Mikroalbüminürisi Olan Esansiyel Hipertansif Hastalarda Plazma Total Homosistein Düzeyleri ve Diğer Kardiyovasküler Risk Faktörleri

Plasma Total Homocysteine Levels and Other Cardiovascular Risk Factors in Essentially Hypertensive Patients With Microalbuminuria

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ÖZET

Bu çalışmanın amacı, mikroalbüminürisi olan esansiyel hipertansiyonlu hastalardaki plazma total homosistein düzeyleri ile kardiyovasküler hastalık için diğer risk faktörlerini araştırmak ve bu verileri mikroalbüminürik olmayanlarınkilerle karşılaştırmaktı.

Esansiyel hipertansif hastalarda (n=45) plazma total homosistein düzeyleri belirlendi ve kontrollerinki (n=14) ile kıyaslandı. Daha sonra, hasta grubu mikroalbüminürik olanlar ve olmayanlar olmak üzere iki alt gruba bölündü. Her iki alt grup birbiriyle –plazma total homosistein düzeylerini de içeren– çalışılan parametreler açısından kıyaslandı. Hastaların plazma total homosistein düzeyleri (12.5±6.58 µmol/L) kontrollerinkinden (13.28±11.11 µmol/L) daha düşüktü; fakat fark anlamlı değildi (p=0.795). Mikroalbüminürik olan (12.82±6.01 µmol/L) ve olmayan (12.06±7.45 µmol/L) hastalar, plazma total homosistein düzeyleri bakımından birbirlerinden istatistiksel olarak anlamlı bir farklılık göstermediler. Benzer şekilde, gerek ana grupta (p=0.648) gerekse mikroalbüminürik olan (p=0.721) ve olmayan (p=0.246) hasta gruplarında, hipertansif retinopatisi olan ve olmayan hastalar arasında da plazma total homosistein düzeyleri farklı değildi. Ancak, mikroalbüminürik hastaların serum LDL kolesterol düzeyleri (111.76±30.10 mg/dL) mikroalbüminürik olmayan hastalarınkinden (87.43±30.39 mg/dL) anlamlı olarak (p=0.037) daha yüksekti. Verilerimiz, plazma total homosistein düzeylerinin esansiyel hipertansiyonlu hastalardaki mikroalbüminüri ve retinopatinin patogenezinde bir rolü olmadığı izlenimini vermektedir; fakat bu hastalardaki mikroalbüminüri serum LDL kolesterol düzeylerinden etkilenmiş görünmektedir.

Anahtar sözcükler: homosistein, mikroalbüminüri, esansiyel hipertansiyon

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ABSTRACT

The aim of the present study was to investigate the plasma total homocysteine levels and other risk factors for cardiovascular disease in microalbuminuric essentially hypertensive patients and to compare with those of non-microalbuminuric ones.

Plasma total homocysteine levels were determined in essentially hypertensive patients (n=45) and compared with those of the controls (n=14). Then, the patient group was divided into two subgroups as microalbuminuric and non-microalbuminuric. Both subgroups were also compared to each other with respect to determined parameters including plasma total homocysteine levels. Plasma total homocysteine levels of the patients (12.5±6.58 µmol/L) were lower, but not significantly (p=0.795), than those of the controls (13.28±11.11 µmol/L). Microalbuminuric (12.82±6.01 µmol/L) and non-microalbuminuric (12.06±7.45 µmol/L) patients did not show a statistically significant difference from each other with respect to plasma total homocysteine levels. Similarly, plasma total homocysteine levels were also not different between the patients with and without hypertensive retinopathy in the main (p=0.648), microalbuminuric (p=0.721) and non-microalbuminuric (p=0.246) patient groups. However, serum LDL cholesterol levels of microalbuminuric patients (111.76±30.10 mg/dL) were significantly higher than those of the non-microalbuminuric patients (87.43±30.39 mg/dL) (p=0.037). It suggests that plasma total homocysteine levels do not involve in the pathogenesis of microalbuminuria and retinopathy in patients with essential hypertension, but microalbuminuria in these patients seems to be affected by serum LDL cholesterol levels.

