|----------------------------|--------|
| Age (yr)                    | 51.19 ± 15.08           | 51.57 ± 17.01              | NS     |
| Sex (male, %)               | 100                     | 100                        | NS     |
| Disease duration (yr)       | 3.2 ± 1.4               |                            |        |
| CRP (mg/dL)                 | 8.12 ± 20.45            |                            |        |
| Uric acid (mg/dL)           | 6.15 ± 2.23             | 5.82 ± 1.22                | NS     |
| BUN (mg/dL)                 | 16.44 ± 5.62            | 14.42 ± 3.83               | 0.015  |
| Cr (mg/dL)                  | 1.13 ± 0.25             | 1.06 ± 0.16                | NS     |
| eGFR (mL/min/1.73 m²)       | 75.35 ± 17.15           | 75.80 ± 13.45              | NS     |
| Glucose (mg/dL)*            | 97.0 (12.0)             | 96.0 (13.0)                | NS     |
| AST (IU/mL)*                | 26.48 ± 8.69            | 29.21 ± 18.98              | NS     |
| ALT (IU/mL)*                | 22.0 (23.0)             | 24.00 (17.0)               | NS     |
| TC (mg/dL)                  | 196.80 ± 36.39          | 204.47 ± 34.11             | NS     |
| LDL (mg/dL)                 | 115.00 ± 28.60          | 127.20 ± 29.76             | 0.004  |
| HDL (mg/dL)                 | 48.65 ± 10.23           | 49.56 ± 12.13              | NS     |
| TG (mg/dL)                  | 192.68 ± 115.16         | 135.70 ± 82.07             | < 0.001|
| sBP (mmHg)                  | 130.75 ± 16.13          | 123.93 ± 13.56             | 0.002  |
| dBP (mmHg)                  | 79.34 ± 10.68           | 74.42 ± 10.26              | 0.001  |

Normally distributed values were represented as mean ± standard deviation (SD). *For the non-normal distribution, median (interquartile range) expression was used.

*For the non-normal distribution, median (interquartile range) expression was used. CRP, C-reactive protein; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated Glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; sBP, systolic blood pressure; dBP, diastolic blood pressure; NS, not significant.

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Correlations between serum homocysteine and other parameters
In patients with gout, serum Hcy positively correlated with serum BUN and Cr (γ = 0.403, P < 0.001; γ = 0.433, P < 0.001, respectively) (Fig. 2A and B). Serum Hcy showed a negative correlation with eGFR and sBP (γ = -0.426, P < 0.001; γ = -0.265, P = 0.017, respectively) in gout group (Fig. 2C and D). However, there were no correlations between serum Hcy and uric acid (γ = -0.002, P = 0.988) or lipid profile. In multivariate linear regression analysis, serum Hcy was associated with eGFR (β = -0.107, P < 0.001; adjusted R² = 0.272), whereas it was not correlated with other parameters (Table 3).

DISCUSSION
In this study, serum Hcy was significantly higher in patients with gout compared with healthy controls. However, there was no difference in serum uric acid levels between these two groups. There are some studies of serum Hcy in gout, suggesting that this marker is not elevated in gout (11-14). Unlike previous studies, uric acid in gout group was not high in our case-control study, possibly because most of the enrolled patients were treated with uric-acid-lowering agents (disease duration 3.2 ± 1.4 yr).

Gout is associated with several comorbidities such as metabolic syndrome and CVD (2); however, mechanisms of the association have not been fully explored. Serum uric acid has been considered as one of the independent predictors of CVD in chronic gout (3, 18). Higher serum Hcy levels in chronic gout along with relatively well-controlled serum uric acid levels suggest that Hcy may be a key factor of the increased CVD risk. Hcy has atherogenic and prothrombotic properties. Hcy triggers the production of reactive oxygen species and impairs endothelial function (4). Hcy-induced vascular injuries lead to the intimal thickening, elastic lamina disruption, smooth muscle hypertrophy, marked platelet dysfunction, and formation of platelet-rich occlusive thrombi (19, 20), which are all associated with CVD.

Serum Hcy is increased in CKD due to decreased renal clearance and impaired metabolism (21). In this study, elevated serum Hcy was not correlated with uric acid. There was an inverse correlation between serum Hcy and renal function (Fig. 2). In gout group, patients with early stages of CKD had significantly lower serum Hcy levels than those with advanced CKD (Table 2). Serum Hcy and renal function correlations depicted in Fig. 2A-C. Multivariate linear regression analysis showed that decreased eGFR was the only factor associated with hyperhomocysteinemia (Table 3).

Cheug et al. reported that serum Hcy was elevated in gout, but there were no significant differences in BUN and Cr levels between gout and control groups (13). A major limitation of that study was its small sample size (gout group, n = 51). In another study with a larger cohort of gout patients, no significant differences in serum Hcy were observed between disease cases and controls, and creatinine clearance was also not significantly different (14), similarly to our results. Considering that gout is associated with CKD (22, 23), we can suggest that decreased renal function confounds the elevated serum Hcy in gout. Hyperhomocysteinemia can be one of the possible triggers of increased CVD in the course of chronic gout. Hcy may be an important risk factor for CVD in chronic gout with impaired renal function, despite well-controlled serum uric acid levels.

In this study, there were significant differences in low-density lipoprotein (LDL) and triglyceride (TG) levels between gout and control groups. Hyperhomocysteinemia may have an addi-
Fig. 2. Correlations between plasma concentrations of homocysteine and (A) BUN, (B) serum creatinine (Cr), (C) estimated glomerular filtration rate (eGFR), and (D) systolic blood pressure (sBP). Plasma concentrations of homocysteine are positively correlated with BUN and Cr, and negatively correlated with eGFR and sBP.

Table 3. Multivariate linear regression analysis model including eGFR and sBP

| Variable   | β    | P value | Adjusted R² |
|------------|------|---------|-------------|
| Homocysteine Constant | 28.076 | 0.272   |             |
| eGFR       | -0.107 | < 0.001 |             |
| sBP        | -0.047 | NS      |             |

eGFR, estimated glomerular filtration rate; sBP, systolic blood pressure; NS, not significant.

Our study has several limitations. First, only male patients were included in this study. However, one should bear in mind that gout is much more common in men than in women (26, 27). We could exclude the role of sex hormones in our study by enrolling only male patients. Second, we did not record data on folate and cobalamin use, which could influence the levels of serum Hcy. Third, this was a relatively small cross-sectional study, and we did not investigate the incidence of CVD in relation to serum Hcy in gout. A prospective study is warranted to assess the relationships between Hcy and gout-related CVD.

In conclusion, serum Hcy was higher in patients with gout compared with healthy controls. Hyperhomocysteinemia was not correlated with serum uric acid in patients with gout, but there was an inverse association between Hcy and renal function. Our results suggest that hyperhomocysteinemia can be one of the potential triggers of increased CVD risk in patients with chronic gout, especially if their uric acid levels are not elevated.

DISCLOSURE

The authors declare no conflicts of interest.

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