Serum homocysteine levels and sildenafil 50 mg response in young-adult male patients without vascular risk factors

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The aim of the present study was to investigate serum homocysteine levels in patients with erectile dysfunction and to evaluate the relationship between serum homocysteine levels and response to the standard 50 mg phosphodiesterase 5 inhibitor treatment. Twenty-eight erectile dysfunction patients having normal vascular parameter according to Penile Doppler Ultrasonography and twenty healthy subjects were enrolled in the study. All subjects filled The International Index of Erectile Function (IIEF) questionnaire. A total of 4-6 doses of phosphodiesterase 5 inhibitor (sildenafil 50 mg) were given to patients. Later, they were divided into two groups as sildenafil responder and non-responder. Serum homocysteine levels were compared in groups based on sildenafil response. Compared with healthy subject, higher homocysteine levels were observed in patients with erectile dysfunction (p = 0.005), especially in sildenafil non-responder group (p = 0.005). There was significant negative correlation between homocysteine and IIEF scores in group responder to sildenafil treatment (r = -0.698, p = 0.008). Mean IIEF scores of patients with non-responder to sildenafil 50 mg were lower than those of controls (p = 0.001), but mean IIEF scores of patients with responders approached values observed in control subjects (p = 0.002). The results indicated that measurement of serum homocysteine levels could be used as a marker for the evaluation of efficacy of phosphodiesterase 5 inhibitor and the selection of efficacious alternative therapies.

Keywords: Erectile dysfunction, Homocysteine, Peak systolic velocity, Penile Doppler, Sildenafil.

Erectile dysfunction has been considered a complication which is developed in patients having cardiovascular disease (CVD). On the contrary, it has been thought that erectile dysfunction may be an early manifestation of systemic cardiac and atherosclerotic process in all organs. In one study, it is shown that 64% of men who presented with myocardial infarction (MI) reported erectile dysfunction before onset of heart problems1. In another study, 57% of men undergone coronary bypass surgery have reported having erectile dysfunction prior to the operation2. Recent studies have shown that the presence and severity of penile vascular disease correlate with cardiovascular risk factors, such as MI, hypertension, cigarette smoking, diabetes mellitus, or dyslipidemia3-5.

Endothelial dysfunction has been considered as a major cause of erectile dysfunction and as the triggering factor for the development of atherosclerosis and subsequent cardiovascular disease6-8. A reduction of endothelial nitric oxide (eNO)-mediated vascular relaxation is the major pathologic condition in endothelial dysfunction. Also, this condition is observed in patients with vascular erectile dysfunction. Relationship between the severity of penile vascular disease and CVD risk factors is also reported9,10.

Homocysteine is accepted as an independent cardiovascular risk factor and its altered levels lead to endothelial dysfunction, increase in oxidative stress, impairment in the endothelium-dependent vasomotor regulation and decrease in NO availability11-13. Therefore, it can be thought that...
increased homocysteine level is an important risk factor for vascular erectile dysfunction. It is reported that slightly elevated levels of homocysteine are significantly related with arterial and probably endothelial dysfunction in patients suffering from erectile dysfunction. Nowadays, phosphodiesterase type 5 inhibitors (PDE5i) are the first line treatment of erectile dysfunction. They have been found effective in around 80% of men having erectile dysfunction, regardless of etiology. On the contrary, while these medications are highly effective and suggested as first step medical treatment, around 20% of men have not shown any benefit; the majority of these men having diabetes mellitus or severe systemic vascular pathologies.

While there are several biochemical markers in the evaluation of systemic disease such as C-reactive protein (CRP), prostate specific antigen (PSA), β-human chorionic gonadotropin (β-hCG), α-alpha-fetoprotein (α-AFP), prothrombin time/international normalized ratio (PT/INR) etc., none has been described in the evaluation of erectile dysfunction. In the present study, we have investigated serum homocysteine levels in patients with erectile dysfunction compared to healthy subjects and have evaluated their response to the standard 50 mg PDE5i treatment.

Materials and Methods

Study subjects

All patients suffered from erectile dysfunction were evaluated in Andrology outpatients in Kirikkale University Hospital. After taking detailed history, biochemical and hormonal analyses were performed including determination of luteinizing hormone, total and free testosterone, prolactin, dehydroepiandrosterone sulfate and lipid parameters were measured with auto analyzer within day. After centrifugation 3000 g for 5 min, plasma specimens were stored at -80°C for homocysteine measurements.

