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SERUM PARAOXONASE ACTIVITY IN CHRONIC HEMODIALYSIS PATIENTS

KRONİK HEMODİYALİZ HASTALARINDA SERUM PARAOKSANAZ AKTİVİTESİ

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ÖZET

Yüksek dansiteli lipoproteinin (HDL) düşük dansiteli lipoproteinin (LDL) oksidasyonunu önlediği gösterilmiştir. HDL 'nin antioksidan etkisini özellikle paraoksanaz (PON) enzimi olmak üzere, taşıdıkları enzimler aracılığıyla yaptığına inanılmaktadır. Son zamanlarda, PON'un aterosklerozım patogenezinde rol oynadığı düşünülmektedir. Biz 76 kronik hemodiyaliz hastası ve 66 sağlıklı kişide serum PON aktivitesi ve fenotip dağılımını araştırdık. Serum PON aktivitesi, HDL ve apolipoprotein Al (Apo-Al) seviyeleri hasta grubunda kontrol grubuna göre önemli derecede azdı (sırasıyla, p<0.0001, p<0.0001 ve p<0.0001). PON/HDL ve PON/Apo-Al oranlan hasta grubunda ***kontrol*** *grubuna göre önemli derecede düşük idi* ***(sırasıyla, p<0.0001*** *ve p<0.0001). PON'un fenotipik dağılım (AA, AB, BB) farkı hasta ve kontrol gruplarında arasında önemli değildi. Biz, kronik hemodiyaliz hastalarında PON aktivitesinin PON'in fenotip inden bağımsız olarak, kontrol grubuna göre düşük olduğu sonucuna vardık.*

Anahtar kelimeler: Kronik hemodiyaliz hastası, Paraoksanaz

ABSTRACT

High-density lipoprotein (HDL) has been shown to prevent the oxidation ***of*** *low-density lipoprotein (LDL). The antioxidant activity of HDL is believed to reside in its enzymes, particularly paraoxonase. Recently, paraoxonase (PON) has been implicated in the pathogenesis of atherosclerosis. We have examined the activity, and phenotype distribution of serum PON in 76 patients with chronic renal failure undergoing maintenance hemodialysis as patient group and 66 healthy subjects as control group. The serum paroxonase activity in this patient group were significantly reduced when compared to the control group (p<0.0001). The levels of HDL and apolipoprotein A1 (Apo-A 1) in the patient group were* ***also lowest when*** *compared* ***to the control*** *group (p<0.0001 and p<0.0001, respectively). The PON activity for* ***HDL*** *(PON/HDL ratio) and apoAl (PON/ Apo-Al ratio) levels* ***in the patient*** *group were significantly reduced when compared to the control (p<0.0001) and p<0.0001, respectively). The phenotypic distribution of PON (AA,AB,BB) was not significantly different between patient and the control groups. We conclude that serum paraoxonase activity was significantly lower in the chronic hemodialysis patients as independent of paraoxonase phenotypic.*

Key words: Chronic hemodialysis patients, paroxonase

INTRODUCTION

Accelerated atherosclerosis is a frequent complication in chronic renal failure patients, and the main causes of mortality and morbidity in these patients are cardiovascular disease (1-5). The serum concentration of high-density lipoprotein (HDL) has long been known to have an inverse correlation with the development of atherosclerosis (3-5,6). Attention has thus focused on factors that may protect low-density lipoprotein from oxidation and this has highlighted a potential role for HDL (2,5).

Human serum paraoxonase (PON) is a polymorphic enzyme that is capable of catalysing the hydrolysis of organophosphate insecticides, nerve gases,

and is widely distributed among tissues such as liver, kidney, intestine, and also serum (5,7). The human serum PON is located on HDL (7-10). Biochemical studies of this enzyme indicated that PON could prevent lip id-peroxide accumulation on LDL (2,3,7). The serum PON activity is reduced. in diabetes and familial hyperlipoproteinemia that are associated with accelerated atherogenesis (5,7,10). Recently, it is reported that serum paraoxonase activity was also significantly reduced in uremic patients (2,3).