Keywords: homocysteine, microalbuminuria, essential hypertension

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Introduction

Microalbuminuria has been reported as a predictor of the risk for cardiovascular disease in both diabetic and nondiabetic individuals (1-4). However, the association between microalbuminuria and cardiovascular disease has not been elucidated clearly (1,2). The cardiovascular disease risk in patients with microalbuminuria may be resulted from the higher prevalence of well known other risk factors such as diabetes, hypertension, smoking and dyslipidemia (2). An elevated plasma total homocysteine (Hcy) concentration is also considered as an independent risk factor for cardiovascular disease in nondiabetic population (5,6). Many studies investigating the relationship between plasma total Hcy levels and microalbuminuria in both general population (2) and particularly diabetic patients (7,8) have been reported. On the other hand, microalbuminuria prevalence in patients with essential hypertension (EH) has been reported from 5% to 37% (9) and patients with EH (10,11) have been reported to have high serum concentrations of Hcy. In the present study, essentially hypertensive patients were investigated with respect to their plasma total Hcy levels and it was aimed to establish the contribution of the plasma total Hcy levels and other cardiovascular risk factors in the development of microalbuminuria in these patients.

Materials and Methods

Forty-five essentially hypertensive patients were included into the study. Of them, 33 were males (73.3%) and 12 were females (26.7%). Their mean age was 37.1±17.3 years (range, 20-75 years). Hypertension was diagnosed when the patients had been on a antihypertensive therapy and/or they met the following criteria recommended by The Seventh Report of the Joint National Committee: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. All of the hypertensive patients were investigated whether they had a clinical and/or laboratory clue suggesting the presence of any form of secondary hypertension. If they had no clue or evidence for secondary hypertension in their history, physical examination and standard laboratory tests, they were accepted as having EH. Weights and heights were measured in all the patients enrolled in the study group. To calculate the body mass index (BMI) of the patients, weight (kg) is divided by height (meter) square. Body fat ratios of the patients were determined with a commercially available electronic device (Tanita body fat monitor, TBF-531A, Japan), using bioelectrical impedance analysis method. Following an overnight fast, venous blood samples from the patients and the controls were drawn into the appropriate tubes to determine the serum creatinine, glucose, uric acid, total cholesterol, high density lipoprotein (HDL) cholesterol, triglyceride, total protein, albumin, vitamin B12, folate and plasma fibrinogen and total Hcy levels. After the collection, blood samples taken for serum studies were centrifuged and they were stored at -80°C until the assay. Serum creatinine, glucose, uric acid, total cholesterol, HDL cholesterol, triglyceride, total protein, albumin levels were determined in a DAX-48 analyzer (Bayer Diagnostica, Germany) using the original kits. Low-density lipoprotein (LDL) cholesterol levels were calculated by using Friedewald formula (12). Serum vitamin B12 and folate levels were measured with automated chemiluminescence system (ACS: 180, Bayer, Germany) method. Blood samples obtained for plasma fibrinogen levels were collected into the tubes containing sodium citrate. Following centrifugation, fibrinogen levels in plasma were determined by using AMAX CS 190 analyzer (H. Amelung GmbH, Germany). Plasma total Hcy levels were also measured. Blood samples (5 mL) collected into tubes containing sodium fluoride, EDTA and potassium were centrifugated in one hour to obtain plasma and stored at -40°C until the assay. A reagent kit (Chromsystems, 39000, Germany) was used for high performance liquid chromatography (HPLC) analysis of Hcy in plasma. The linearity of the kit was in total therapeutic range. Detection limit was 1 µmol/L, analysis time less than 5 minutes and intraassay reproducibility <5%. Internal standard (50 µL) and reduction solution (50 µL) were added into 400 µL plasma obtained from the patients. This mixture was shaken for 2 seconds on a Vortex and then it was incubated for 5 minutes at room temperature. After incubation, 500 µL precipitation reagent was added into the mixture and it was shaken on Vortex for 1 minute. The sample was centrifuged for 5 minutes at 10000 × g. Two-hundred reaction buffer was added into 100 µL supernatant from the last centrifugation and 50 µL derivatisation solution in a separate reaction vial. Subsequently, the sample was incubated for 10 minutes at 50°C in a water bath and then the samples were cooled to room temperature. HPLC with flu-

