

ORJINAL ARAŞTIRMA / ORIGINAL RESEARCH

Serum Homocysteine, Lipoprotein (a), Tumor Necrosis Factor-Alpha, Total Cholesterol and Triglyceride Levels in Hemodialysis Patients

HEMODİYALİZ HASTALARINDA SERUM HOMOSİSTEİN, LİPOPROTEİN (a), TÜMÖR NEKROZİS FAKTÖR-ALFA, TOTAL KOLESTEROL VE TRİGLİSERİD DÜZEYLERİ

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Abstract

Objective: Hyper-homocysteinemia and lipoprotein (a) (Lp(a)) are accepted independent risk factors for atherosclerotic process. Lipid abnormalities are important variables in patients with end-stage renal disease (ESRD) in the development of vascular atherosclerotic lesions. In most hemodialysis (HD) patients, serum levels of Lp(a) and homocysteine are markedly elevated and contribute to premature atherosclerosis in these patients. Cytokines such as tumor necrosis factor-alpha (TNF- α) can alter lipid metabolism and produce hyperlipidemia. In this study we purposed to evaluate the changes of these parameters with the application of hemodialysis.

Material and Methods: In this study thirty patients undergoing dialysis were selected. Serum levels of Lp(a), TNF- α , total cholesterol and triglyceride levels were measured before and after hemodialysis.

Results: Serum Lp(a) and TNF- α were significantly increased after hemodialysis. However, there was no difference statistically in homocysteine, total cholesterol and triglyceride levels between the pre- and post-HD period.

Conclusion: These results indicate that Lp(a) and TNF- α levels were markedly elevated in ESRD patients after HD and contributed to possible atherosclerotic process. The acute rise of TNF- α , however, had no effect on other serum lipid and homocysteine levels.

Key Words: Hemodialysis, homocysteine, lipoprotein (a), tumor necrosis factor-alpha, lipid

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Özet

Amaç: Hiperhomosisteinemi ve lipoprotein (a) (Lp(a)) aterosklerotik olaylarda bağımsız risk faktörleri olarak kabul edilmektedir. Son evre renal hastalığı olanlarda vasküler aterosklerotik lezyonların gelişiminde lipid anomalileri önemli değişkenlerdir. Pek çok hemodiyaliz hastasında, Lp(a) ve homosisteinin serum düzeyleri anlamlı olarak yüksektir ve bu hastalarda prematüre ateroskleroz gelişimine katkıda bulunurlar. Tümör nekrozis faktör-alfa (TNF- α) gibi sitokinler hiperlipidemiye neden olarak lipid metabolizmasını etkileyebilmektedir. Bu çalışmada bu parametrelerde hemodiyaliz uygulamasına bağlı değişikliklerin gösterilmesi amaçlanmıştır.

Gereç ve Yöntemler: Düzenli olarak hemodiyaliz uygulanan 30 hasta bu çalışmaya dahil edilmiştir. Serum Lp(a), TNF- α , total kolesterol ve trigliserid düzeyleri hemodiyaliz öncesi ve sonrasında ölçülmüştür.

Bulgular: Hemodiyalizden sonra serum Lp(a) ve TNF- α düzeyleri anlamlı olarak yükselmiştir. Fakat homosistein, total kolesterol ve trigliserid düzeylerinde hemodiyaliz öncesi ve sonrasında anlamlı bir fark ortaya çıkmamıştır.

Sonuç: Bu sonuçlara göre, kronik böbrek yetmezliği olan hastalarda hemodiyaliz sonrasında Lp(a) ve TNF- α düzeyleri yükselmekte ve muhtemel aterosklerotik olaylara katkıda bulunmaktadır. Fakat TNF- α düzeyindeki yükselmenin serum lipidleri ve homosistein üzerine bir etkisi olmamaktadır.

