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Hyperhomocysteinemia: a novel risk factor for erectile dysfunction

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Abstract

Nitric oxide (NO), the key mediator synthesized by different NO synthase isoenzymes, plays an important role in endothelial function. It was recently shown that hyperhomocysteinemia is an important regulator of NO synthase. We investigated the role of homocysteine (Hcys) in erectile dysfunction (ED), which is associated with the defect in NO generation. Thirty-one nondiabetic patients and 33 control cases were evaluated. Patients with diabetes, coronary artery disease, vitamin B_{12}, or folate deficiency were excluded in the study. The International Index of Erectile Function questionnaire was used to gauge identified erectile quality. Fasting plasma glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, vitamin B_{12}, folic acid, and Hcys levels of patients were measured. Penile color Dupplex ultrasound was used to detect vascular abnormalities in nondiabetic patients with ED. Patients with ED were older than the control subjects (55.6 ± 8.4 vs 44.5 ± 4.7 years, respectively; $P < .001$). Patients with ED had higher fasting plasma glucose, total cholesterol, low-density lipoprotein cholesterol, and Hcys levels. There was a significant negative correlation between mean Hcys level and mean International Index of Erectile Function domain score ($P < .001$). The penile color Doppler ultrasound findings showed that there was a negative significant correlation between mean Hcys level and the 1st, 5th, and 10th minute's peak-systolic velocity. Logistic regression analysis revealed that age and Hcys levels were the main determinants in ED. Hyperhomocysteinemia, known to be an important risk factor in endothelial dysfunction, seems to be an important determinant in ED. These data suggest that slightly elevated Hcys levels are significantly related with arterial and probably endothelial dysfunction in patients with ED.

1. Introduction

The inability of endothelial cells to properly stimulate vasodilation is referred to as endothelial dysfunction. Endothelial dysfunction is believed to be one of the earliest stages of vascular pathologies such as atherosclerosis. It is correlated with subclinical measures of cardiovascular disease and prospectively associated with an increased risk for erectile dysfunction (ED) events [1].

The mechanisms of endothelial dysfunction are likely multifactorial; one of the contributing abnormality appears to be increasing levels of homocysteine (Hcys) [2]. Both prospective and case-control studies have shown that an elevated plasma total Hcys level is an independent risk factor for atherosclerotic vascular disease [3,4]. Homocysteine promotes oxidant injury to the vascular endothelium, impairs endothelium-dependent vasomotor regulation, and may also alter the coagulant properties of blood [5].

These studies report that Hcys impairs vascular function and is a putative risk factor for endothelial dysfunction. Homocysteine may be a potential mechanism linking ED and atherosclerosis because of its role as an atherogenic mediator in the vasculature. Therefore, we investigated the Hcys levels in patients with ED and searched for the correlation between Hcys and penile vascular dysfunction parameters.

2. Materials and methods

2.1. Patient population

Thirty-one patients with ED who came to our urology outpatient clinics and 33 control cases were evaluated

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Table 1
Clinical characteristics and laboratory findings of patients with ED and control subjects

	ED Patients	Control	*P*
n	31	33	–
Age (y)	55.6 ± 8.4	44.5 ± 4.7	<.001
BMI (kg/m^2)	26.6 ± 3.4	25.7 ± 2.6	.207
Hypertension (%)	22.6	–	.003
Smoking (%)	36.4	38.7	.846
FPG (mg/dL)	90.8 ± 9.3	84.7 ± 9.9	.013
Triglyceride (mg/dL)	151.3 ± 65.4	144.7 ± 80.7	.724
Total cholesterol (mg/dL)	200.7 ± 41.7	170.6 ± 28.5	.001
LDL cholesterol (mg/dL)	128.5 ± 32.6	107.9 ± 29.7	.012
HDL cholesterol (mg/dL)	41.3 ± 10.9	39.9 ± 11.4	.624
Hcys (μmol/L)	16.4 ± 5.4	10.7 ± 1.6	<.001

Values are expressed as mean ± SD.
BMI indicates body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