orescence detector (Hewlett-Packard 1100, USA) was used to determine plasma total Hcy levels. The studies were performed isocratically. Flow rate was 1.7 mL/minute and 20 µL sample was injected into 20 µL Rheodyne injection module. In elution, reverse phase ion exchange was used. C18 column in size of 150 × 4,6 mm was used as analytic column. The column had 5 µm particle size. C18 column in size of 50 × 4.6 mm, as guard column, was settled between the injection module and the analytic column. Detection for 5 minutes was performed with fluorescence detector at 385 nm excitation and 515 nm emission wavelengths. Based upon the calculated slope values in regression analysis, which was applied to calibration curve drawn from the peak heights, established in standardization studies, total plasma concentrations of the samples were obtained from their peak heights. The concentrations were expressed as µmol/L.

All the patients tested with dipstick for urinalysis and the patients with abnormal urinalysis were excluded. In patients with a normal urinalysis, microalbuminuria was investigated with a semiquantative test (Micral-Test II, Boehringer Mannheim GmbH, Germany). First morning urine samples of the patients were tested for microalbuminuria in three consecutive days. When the test was positive on two occasions, the patient was accepted as microalbuminuric.

Hypertensive retinopathy was detected by direct fundoscopic examination by a family practice specialist who had experience on this examination. Keith-Wagener (13) classification was used to categorize the hypertensive retinopathic changes.

The control group was consisted of 14 healthy voluntary subjects (4 females and 10 males) working as hospital staff. Their mean age was 34.3 ± 5.2 years (range, 27-45). None had any disease history and microalbuminuria was not detected in their urinalyses. All the serum and plasma parameters studied in the patients were also determined in the controls simultaneously with the same techniques, by the same workers.

Following the comparison of the essentially hypertensive patients with the controls with respect to studied parameters, microalbuminuric and non-microalbuminuric patients were also compared to each other for these parameters. Then, correlations of plasma total Hcy levels with other parameters were investigated both in the main group and subgroups.

The results were expressed as percentage and mean ± standard deviation. Statistical analyses were done by SPSS 8.0 (Statistical Package for the Social Sciences Program) for Windows. Fisher's exact, Mann-Whitney U and Pearson's correlation tests were used. P values less than 0.05 are considered as statistically significant.

Results

The patients and the control group were not different from each other with respect to gender (x^2=0.020, p=1.000). The mean age of the patients (37.1 ± 17.3 years) and the controls (34.3 ± 5.2 years) did not have a statistically significant difference (p=0.363). Except serum fasting glucose and folate levels, the patients and the controls had no significant difference in regard to the studied parameters as shown in Table I.

Although there was statistically significant difference between the two groups with respect to serum fasting glucose and folate levels, the mean values of these parameters were within the normal ranges (less than 126 mg/dL and 2.76-20.0 ng/mL for serum fasting glucose and folate levels, respectively) in both of the groups. Furthermore, none of the patients or controls had diabetes mellitus.

When the patient group was divided into two subgroups as microalbuminuric (26 patients, 57.7%) and non-microalbuminuric (19 patients, 42.2%), no significant difference was established between these two subgroups with respect to age (p=0.193) and gender (x^2=0.002, p=1.000). Table II shows the comparison of both subgroups in regard to the studied parameters.

