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Son Dönem Böbrek Yetmezlikli Hastalarda Plazma Homosistein ve Malondialdehid Düzeyleri ve Bu Parametreler Üzerine Hemodiyalizin Etkisi

[Plasma Homocysteine and Malondialdehyde Levels in Patients with ESRD and The Effect of Hemodialysis on These Parameters]

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ABSTRACT

The purpose of the present study was to investigate plasma total homocysteine and malondialdehyde levels (malondialdehyde was expressed as Thiobarbituric Acid Reactive Substances=TBARS) in patients with End Stage Renal Disease (ESRD), and to investigate the changes in levels of these parameters after a turn of hemodialysis treatment. In the study 15 patients with ESRD who received hemodialysis treatment and 20 healthy volunteers were included.

Plasma total homocysteine and TBARS levels in patients with ESRD significantly increased compared with the control group. Although plasma total homocysteine levels after dialysis were lower than before dialysis, plasma TBARS levels after dialysis were not significantly lower than before dialysis. In addition, positive correlations were observed between plasma total homocysteine and TBARS levels, and between plasma total homocysteine and serum creatinine levels.

It is concluded that both homocysteine and TBARS levels rise in ESRD. Hemodialysis were found to have a dimishing effect on elevated homocysteine content while TBARS were not affected by hemodialysis. It was also found that elevated homocysteine levels were not return to those of normal subjects.

Key Words: Homocysteine; Thiobarbituric Acid Reactive Substances; End Stage Renal Disease; Hemodialysis

ÖZET

Bu çalışmanın amacı, son dönem böbrek yetmezlikli hastalarda plazma total homosistein ve malondialdehid düzeylerini (malondialdehid, tiyobarbütirik asid reaktif maddeler=TBARS olarak ifade edildi) ve hemodiyalizden sonra bu parametrelerdeki değişiklikleri araştırmaktı. Çalışmaya hemodiyaliz tedavisi alan son dönem böbrek yetmezlikli 15 hasta ve 20 sağlıklı gönüllü dahil edildi.

Kontrol grubu ile karşılaştırıldığında son dönem böbrek yetmezlikli hastalarda plazma total homosistein ve TBARS düzeyleri anlamlı derecede artmış bulundu. Diyaliz sonrası plazma total homosistein düzeylerinin diyaliz öncesinden daha düşük olmasına rağmen, plazma TBARS düzeyleri diyaliz öncesinden daha düşük değildi. Ayrıca plazma total homosistein ile TBARS düzeyleri arasında ve plazma total homosistein ile serum kreatinin düzeyleri arasında pozitif korelasyonlar gözlendi.

Son dönem böbrek yetmezlikli hastalarda plazma total homosistein ve TBARS düzeylerinin artmış olduğu sonucuna varıldı. Hemoliz, artmış homosistein düzeylerinde azalmaya yolaçarken TBARS düzeylerinin hemolizden etkilenmediği görüldü. Ayrıca homosistein düzeylerinin hemodiyaliz ile normal bireylerin seviyesine düşürülemediği gözlendi..

Anahtar Kelimeler: Homosistein; Tiyobarbütirik Asid Reaktif Maddeler; Son Dönem Böbrek Yetmezliği; Hemodiyaliz

INTRODUCTION

Atherosclerotic diseases are the main cause of mortality and morbidity in patients with end-stage renal disease (ESRD). The high prevalence of atherosclerotic diseases have been believed to be caused by increased rates of known risk factors, such as hypertension, hypercholesterolemia and diabetes (1-4). However, these risk factors do not completely explain the increased risk for atherosclerotic diseases in patients with ESRD.

Homocysteine (Hcy) is an intermediate of methionine metabolism that has been identified as a potential atherogenic agent and a risk factor for atherosclerosis in healthy subjects (5,6) and patients with ESRD (1-4,6,7). The induction of the atherogenic process by hyperhomocysteinemia seems to be associated with an alteration of endothelial and smooth muscle cell function leading to an accelerated formation of reactive oxygen species (ROS) with a disturbance of coagulation system (5,6). Consequently, the formed ROS lead to lipid peroxidation, which may damage cell membrane. Malondialdehyde (MDA), which is a marker of lipid peroxidation is usually measured as thiobarbituric acid reactive substances (TBARS) (8).

Since Hcy is a risk factor for atherosclerosis in patients with ESRD, we aimed to search plasma total homocysteine (tHcy) and TBARS levels in patients with ESRD and whether there is an effect of hemodialysis (HD) on these parameters. For this purpose, the tHcy and TBARS concentrations were determined in ESRD subjects before and after dialysis.