(40-70 years old). Control cases consisted of volunteer healthy hospital staff and their relatives. Patients and control subjects filled in a self-report International Index of Erectile Function (IIEF) questionnaire [6]. Erectile function (EF) status was determined by using the IIEF-EF domain (IIEF 1-5, 15). Scoring the IIEF domain of EF allowed the classification of each patient as control (26-30) or ED (≤25). Patients and control subjects with a known history of diabetes, coronary artery disease, neurological disease, pelvic trauma, major psychiatric disorder, thyroid disease, end-stage renal disease, or vitamin deficiency were not included in this study. All subjects underwent physical examination including measurement of height and weight. Body mass index was calculated as weight divided by the square of height (kg/m^2). To rule out diabetes, all ED patients and control subjects were asked to take the standard oral glucose tolerance test. Informed consent was obtained from all participants. The study protocol was approved by the local ethics committee.

Table 2
Logistic regression analysis of plasma Hcys levels and traditional risk factors for ED

Risk factors	β coefficient	SE	OR	95% CI	*P*
Age	0.41	0.17	1.51	1.09-2.09	.013
Hcys (μmol/L)	1.11	0.40	3.04	1.39-6.63	<.001
Smoking (packet/y)	0.03	0.04	1.03	0.95-1.12	.457
BMI (kg/m^2)	−0.16	0.30	0.86	0.48-1.53	.594
FPG (mg/dL)	0.16	0.12	1.18	0.94-1.49	.157
Triglyceride (mg/dL)	−0.01	0.02	0.99	0.95-1.03	.563
Total cholesterol (mg/dL)	0.20	0.14	1.23	0.94-1.61	.136
LDL cholesterol (mg/dL)	−0.17	0.14	0.85	0.65-1.10	.215
HDL cholesterol (mg/dL)	−0.11	0.13	0.90	0.69-1.16	.407

Logistic regression was used for adjusted odds ratios.
OR indicates odds ratio.

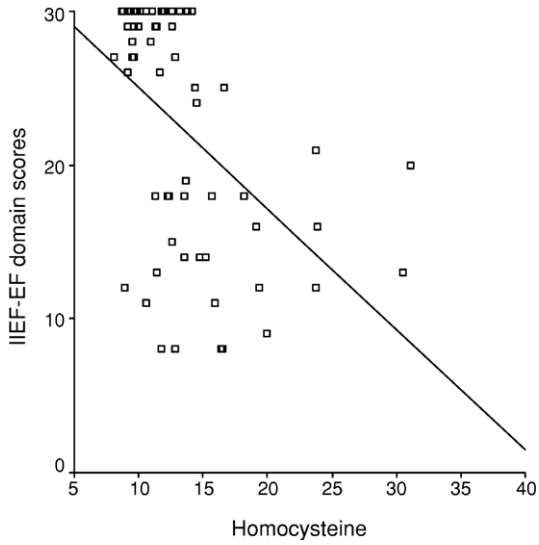

Fig. 1. Correlation between IIEF-EF domain score and Hcys levels ($P < .001$, $r = -0.49$).

2.2. Laboratory tests

Fasting blood analyses included the lipid panel (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), oral glucose tolerance test, Hcys, vitamin B_{12}, and folic acid. All blood samples were drawn into serum EDTA–containing tubes with 3.2% buffered citrate and separated within 1 hour at +4°C. Plasma aliquots were immediately frozen at −87°C until analysis. Total cholesterol, low- and high-density lipoprotein cholesterol, triglyceride, and fasting plasma glucose (FPG) were measured in serum by colorimetric reflectance spectrophotometry (Hitachi 747, Tokyo, Japan). Fasting Hcys levels were measured in EDTA plasma by a fluorescence polarization immunoassay (IMx Homocysteine

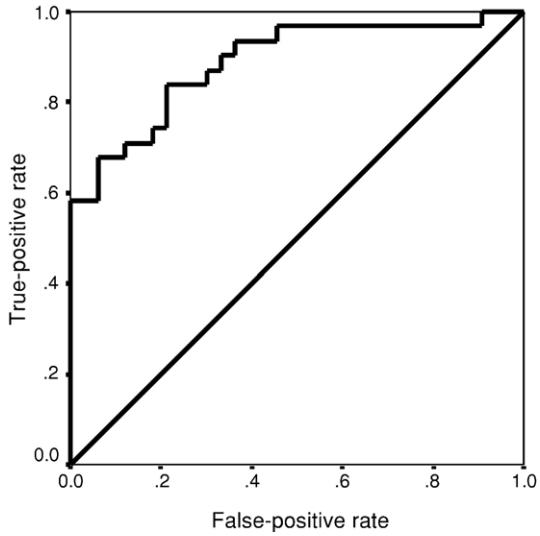

Fig. 2. Receiver operating characteristic curve predicting the impact of Hcys levels on erectile dysfunction. Area under the curve, 0.888; SE, 0.04; 95% CI, 0.806 to 0.969; significance level, $P < .001$.