Both subgroups did not have statistically significant difference from each other with respect to plasma total Hcy levels (12.82 ± 6.01 µmol/L in microalbuminuric patients, 12.06 ± 7.45 µmol/L in non-microalbuminuric patients and p=0.460). Plasma total Hcy levels did not have any significant correlation with the other parameters in the main group of the patients with EH. However, it correlated with serum triglyceride levels as positively and significantly (r=0.473, p=0.017) in microalbuminuric essentially hypertensive patients while age (r=0.513, p=0.030) and serum albumin (r=0.518, p=0.028) levels had significant positive correlations with plasma total Hcy levels in the non-microalbuminuric patients.

On the other hand, microalbuminuric and non-microalbuminuric essentially hypertensive patients

Table I. The comparison of serum and plasma parameters studied in the patient and control groups

	Patients (n=45)	Controls (n=14)	p value
Serum creatinine (mg/dL)	0.72 ± 0.11	0.71 ± 0.10	0.607
Fasting serum glucose (mg/dL)	91.34 ± 18.09	76.69 ± 15.58	0.004*
Serum uric acid (mg/dL)	5.84 ± 1.15	5.32 ± 0.99	0.117
Serum total cholesterol (mg/dL)	174.97 ± 36.51	187.35 ± 35.20	0.285
Serum HDL cholesterol (mg/dL)	43.29 ± 6.91	41.00 ± 5.18	0.263
Serum LDL cholesterol (mg/dL)	102.26 ± 32.16	114.00 ± 22.88	0.184
Serum triglyceride (mg/dL)	163.28 ± 93.22	158.92 ± 132.72	0.504
Serum total protein (g/dL)	6.86 ± 0.39	6.65 ± 0.35	0.067
Serum albumin (g/dL)	4.22 ± 0.27	4.24 ± 0.26	0.660
Plasma fibrinogen (mg/dL)	268.02 ± 73.78	261.61 ± 35.94	0.805
Serum vitamin B12 (pg/mL)	220.11 ± 155.77	182.60 ± 87.48	0.720
Serum folate (ng/mL)	7.06 ± 3.58	4.80 ± 1.91	0.017*
Plasma total homocysteine (µmol/L)	12.5 ± 6.58	13.28 ± 11.11	0.795

* Statistically significant

Table II. The comparison of both subgroups in regard to the studied parameters

	Microalbuminuric patients (n=26)	Non-microalbuminuric patients (n=19)	p value
Body mass index (kg/m^2)	27.78 ± 4.18	28.00 ± 4.32	0.954
Fat percentage	28.39 ± 8.40	27.00 ± 9.47	0.520
Systolic blood pressure (mmHg)	156.80 ± 18.86	162.77 ± 12.15	0.162
Diastolic blood pressure (mmHg)	93.84 ± 8.40	97.22 ± 12.62	0.213
Serum creatinine (mg/dL)	0.73 ± 0.13	0.72 ± 0.09	0.990
Fasting serum glucose (mg/dL)	92.08 ± 18.68	90.33 ± 17.73	0.777
Serum uric acid (mg/dL)	5.80 ± 1.23	5.90 ± 1.07	0.739
Serum total cholesterol (mg/dL)	182.15 ± 40.66	165.15 ± 28.04	0.171
Serum HDL cholesterol (mg/dL)	43.40 ± 7.40	43.12 ± 6.29	0.748
Serum LDL cholesterol (mg/dL)	111.76 ± 30.10	87.43 ± 30.39	0.037*
Serum triglyceride (mg/dL)	146.61 ± 70.94	186.10 ± 115.34	0.280
Serum total protein (g/dL)	6.93 ± 0.41	6.91 ± 0.36	0.729
Serum albumin (g/dL)	4.22 ± 0.28	4.22 ± 0.26	0.772
Serum vitamin B12 (pg/mL)	200.53 ± 157.82	246.89 ± 153.00	0.129
Serum folate (ng/mL)	7.50 ± 4.35	6.44 ± 2.10	0.581
Plasma fibrinogen (mg/dL)	276.30 ± 76.17	256.05 ± 70.57	0.659
Plasma total homocysteine (µmol/L)	12.82 ± 6.01	12.06 ± 7.45	0.460