Anahtar Kelimeler: Hemodializ, homosistein, lipoprotein (a), tümör nekrozis faktör-alfa, lipid

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Cardiovascular diseases are the leading cause of death in hemodialysis patients. Excess mortality due to atherosclerotic vascular disease has been increasingly recognized as a major problem in patients with end stage renal disease (ESRD). Cardiovascular mortality is 10- to 30-fold

higher in ESRD patients than in the general population after adjustment for age, gender, ethnic origin and diabetes. Left ventricle hypertrophy, heart failure, and arterial atheroma are the main causes of cardiovascular morbidity-mortality.¹

According to some resources, since the classical risk factors are insufficient to explain the cardiovascular morbidity-mortality in the dialysis population, the other factors related to chronic renal failure and its treatment have been proposed. These factors include hydroelectrolytic disorders, anemia, elevated lipoprotein (a) (Lp(a)), homocysteine, total cholesterol, and triglyceride levels, a stage of microinflammation, and elevated thrombogenesis factors.²

Elevated Lp(a) levels and hyperhomocysteinemia are accepted as independent risk factors for atherosclerosis.³⁻⁷ Lipid abnormalities are important variables in the development of vascular atherosclerotic lesions in ESRD patients.^{8,9} It has been reported recently that a number of cytokines, mainly tumor necrosis factor-alpha (TNF- α), interleukin (IL)-1 β and IL-6 can alter lipid metabolism and produce hyperlipidemia.¹⁰ Studies in hemodialysis (HD) patients have demonstrated increased production of these cytokines during HD.^{11,12}

In this study, in order to investigate any possible relationship among changes TNF- α , Lp(a), homocysteine, total cholesterol and triglyceride levels, we measured these parameters in hemodialysis patients before and after HD.

Material and Methods

This study was planned at Gazi University, Medical Faculty as a prospective and randomized trial. The study protocol was approved by the local review board of the University (July 2003-No. 2003/145). Thirty consecutive patients with ESRD for three years, on regular maintenance, HD performed one time monthly were included in the study. The median age of patients was 49 (22-69). For HD, hemophan membranes were used. Venous blood samples were collected and centrifuged at 3500 g for 5 minutes. Serum

samples were frozen at -70°C until applying the tests. Serum levels of Lp(a), TNF- α , total cholesterol and triglyceride were measured before and one day after HD.

TNF- α levels were measured by radioimmunoassay method using Biosource kit. Lp(a) levels were determined by nephelometric assay using Beckman 360 protein array nephelometry. Serum homocysteine levels were measured using Chromsystems HPLC with Fluorescence detector. Serum total cholesterol and triglyceride levels were determined with Abbott Aeroset autoanalyzer using original kits.

The statistical differences of Lp(a) and homocysteine levels between pre and post HD samples were analyzed using Wilcoxon test. Paired-t test was performed in order to evaluate the differences of TNF- α , total cholesterol and triglyceride levels between pre and post HD samples and $p < 0.05$ was considered significant.

Results

Both serum TNF- α and Lp(a) levels were higher in post HD period ($p < 0.001$) than those in pre HD (Figure 1).

There was no statistical difference on serum total cholesterol and triglyceride levels between pre and post HD period. Similarly, serum homocysteine levels did not change after HD. Results were given at (Table 1).

Discussion

Several studies have demonstrated that elevated plasma homocysteine, Lp(a) and lipid levels are now considered risk factors for atherosclerosis.¹³⁻¹⁵ Currently there is a great interest in hyperhomocysteinemia as an independent risk factor for cardiovascular events.¹⁶⁻²¹

There is a higher prevalence of hyperhomocysteinemia in ESRD patients who are in predialysis and on dialysis,^{22,23} as well as in renal transplant patients.^{24,25} In both populations, the increased incidence of atherosclerotic vascular

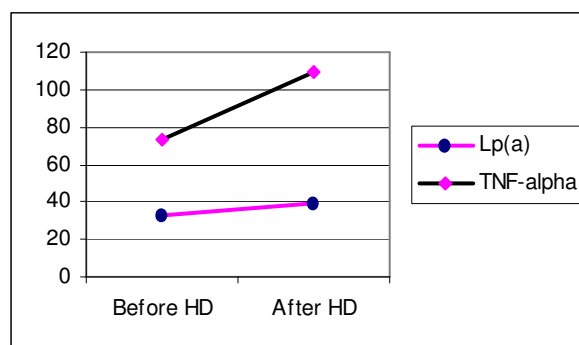
Table 1. Results of the patients. Results were expressed mean \pm SD.