Human PON has two genetic polymorphism both due to amino acid substitution, one involving glutamine (A or Q) and arginine (B or R genotype) at position 192 and the other leucine (L genotype) and methione (M genotype) at position 55. The serum activity of PON varies among individuals and this difference is related with the position-192 polymorphism of the paraxonase gene (4,9). The population can be subdivided into three phenotypic groups: A A represents low; AB intermediate, and BB high enzyme activity of PON (1-3,7). The hypothesis that PON polymorphism might be a risk factor for cardiovascular disease (1,7).

In this study, we report the activity and phenotype distribution of serum PON in the chronic renal failure patients on long-term hemodialysis treatment.

PATIENTS AND METHODS

76 patients with chronic renal failure undergoing maintenance hemodialysis treatment for 4h, 3 times/week as patient group (31 male and 45 female; mean age 46,28±13,84 years) and 66 healthy subject as control group (24 male and 42 female; mean age 48,12±11,99 years) were evaluated. None of the patients had received any medication known to affect serum lipoprotein levels.

Blood samples were drawn from the venous line before dialysis after an overnight fast (12 h). The blood samples were collected using commercially available collection test tubes (glass tube without additives). For preparation of venous serum the collection tubes were centrifuged unopened 30 min after 3,000 g for 10 min, and aliquots of serum were immediately stored at - 80 ˚C until analyzed.

Lipid measurements

Levels of serum triglyceride and total cholesterol were determined enzymatic kit method (Boehringer-Mannheim) using auto analyser (Hitachi 717). Levels of HDL-cholesterol were also determined enzymatic kit method (Sigma) using auto analyser (Hitachi 717). The LDL-cholesterol fraction was calculated indirectly using the Friedewald equation. Apolipoproptein B (Apo-B) and Apo-Al titreş were determined by nephelometry (Beckman Array 360 System) using commercial kit (Beckman'reagents).

Analysis of PON activity

Paraoxonase assay were made either without any added NaCl (basal activity) or with 1 M NaCl included (salt-stimulated activity) (11). The basal PON activity was measured by adding 50 µl serum to 1 ml Tris/HCL buffer (100 mmol/1, pH 8.0) containing 2 mmol/1 CaC12 and 5.5 mmol/1 paraoxon (O,O-diethyl-O-/?-nitrophenyl phosphate; Sigma Chemical, St. Lois, Mo., USA), "and salt-stimulated PON included 1.0 M NaCl in addition to this mixture. The rate of hydrolysis of paraoxon was assessed by measuring the liberation of 4-nitrophenol at 412 nm at 25 oC, by the use of spectrophotometer. Enzymatic activity was calculated from the molar extinction coefficient 17,1000 M-l cm-1. One unit was defined as the amount of PON producing 1 nmol of 4-nitrophenol formed per minute per millilitre serum. The percent stimulation of PON was calculated as

$$\frac{\text{Paraoxonase activity with 1 M NaCl - Basal paraoxonase activity}}{\text{Basal paraoxonase activity}} \text{x-100\%}$$

Individuals were classified for PON phenotype using the antimode of PON as proposed by Eckerson et al (12). Individuals below the limit of 60% stimulation were considered A phenotypes, between 60% and 200% AB phenotypes, and above the level 200% stimulation BB phenotypes.

Statistical methods

The statistical analysis was performed by the SPSS 7.5 for Windows computer program. The comparison between groups were performed by Student's t test.

RESULTS

Lipid parameters

The data including lipid parameters of patients and control groups are shown in **Table 1.** The serum triglyceride, total cholesterol, LDL-cholesterol, and Apo-B levels in the patient group were significantly elevated when compared to the control group ($p<0.0001$ $p<0.0001$ and $p<0.0001$, respectively).The levels of HDL-cholesterol and Apo-Al in the patient group were lowest when compared to the control group ($p<0.0001$ and $p<0.0001$, respectively).

PON activity changes

The HDL-associated PON activities are shown **Table 2** and **Figure 1.** Basal and salt-stimulated PON activity in the patient group were significantly reduced

when compared to the control group. HDL and PON activity are decreased in chronic hemodialysis patients. These results suggest that one of the possible causes of the decreased PON activity may be lower HDL and Apo-Al levels in patient group. Therefore, we standardized the PON activity for HDL (PON/HDL ratio) and Apo-Al (PON/Apo-Al ratio) concentrations. We found that the PON/HDL and PON/ Apo-Al ratios in patient group were significantly reduced when compared to the control group ($p<0.0001$ and $p<0.0001$, respectively) (Table 2).