MATERIALS AND METHODS

We evaluated 15 patients with ESRD who received HD for three times weekly, 4 hours each, using a low-flux 1.4 m^2 dialyser of cellulosynthetic membrane (Hemophane®, Kavasumi Laboratories, Japan), glucose-containing dialysate with bicarbonate as the buffer, blood flow between 300-350 mL/min, and a dialysate flow of 500 mL/min (8 men and 7 women; aged 40.2 ± 2.2 years), and 20 healthy volunteers (10 men and 10 women; aged 38.3 ± 2.5 years). The mean duration of HD in patients was 8.1 ± 1.9 years. None of the patients and controls was smoker, alcohol consumer, and supplemented with folate or vitamin B_{12}. This study was conducted according to the rules of the Ethics Committee.

Fasting plasma total Hcy levels before and after HD were measured as the total amount of free and protein-bound Hcy by the flourimetric high performance liquid chromatography (Hewlett Packard 1100) by using Chromosystems® calibrators and kits. Intraassay CV was 2.5%, and interassay CV was 3.2%. Blood samples were collected and spun to serum and plasma within 30 minutes of collection. Samples were stored at – 80 °C, and all assays were conducted in one batch. Fasting plasma MDA levels before and after HD were measured by the thiobarbituric acid method (9). 0.2 mL of aliquots from plasma were mixed thoroughly with 0.8 mL phosphate-buffered saline (pH 7.4) and 0.025 mL butylated hydroxytoluene solution. After adding 0.5 mL 30% trichloroacetic acid, the samples were placed on ice for 2 hours and then centrifuged at 2000x g at 25 °C for 15 minutes. One mL of supernatant was mixed with 0.075 mL 0.1 mol/L ethylenediamine tetraacetic acid and 0.25 mL 1% thiobarbituric acid in 0.05 N sodium hydroxide and placed on boiling water for 15 minutes, cooled to room temperature and absorbance at 532 nm was determined. We used 1.56 x 10^5 cm^{-1} M^{-1} as a molar extinction coefficient for TBARS. Plasma TBARS levels were expressed as nmol/mL. It has been reported that there is the close relationship between serum creatinine and tHcy, and that homocysteine metabolism is linked directly to creatinine generation system (6,10). Therefore, we measured serum creatinine levels. Serum creatinine, cholesterol (Chol) and triglycerides (TG) levels were measured before HD with a chemistry analyser by using specific Boehringer calibrators (Hitachi 717 Analyser, Boehringer-Mannheim, Germany) by commercial kits. Some characteristics of the study subjects are given in Table I.

Statistical analysis

SPSS for windows (Version 10.0, Chicago, IL, USA) was used for statistical analyses. Data were expressed as mean value ± SD. Comparison between two groups was performed using the non-parametric Mann-Whitney U-test. Pearson's rank correlation test was used for correlation analysis. $P<0.05$ was accepted as statistically significant.

RESULTS

Table 1. Some characteristics and biochemical parameters of the study subjects

	Controls	Patients	
n	20	15	
Duration of HD (years)	-	8.1 ± 1.9	
Age (years)	38.3 ± 2.5	40.2 ± 2.2	
Sex (Male/Female)	10 / 10	8 / 7	
TG (mg/dL)	97.6 ± 36	152.3 ± 53	
Chol (mg/dL)	158.4 ± 31	204.4 ± 51	
Creatinine (mg/dL)	1.79 ± 0.49	7.96 ± 2.9	
		Before dialysis	After dialysis
Plasma tHcy (μmol/L)	9.8 ± 2.8	18.8 ± 6.7$^{*,\Phi}$	13.0 ± 2.7*
Plasma TBARS (nmol/mL)	4.4 ± 0.9	6.2 ± 2.1$^{\blacklozenge}$	5.0 ± 0.7$^{\blacklozenge}$

* $p<0.001$; ♦: $p<0.05$ when compared with the control group.

Φ: $p<0.005$ when compared with those of determined after dialysis.

Results are shown in Table I.

When plasma tHcy and TBARS levels were compared with those of the control group before and after dialysis; plasma tHcy (both before and after HD $p<0.001$) and TBARS (both before and after HD $p<0.05$) levels were found to be significantly increased as shown in Table I. Plasma tHcy levels were significantly were found to be decreased after dialysis ($p<0.005$) (Figure 1) when compared with those of before dialysis. There was no significant change in TBARS levels of the patients by dialysis. TBARS levels did not correlated with TG or Chol.