Table 3
Doppler measurements of the patients with ED

Hcys levels	n (%)		Time table in PDU				
			0 min	1 min	5 min	10 min	20 min
Normal	16	PSV	12.7	59.8	68.5	73.6	67.4
(≤15.0	(52%)	EDV	−0.1	16.2	8.8	0.9	0.1
μmol/L)		RI	1.0	0.7	0.9	1.0	1.0
High	15	PSV	12.6	53.1	59.1	56.4	58.0
(>15.0	(48%)	EDV	0.0	10.2	5.4	3.0	0.0
μmol/L)		RI	1.0	0.8	0.9	0.9	1.0

PDU indicates penile Doppler ultrasonography.

Assay; Axis Biochemicals, Oslo, Norway) using the IMx Analyzer (Abbott Diagnostics, Abbott Park, IL).

2.3. Penile Doppler studies

Penile Doppler ultrasonography was performed on patients who had ED complaints. Doppler examinations were performed by the same investigator (AG) with standard protocol in all patients, using the L5-12 MHz broadband transducer of HDI 5000 scanner (ATL-Philips, Bothell, WA). Patients were examined in an isolated, quiet, and semidarkened room, and requested to stimulate the penis without ejaculating, and baseline measurements were made immediately after tactile stimulation. Subsequently, 50 mg papaverine hydrochloride was injected into the corpus cavernosum. Penile vascular flow parameters were recorded at 1, 5, 10, and 20 minutes after injection. The patients were left alone between the evaluations to avoid the loss of concentration for sexual arousal and were asked to provide the best possible erection by tactile stimulation.

The patients were evaluated in supine position while the penis was slightly stretched onto the abdomen. The transducer was placed on the ventral penile surface and real-time imaging of corpus cavernosum and cavernous artery was maintained. Cavernous artery was detected by color Doppler encoding at the level of penoscrotal junction, and spectral waveforms were obtained at the proximal part of the artery before its initial branching. Doppler angle was maintained between 30° and 60° during the examinations. Penile Doppler measurements of the spectral waveforms were performed including peak-systolic velocity (PSV), end-diastolic velocity (EDV), and resistance index (RI = PSV − EDV/PSV). Values of PSV higher than 35 cm/s and EDV less than 5 cm/s were considered normal response. Diagnosis criteria for abnormal response include arterial insufficiency for PSV of less than 35 cm/s, cavernous venous occlusive disease for PSV of 35 cm/s or more, EDV of 5 cm/s or more, and RI of less than 0.9; and combined insufficiency for PSV of less than 35 cm/s, EDV of 5 cm/s or more, and RI of less than 0.9.

2.4. Statistics

Demographic data and the laboratory results were compared by using independent Student t test and analysis of variance. Data were given as mean ± SD. We used multivariate logistic regression analyses to assess the effects of traditional risk factors and serum Hcys levels on ED. Adjustments were made for potential confounding factors such as age, hypertension, smoking, and lipid levels. We used receiver operating characteristic curves to evaluate the impact of Hcys as risk factors for ED. Bivariate correlations between penile Doppler flow rates and demographic and laboratory values were determined by using Pearson (parametric) and Spearman (nonparametric) correlation coefficients as appropriate. Covariates were included if they were shown to be statistically significant via analysis of variance (statistical significance was defined as $P < .05$). Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS.11.0) software (SPSS, Chicago, IL).

3. Results

Clinical and laboratory findings on patients with ED and the control group are presented in Table 1. Patients with ED were significantly older (55.6 ± 8.4 vs 44.5 ± 4.7 years, respectively; $P < .001$) and had significantly higher FPG, total and low-density lipoprotein cholesterol, and Hcys levels than control subjects (Table 1). Body mass index and smoking status were not significantly different between the patients with ED and the control group ($P > .05$).