The phenotypic distribution of PON in the patient group was 46.5% AA, 48.08% AB, and 5.77% BB. In the control group the distribution was 54.5% AA, 36.7% AB, and 9.08% BB. The phenotypic distribution of PON (AA,AB,BB) was not significantly different between patient and the control groups.

Table 1: Clinical data and lipid parameters in the both groups

Parameters	**Patient group**	**Control group**
n (male/female)	76	66
Age, years	46.28 ±13.84	48.12 ± **11.99**
Body mass index, kg/m^2	22.39 ±2.61	25.46 ±4.61
Triglyceride, mg/dl	186.47 ±12.27*	108.61 ±38.45*
Total cholesterol, mg/dl	229.04 ± 23.59*	163.82± 15.71*
HDL-cholesterol, mg/dl	36.50 ±5.26*	54.07 ±6.28*
LDL-cholesterol, **mg/dl**	155.29 ±23.55*	88.02 ±13.80*
Apo-B, g/I	1.10±0.15*	0.94 ±0.15*
ApoAl, g/I	0.88 ± 0.01	1.41 ±0.19*

Values are mean ±SD, Significance of difference: *: $p<0.0001$

Table 2. Parameters with paraoxonase in the both groups

Parameters	**Patient group**	**Control group**
n (male/female)	76	**66**
Basal PON activity, U/ml	36.15 ±19.99*	**68,52 ±31.34***
Salt-stimuleted PON activity, U/ml	63.15 ±42.35*	**157,60 ±113.33***
PON /Apo-Al ratio	**40.43±** 21.20*	48.18+ 18.62*
PON/HDL ratio	0.98±0.52*	1.27±0.55*

Values are mean ±SD, Significance of difference: *: $p<0.0001$

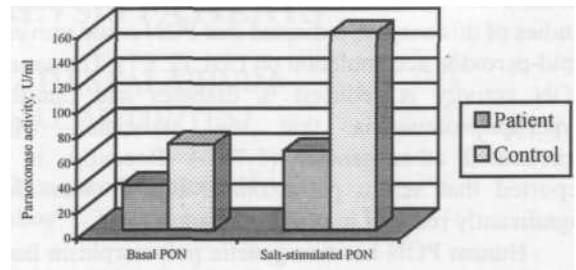

Figure 1: Paraoxonase activity in the patient and control group

DISCUSSION

The high incidence of atherosclerotic deaths in patient with chronic renal failure has been a growing problem. Hyperlipidemia is a major risk factor in the development of cardiovascular disease. Oxidation of LDL appears, that initiates foam-cell development, play an important role in the development and progression of the atherosclerotic lesion (4,5,8,13). There is also evidence for an increased prevalence of oxidized LDL in chronic renal failure (2,3).

It is reported that HDL protects against LDL oxidative modification which is believed to be the central to the initiation and progression of atherosclerosis. The antioxidant activity of HDL is believed to reside in its enzymes, particularly PON (2,5,14,15).

Our current findings have shown that serum PON activity was lower in patients group than in control group. The low PON activity was likely to have been due to decreased synthesis and/or enhanced catabolism (4,10). Low concentrations of HDL increase susceptibility to atherosclerosis and consequently coronary heart disease (2,3). In this study, the levels of HDL and Apo-A 1 of patient group were also lower when compared to the control group.

PON present in serum is located on HDL, being tightly bound to a HDL subtraction containing apoAl and clusterin. The PON containing HDL particles constitute a very small fraction of total plasma HDL (4). To asses whether the altered PON activity was due to HDL or Apo-Al level decrease, we standardized the enzyme activity for HDL and Apo-Al concentration (PON (HDL and PON/ Apo-Al ratios). We found that the standardized enzyme activities were lower in patient group compared to control group ($p<0.0001$ and $p<0.0001$, respectively). This suggested that the decreased in PON activity is independent of changes in HDL and Apo-Al level in chronic renal failure patients (3,8,16).