Measurement of homocysteine levels

Total homocysteine (free and oxide) levels were measured using fluorometric HPLC methods with some modification. This method depends on the derivatization of thiols with 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) after reduction with tris-(2-carboxyethyl) phosphine hydrochloride (TCEP). After derivatization, samples were cooled on ice and applied to an HPLC system (HP 1050 series). Supelco™ LC18DB (15 cm × 4.0 I.D., 3 µm) analytical column and Supelguard™ LC18DB (2 cm, 3 µm) guard column were used for analysis. Acetate buffer (0.1 M, pH 4) containing 2% methanol was used as a mobile phase. The fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm.

Statistical methods

Statistical analysis was performed with SPSS Statistical Package program (SPSS version 8.0;
Results

The study group consisted of 28 patients and 22 healthy controls having mean age of 40.2 ± 7.5 yrs in patients with erectile dysfunction and 38.6 ± 8.2 yrs in healthy controls (p = 0.482). Cholesterol, triglyceride and LDL levels of patients (184.6 ± 20.6, 100.5 ± 13.3, 118.4 ± 11.2 mg/dL, respectively) were not statistically different from controls (173.5 ± 26.3, 100.5 ± 38.4, 116.2 ± 9.3 mg/dL, respectively) (p>0.05). However, measured serum hormone levels were in normal limit in all cases and there were not any statistical differences between two groups.

All patients enrolled into the study had normal sufficient arterial velocity on PDU; the mean PSV and EDV values were 32.3±2.2 and 0.3±0.8 cm/sec, respectively. Patients had significantly higher homocysteine levels (p = 0.005) and significantly lower IIEF score (p = 0.0001) at baseline than healthy controls (Table 1).

While 13 patients (46.4%) achieved sufficient erection for sexual intercourse with sildenafil 50 mg (PDE5i + group), 15 patients (53.6%) (PDE5i - group) did not achieve sufficient erection. Mean age, IIEF scores, serum homocysteine levels and penile doppler parameters of PDE5i (+) and PDE5i (-) groups are given in Table 2. There was no statistically significant difference between PDE5i (+) and PDE5i (-) groups in terms of age and homocysteine levels. PDEi5 (-) group had significantly higher homocysteine levels than controls (p = 0.005), but homocysteine levels of PDEi5 (+) group did not show statistically significant difference from controls’ levels (p = 0.137). In addition, PDE5i (+) patients had significantly higher PSV and IIEF scores and lower EDV values than PDE5i (-) patients. Mean IIEF scores at baseline were significantly lower both in PDEi5 (+) and PDEi5 (-) patients groups than in controls. After treatment, mean IIEF scores of patients with non-responder to sildenafil 50 mg were lower than those of controls (p = 0.0001), but mean IIEF scores of patients with responders approached values observed in control subjects (p = 0.002).

Median values of homocysteine were 12.46 µmol/ L and 10.40 µmol/ L for patients and controls, respectively. When 12 µmol/L homocysteine level was used as a cut-off value, 39% of participants (n = 19) had higher homocysteine levels than 12 µmol/L and 79% of them (n = 15) had erectile dysfunction. In sildenafil responder group (PDE5i + group), 53.8% of patients had higher homocysteine levels than cut-off value. In sildenafil non-responder group (PDE5i - group), 53.3% of patients had higher homocysteine levels than cut-off value.

Serum homocysteine levels showed negative and significant correlation between IIEF score (rs = -0.470, p = 0.001) in all cases. When patients were divided into two groups as PDE5i (+) and PDE5i (-), serum homocysteine levels showed significant correlation with IIEF scores in only PDE5i (+) group (rs = -0.698, p = 0.008) (Fig. 1). While homocysteine levels showed negative correlation with PDU parameters and these were not statistically meaningful.
Discussion

There is increasing evidence of endothelial dysfunction as the common underlying mechanism for the development of erectile dysfunction. Endothelial dysfunction is an important pathophysiologic factor underlying both vasculogenic erectile dysfunction and atherosclerosis in other vascular beds\textsuperscript{3,20,21}. Erectile dysfunction is associated with impaired endothelial-dependent flow-mediated vasodilatation in the brachial artery, suggesting that penile endothelial dysfunction is associated with generalized endothelial dysfunction\textsuperscript{22,23}.

Since penile erection is vascular process, risk factors can be grouped into those that affect vascular system. Therefore, impaired endothelial function and decreased vascular smooth muscle relaxation result in impaired cavernosal perfusion due to atherosclerosis. It has been recently shown that increased homocysteine level is a risk factor for endothelial dysfunction in animal studies\textsuperscript{24,25}, and later this finding has also been observed in patients suffering from erectile dysfunction in another study\textsuperscript{14}.