Table 2 shows multiple logistic regression analyses for different parameters in predicting ED. Age and Hcys were the only significant predictors of ED. Mean plasma Hcys level was significantly negatively correlated with IIEF-ED domain score ($P < .001$, $r = -0.49$) (Fig. 1).

Predicting the impact of Hcys levels on ED is demonstrated in Fig. 2. The area under the receiver operating characteristic curve was 0.888 (95% confidence interval [CI],

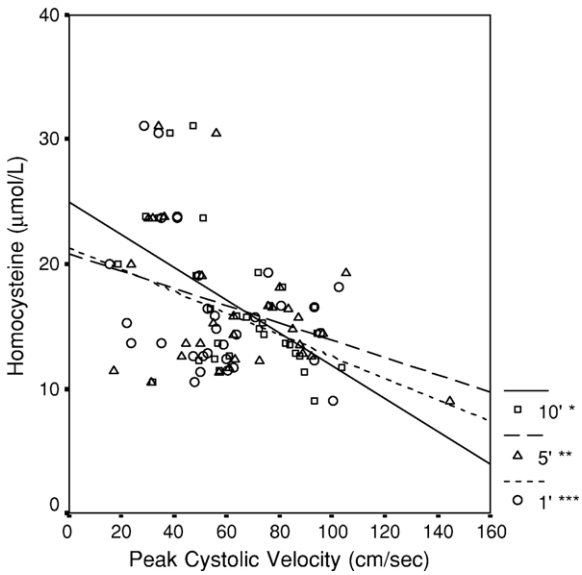

Fig. 3. Homocysteine was significant inversely correlated with penile Doppler ultrasonography flow rates. *$P = .002$, $r = -0.531$; **$P = .049$, $r = -0.357$; $P = .037$, $r = -0.376$.

0.806-0.969; SE, 0.04) for Hcys ($P < .001$). The cutoff point for Hcys to discriminate between the subjects with and without ED was determined as 12.1 μmol/L or more. For this value, a sensitivity of 84% and a specificity of 79% were calculated.

The penile Doppler ultrasonography measurement results for patients with ED are shown in Table 3. According to penile Doppler ultrasonography results, 18 (58.1%) patients had nonvasculogenic ED and 13 (41.9%) patients had vasculogenic ED. Among these 13 patients, 11 had veno-occlusive insufficiency and 2 had arterial or combined insufficiency. Significant inverse correlation was determined between Hcys levels and 1st, 5th, and 10th minute's PSV. The highest correlation was observed between the mean values of the 10th minute PSV and Hcys levels ($P < .01$, $r = -0.53$) (Fig. 3).

4. Discussion

Erectile dysfunction is a common medical disorder that has a negative impact on the quality of life of millions of men worldwide. Because penile erection is a vascular process, risk factors for ED can be grouped into those that affect the vascular system and those that do not. Causes of vasculogenic ED include diabetes mellitus, hypertension, coronary artery disease, peripheral vascular disease, and smoking [7]. There is increasing evidence demonstrating that these diseases and conditions are associated with endothelial dysfunction. Impaired endothelial function and the consequent decreased capacity of the vascular smooth muscle to relax are regarded as precursors of atherosclerosis and subsequent impaired cavernosal perfusion.

A number of novel plasma markers have been associated with endothelial dysfunction. The elevated plasma level of Hcys may be another risk factor for endothelial dysfunction. It was also recently shown that hyperhomocysteinemia is a novel risk factor for ED in an experimental model [8,9]. Our findings of an association between elevated levels of Hcys and ED are unique among the traditional and emerging risk factors assessed in the clinical setting. Plasma Hcys levels of patients with ED were significantly higher than those of control subjects, and there was a significant negative correlation between plasma Hcys level and mean IIEF-EF domain score ($P < .001$). In our study, patients with elevated levels of Hcys had a 3-fold increased risk for ED. Our results are concordant with previous studies that investigated the association between coronary artery diseases and Hcys [10]. In those studies, it has been established that mild to moderate high levels of Hcys were associated with 2- to 3-fold elevated risk for vascular disease. In our study, we found that mild to moderate high levels of Hcys may increase the chance of developing ED. However, there is no distinct threshold concentration for Hcys that correlates with the increase in risk of vascular events. The reference range for Hcys concentrations has been proposed to be between 5 and 15 μmol/L. Based on these data, we speculate that Hcys levels greater than 12.1 μmol/L increase the risk of ED. Based on our results, the chance of developing ED is 80% in this cutoff point of patients with hyperhomocysteinemia. In their recent work, Rasouli et al [11] reported similar findings in patients with coronary artery disease. They showed that the presence of elevated Hcys (>12 μmol/L) strongly and independently predicts progression of atherosclerosis.