There were positive correlations between plasma tHcy and TBARS levels in the control ($p<0.001$, $r=0.78$), and in the patient groups before ($p<0.005$, $r=0.70$) and after dialysis ($p<0.01$, $r=0.63$) (Figure 1). In addition, positive correlations were observed between plasma tHcy and serum creatinine levels in the control ($p<0.001$, $r=0.83$) and in the patient groups ($p<0.001$, $r=0.83$ before dialysis (Figure 2).

DISCUSSION

Plasma tHcy levels were found significantly higher in the ESRD group than those of in the healthy group in agreement with several studies (1-4,6),. The major reason for increased serum creatinine levels in patients with ESRD is attributed to decreased glomerular filtration rate which also leads to increased plasma tHcy levels in these subjects. It was previously postulated that transsulfuration and remethylation are the main pathways of Hcy metabolism in kidneys and liver. (6). However, the methylation pathway in methionine metabolism in liver, which is linked directly to creatine generation system, may be disturbed in ESRD. Another reason of increased Hcy levels in ESRD is that one molecule of Hcy is produced per each molecule of creatine formation during creatine synthesis from guanidinoacetate (10). Then, creatinine is formed spontaneously from creatine. The correlation between Hcy and creatinine foun in te present study has confirmed these findings reported.

An another important finding in this study is the decreasing effect of dialysis on plasma tHcy levels. HD decreased plasma tHcy levels, but it failed to bring them to normal levels. It may be related to diffusion of Hcy directly into the dialysate during dialysis (6).

It has been demonstrated that Hcy increases production of ROS by endothelial and vascular smooth cell, and increases lipid peroxidation (5). We found a positive correlation between tHcy and TBARS levels both in the control and the patient groups before and after dialysis. In the present study, TBARS levels were found to be higher in ESRD than in those of control group both before and after dialysis. Although TBARS levels were found as decreased after dialysis, the difference was found to be nonsignificant. This can be partly explained by the fact that MDA can easily pass to dialysate through the dialysis membrane since it is a small molecule.

Haklar et al. (11) reported that MDA production is significantly increased in ESRD. Şekeroğlu et al. (12) have also reported that HD decreases the elevated MDA levels. Contrary, Lougrey et al. (13) claimed that HD does not have any significant effect on lipid peroxidation. Our study confirms the data reported in the papers of Lougrey et al. and Haklar et al.

Nacitarhan et al. (14) reported that lipid status affects MDA levels. However in the present study, TBARS levels did not correlated neither with TG nor with Chol.

In conclusion, tHcy and TBARS levels were found to be elevated in ESRD and HD decreased this increase in Hcy levels. HD did not decrease TBARS significantly and increased Hcy levels could not return to the basal levels of normal subjects.

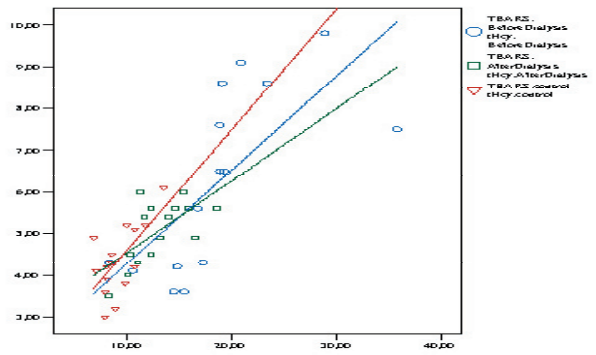

Figure 1 Correalation between TBARS and plasma total homocysteine in study groups.

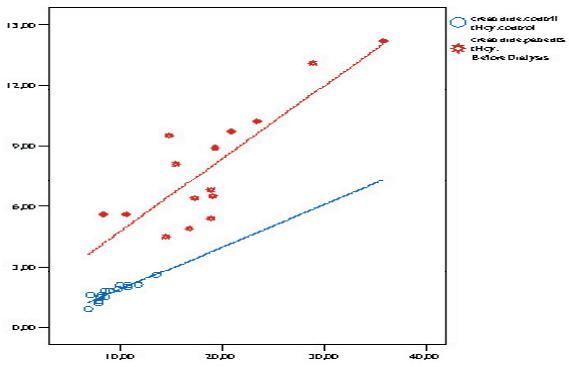

Figure 2 Correlation between serum creatinine and plasma total homocysteine in study groups.

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