Groups (n= 30)	Before HD	After HD	p value
Lp(a) (mg/dl)	32.87 \pm 24.33	38.99 \pm 28.83	<0.001
TNF- α (pg/mL)	73.49 \pm 21.01	109.02 \pm 3.09	<0.001
T. Cholesterol (mg/dl)	161.05 \pm 9.08	165.02 \pm 8.15	>0.05
Triglyceride (mg/dl)	170.42 \pm 24.11	168.51 \pm 12.52	>0.05
Homocysteine (μ mol/L)	25.19 \pm 9.59	28.65 \pm 10.48	>0.05

disease has been shown to correlate with the level of serum homocysteine.^{18,25-28} Moreover, ESRD patients have serum total homocysteine values twofold to threefold higher than those of age-matched controls with normal renal function.^{29,30} Our study demonstrated the similar results for serum homocysteine levels with the previous studies.

The physiological basis for hyperhomocysteinemia in renal failure is not clear. Homocysteine is produced during the breakdown of methionine, and can be metabolized by three separate pathways.³¹ The remethylation pathway is the most important determinant of homocysteine metabolism. Several mechanisms have been postulated to explain the reduction in the activity of remethylation pathway in renal failure, including a subclinical deficiency of folic acid and vitamin B12. There is another explanation for hyperhomocysteinemia that kidney has an important role in the normal metabolism of homocysteine. The clearance of homocysteine is reduced in ESRD patients. The reduction in homocysteine metabolism by the diseased kidneys may be exacerbated by the very low levels of serine, commonly present in the patients with ESRD, since serine is reduced as a methyl donor in the remethylation pathway.³¹

The mechanism responsible for homocysteine dependent atherosclerosis include, direct endothelial damage (due possibly to free oxygen radicals), enhanced oxidation of low-density lipoproteins increased platelet aggregation, and proliferation of vascular smooth muscles.³² When released in plasma, homocysteine rapidly auto-oxidizes. During oxidation of the sulphhydryl group of particle, free radicals, including the superoxide

**Figure 1.** The changes of Lp(a) and TNF- α levels.

anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are generated.³³ Both $O_2^{\cdot-}$ and H_2O_2 cause endothelial cytotoxicity and lipid peroxidation. The oxidative changes in low-density lipoprotein (LDL) particles modify their composition and promote foam cell formation, fatty streak development, and endothelial lesion progression.³⁴

Although the influence of total plasma homocysteine in serum lipids is unknown yet, there are few studies that have shown a correlation between plasma levels of total homocysteine and increased plasma levels of cholesterol and/or triglycerides.^{19,34} In our study, we investigated a possible effect of HD on total cholesterol and triglyceride levels. But we couldn't have found a difference on these parameters. Homocysteine induces hepatocytes endoplasmic reticulum stress which leads to activation SREBs involving to an increased cholesterol biosynthesis.³⁵ On the other hand, homocysteine increases the activation of intracellular 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase resulted in an increased production and excretion of cholesterol.^{36,37} Homocysteine inhibites the fatty

acid oxidation resulted in an increase of triglycerides.³⁸

In experimental renal failure a direct effect of high phosphorus diet in arterial wall thickening was also documented.³⁹ But in the present study we didn't investigated the changes of the atherosclerotic risk factors caused by the diet in dialysis patients.

Dialysis patients are characterized by high incidence of established or presumed risk factors for the development of atherosclerosis such as hypertension,⁴⁰ hypertriglyceridemia and dyslipidemia.⁴¹⁻⁴² Increased serum Lp(a) levels in HD patients were shown in several studies, and Lp(a) was accepted as an independent risk factor for atherosclerosis in HD patients.⁴³⁻⁴⁶

It has been suggested that changes in immune response to infectious agents in patients on HD might be due to impaired monocyte function; uraemic and hemodialysed patients overproduce proinflammatory cytokines, such as IL-1 β , TNF- α and IL-6. Malaponte et al. have showed that monocytes from HD patients spontaneously secreted significantly higher levels of cytokines than those from controls and uraemic patients who had not yet started dialysis.¹¹

Tzanatos et al. investigated the relation between changes of cytokines and lipid concentration during HD. Their results indicated that release of TNF- α and IL-1 β during HD have no effect on serum lipid concentration, except on Lp(a). They explained the acute rise of this lipoprotein during HD may be related with the TNF- α overproduction.¹⁰

In the present study, we showed that Lp(a) and TNF- α levels markedly elevated in ESRD patients after HD and contributed to possible atherosclerotic process. We think that increased plasma concentration of Lp(a) in our patients after HD is not due to decreased catabolism but is caused by increased synthesis. In addition to this, it was shown that hemodialysis have no effect on homocysteine levels, the important risk factor for atherosclerosis.

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