Our results show that slightly elevated plasma levels of Hcys were inversely correlated with penile arterial flow rates that indirectly demonstrate penile arterial smooth muscle relaxation capacity. These data indicate the impairment of the penile arterial distensibility due to hyperhomocysteinemia. This finding suggests that decreased distensibility in hyperhomocysteinemia may be attributable to impaired production of endothelium-dependent vasorelaxant substances. As noted previously, the penis is a vascular organ and abnormal conditions that may cause endothelial dysfunction affect the penile arterial flows. Reduced flow rates, as a response to vasoactive agents, may be a sign of endothelial dysfunction at the penile vasculature. Virag [12] reported that penile endothelial function was strongly impaired in patients with organic ED diagnosed via penile Doppler ultrasound, and this was correlated with impaired flow-mediated vasodilatation. Our results, consistent with previous studies, show that established elevated levels of Hcys were correlated with endothelial dysfunction in both young and old subjects. Hyperhomocysteinemia-induced endothelial dysfunction may occur through the formation of disulfides and the generation of hydrogen peroxide and superoxide anion, which causes an increase in the oxidative degradation of nitric oxide (NO) [8,13]. Other potential effects of Hcys may be causing a decrease in NO synthesis. The synthesis of NO can be inhibited by obviously occurring analogues of the NO precursor L-arginine such as N^G-monomethyl-L-arginine or asymmetric dimethylarginine. Several experimental and clinical studies have shown that plasma concentration of the endogenous NO synthase inhibitor asymmetric dimethylarginine is elevated in methionine loading test to induce hyperhomocysteinemia [14,15].

The first study that investigated the relation between Hcys and ED was reported by Khan et al [8] in a rat model. They reported that Hcys inhibited NO-mediated corpus cavernousum smooth muscle relaxation. This effect was potentiated by copper and reversed by superoxide dismutase or catalase. A similar trend was reported by Jones et al [9] in a rabbit model. They found that Hcys had a marked inhibitory effect on endothelium-dependent relaxation and NO formation in isolated corpus cavernousum in rabbits. However, Bank et al [16] did not report any correlation between ED and Hcys in their study, in which they investigated the relationship between traditional and emerging vascular risk factors and the severity of penile vascular disease in men with ED. Although similar variables are

measured in our work and that of Bank et al, the composition of our patient population is different. For example, we excluded diabetic patients with multiple confounding risk factors. The study of Bank et al evaluated a relatively heterogeneous population (compared to ours), and we have demonstrated similar clinical findings that were published in animal models.

In our study, FPG and lipid levels were slightly elevated in patients with ED, but these values were found to be within reference ranges. Logistic regression analysis did not reveal these factors as potential risk for the development of ED. Among the traditional risk factors, age was the only factor to be significantly associated with ED, as observed in previous studies [17,18]. It was observed in this study that plasma Hcys concentration increased with age, matching documented increased plasma Hcys levels in older subjects. Plasma Hcys concentration increases progressively with age, and it is known that plasma Hcys levels tend to increase in older populations [17,18]. These reported increased Hcys levels are thought to be attributable to several mechanisms including age-related decline in cystathionine synthase and other enzymes involved in Hcys metabolism, progressive decline of kidney function, and decrease in bioavailability of essential cofactors such as vitamin B_{12} and folate. Vascular changes with aging and Hcys-induced endothelial dysfunction may be the most significant factors associated with age-related deterioration in erectile response and have a significant role in the mechanisms of age-related ED.

In conclusion, this study demonstrates a significant association between Hcys and ED, and also penile arterial flow as assessed by penile Doppler ultrasound. Elevated plasma Hcys level may be an independent risk factor for ED. Furthermore, larger prospective studies are required to fully determine the association risk profiles between Hcys and ED.

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