Increased serum homocysteine level has been shown to impair NO-mediated vasodilatation in human vascular tissues. Homocysteine elicits angiopathic effects, especially through auto-oxidation, generating reactive oxygen species such as superoxide and hydrogen-peroxide. Superoxide promotes vasoconstriction and reacts with NO to from peroxynitrite, that limiting endothelial NO availability in vascular tissues. Additionally, increased homocysteine decreases cGMP levels. The effect of homocysteine on NO activity has been shown by several authors in experimental studies\textsuperscript{26-28}. It is reported that hyperhomocysteinemia promotes erectile dysfunction in rabbit by upregulating NADPH oxidase which is intravascular source of superoxide and this upregulation results in augmentation of superoxide and negation of NO bioactivity\textsuperscript{26}. Homocysteine is found to inhibit NO activity in cavernous tissue and this effect is potentiated by copper and reversed by superoxide dismutase or catalase\textsuperscript{27}. It is also shown that homocysteine and copper interact to induce superoxide formation from NADPH oxidase in rabbit cavernosal vascular smooth muscle cells that in turns, leading to an upregulation of PDE5\textsuperscript{28}.

The relationship between homocysteine levels and erectile dysfunction is not clear yet. In one study, no correlation has been found between erectile dysfunction and serum homocystein levels in patients having traditional and emerging vascular risk factors, also having been severe penile vascular pathologies based on Doppler ultrasonography findings\textsuperscript{13}. In another study\textsuperscript{29}, a significant association is reported between erectile dysfunction and homocysteine levels; in this study, serum homocysteine levels are found to be higher in patients having erectile dysfunction than control cases and a strong correlation has been observed between homocysteine level and IIEF score. The elevated serum homocysteine levels in diabetic patients also have a 17-fold increased risk for erectile dysfunction\textsuperscript{30}. Moreover, another study has reported hyperhomocystinemia having 5.2-times the odds ratio for vasculogenic erectile dysfunction in diabetic group\textsuperscript{31}.

In our study, we observed higher homocysteine levels in erectile dysfunction patients without vascular risk factors and having normal vascular parameters according to PDU. Genetic, demographic, acquired factors and lifestyle determined the plasma homocysteine levels. Normal limits of serum homocysteine levels for healthy adults are reported to be between 5-15 µmol/L and patients with mild hyperhomocysteinemia, whose plasma homocysteine levels are within 15-25 µmol/L range suffer from coronary artery, cerebrovascular and peripheral vascular disease\textsuperscript{32-34}.

The upper reference limit of homocysteine levels is 12 µmol/L in population on folic acid fortified diet\textsuperscript{35}. 
However, there has not been clear evidence about cut-off value of homocysteine levels. In the literature, this value has been speculated as 12 µmol/L for erectile dysfunction and severe coronary arterial disease\textsuperscript{14}. In the light of this knowledge and 12 µmol/L was the median value for our patients, we selected this concentration as a cut-off value. In the present study, serum homocysteine level was between 6 and 25 µmol/L and 39% of participants (n = 19) had higher homocysteine levels than 12 µmol/L and 79% of them (n = 15) had erectile dysfunction. After sildenafil treatment, only patients who were non-responder to sildenafil treatment had shown higher homocysteine levels compared with healthy controls.

Abnormal homocysteine concentration results from genetic defect, abnormal vitamin status or both. Methylene tetrahydrofolate reductase (MTHFR) plays crucial role in homocysteine metabolism and higher homocysteine levels are reported to be associated with the MTHFR gene polymorphism\textsuperscript{36}. The 677TT genotype is also reported to be associated with >5-fold increased risk of severe erectile dysfunction\textsuperscript{17}. It is also reported after two months of therapy with folic acid and vitamin B12, non-responder patients show response to sildenafil\textsuperscript{38}. Similar results have been obtained by another study\textsuperscript{39}. These studies imply that administration of sildenafil may fail, if not preceded by the correction of altered levels of homocysteine and folate. In our study, observed higher homocysteine levels and non-response to sildenafil therapy might result from genetic defect and altered vitamin status related with homocysteine and folate metabolism.

In conclusion, higher homocysteine levels were found in patients having erectile dysfunction, especially in non-responder to sildenafil treatment, when compared with control cases. Therefore, homocysteine can be used as a marker of erectile dysfunction. Additionally, the measurement of serum homocysteine levels can be used as a marker for the evaluation of efficacy of PDE5i and the selection of efficacious alternative therapies.

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References
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