Under oxidative stress, not only is LDL susceptible to lipid peroxidation, but all other serum lipids, including those present in HDL, are also prone to oxidation. Reduced level of serum HDL is an independent risk factor for atherosclerosis, and oxidized HDL perhaps increases this risk (6,7). Aviram et al. demonstrated that HDL associated PON inhibited not only LDL oxidation, but also HDL oxidation. The inhibitory effect of PON on HDL oxidation was associated with preservation of the HDL ability to induce cellular cholesterol efflux from macrophages (6).

The protective effect of HDL may not be dependent on the absolute levels of HDL in the blood but rather the abundance of HDL particles which contain protective enzymes relative to the concentration of oxidized LDL proximate to the artery wall cells. This ratio is determined by both genetic and environmental factors. Genetic determinants may include plasma levels and isoforms of PON (16).

The relationship between PON phenotypes, diabetes mellitus, coronary atherosclerosis and the occurrence of coronary heart disease are still controversial (9,11,20). Generally, in case-control studies serum PON activity was also found to be decreased in coronary heart disease independent of PON polymorphism. Serato et al. found that the frequency of the BB phenotype, having higher activity enzyme activity than the other phenotypes, was significantly higher in patient with coronary heart disease than in controls (16). In our study, both AA and AB phenotypes accounted for more than 90% of the patients and controls, without any significant differences between them (3,4,11). Therefore, chronic renal failure patients must have another factor influencing the activity of PON besides the polymorphism (9,10,11,13).

We conclude that serum PON activity was significantly lower in the chronic hemodialysis patients as independent of PON polymorphism. The importance of PON as a predictive risk factor for cardiovascular disease at chronic renal failure patients should be assessed in future studies.

REFERENCES

1. Ruiz J, Blanche H, James RW, Blatter-Garin MC, Vaisse C, Charpentier G, et al. Gln-Argl92 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet 1995;346:869-872.
2. Paragh G, Asztalos L, Seres I, Balogh Z, Löcsey L, Kârpâti I, et al. Serum paraoxonase activity changes in uremic and kidney-transplanted patients. Nephron 1999:83:126-131.
3. Paragh G, Sers I, Balogh Z, Varga Z, Kârpâti I, Mâtyus J, et al. The serum paraoxonase activity patients with chronic renal failure and hyperlipidemia. Nepron 1998;80:166-170.
4. Ayub A, Mackness MI, Arrol S, Mackness B, Patel J, Durrington PN. Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vase Biol 1999;19:330-335.
5. Blatter-Garin MC, James RW, Dussoix P, Blanche H, Passa P, Froguel P. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. J Clin Invest 1997;99:62-66.
6. Aviram M, Rosenblat M, Bisgaiger CL, Newton RS, Primo-Parma SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998;101:1581-1590.
7. Karakaya A, İbiş S, Kural T, Köse SK, Karakaya AE. Serum paraoxonase activity and phenotype distribution in Turkish subjects with coronary heart disease and its relationship to serum lipids and lipoproteins. Chem-Biol Interact 1999;118:193-200.
8. Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, et al. Serum paraoxonase activity in familial hypercholesterolemia and insulin-dependent diabetes mellitus. Atherosclerosis 1991;86:193-199.
9. Suehiro T, Nakauchi Y, Yamamoto M, Arii K, Itoh H. Hamashige N, et al. Paraoxonase gene polymorhism in Japanese subjects with coronary heart disease. In J Cardiol 1996;57:69-73.
10. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the molecular polymorphisms of human paraoxanase (PON1) on the rate of hydrolysis of paraoxon. Br J Pharmacol 1997;122:265-268.
11. La Du BN, Eckerson HW. The polymorphic paraoxonase/arylesterase isozymes of human serum. Fed Proc 1984;43:2338-2341.
12. Eckerson HW, Wythe CM, La Du BN. The human serum paraoxonase/aryesterase polymorphism. Am J Hum Genet 1983;35:1126-1138.
13. Witztum JL, Daniel S. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1995;88:1785-1792.
14. Odawara M, Tachi Y, Yamashita K. Paraoxonase polymorphism (Glnl92-Arg) associated with coronary heart disease in Japanes noninsulin dependent diabetes mellitus. J Clin Endocrinol Metabol 1997;82:2257-2260.
15. Krauss RD, Berkeley MD. The tangled web of coronary risk factors. Am J Med 1991 ;90 (suppl 2A):36-41.
16. Serrato M, Marian AJ. A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. J Clin Invest 1995;96:30005-30008.