* Statistically significant

had no difference from each other with respect to parameters shown in Table II, but serum low density lipoprotein (LDL) cholesterol levels. Since only serum LDL cholesterol levels were significantly different (p=0.037) between the microalbuminuric (111.76 ± 30.10 mg/dL) and non-microalbuminuric (87.43 ± 30.39 mg/dL) patients, its correlations with the other parameters were also investigated in the main group and in the subgroups. Serum LDL cholesterol levels in the study group had statistically sig-

nificant positive correlations with BMI (r=0.392, p=0.011), body fat percentage (r=0.377, p=0.015), total cholesterol (r=0.865, p<0.001) and HDL cholesterol (r=0.432, p=0.005). Serum total cholesterol (r=0.906, p<0.001), HDL cholesterol (r=0.520, p=0.08) and total protein (r=0.460, p=0.021) levels correlated with serum LDL cholesterol levels in microalbuminuric essentially hypertensive patients. In the non-microalbuminuric patients, serum LDL cholesterol levels showed significantly positive [BMI (r=0.630, p=0.009), body fat percentage (r=0.607, p=0.013), systolic blood pressure (r=0.762, p=0.001), total cholesterol (r=0.794, p<0.001)] and negative correlations [triglyceride (r=-0.726, p=0.001), albumin (r=-0.547, p=0.028), vitamin B12 (r=-0.660, p=0.005)] with some of the parameters.

When the patients were evaluated for their hypertensive retinopathy status, 14 (31.1%) patients with EH had hypertensive retinopathy in various grades ranging from 1 to 3 according to the Keith-Wagener classification. Five (35.7%), 8 (57.1%) and 1 (7.1%) of the essentially hypertensive patients with hypertensive retinopathy had first, second and third grade retinopathy, respectively. One patient (2.2%) did not undergo an ophthalmologic examination. Plasma total Hcy levels in essentially hypertensive patients with (13.86±8.22 µmol/L) and without (12.15±5.74 µmol/L) retinopathy were not significantly different from each other (p=0.648). When the microalbuminuric (7/25, 28.0%) and the non-microalbuminuric (7/19, 36.84%) patients with EH were compared to each other for the frequency of hypertensive retinopathy, both subgroups did not show statistically significant difference (x^2=0.389, p=0.745). Plasma total Hcy levels did not show statistically significant difference (p=0.721) in microalbuminuric essentially hypertensive patients with (12.73±6.69 µmol/L) and without (13.28±5.84 µmol/L) retinopathy. Similarly, non-microalbuminuric essentially hypertensive patients with retinopathy (14.84±9.77 µmol/L) had no significantly different (p=0.246) plasma total Hcy levels from the patients without retinopathy (10.30±5.31 µmol/L) in the same group.

Discussion

Elevated plasma total Hcy concentration is considered an independent risk factor for atherosclerotic diseases in subjects with normal glucose tolerance (6). The general theory is that hyperhomocysteinemia causes oxidative injury to the vascular wall, presumably including glomerular capillaries. (14). Patients with EH were reported to have significantly higher mean serum concentrations of Hcy (10,11). In contrast, in another study, no statistically significant difference was established in a hypertensive group compared to the normotensive one in regard to mean plasma Hcy concentration (15). In our study, mean total plasma Hcy concentration of the patients was lower (12.50±6.58µmol/L) than that of the healthy controls (13.28±11.11µmol/L) and the difference was not statistically significant (p=0.795). Although our study and that of the Rodriguez-Perez showed no statistically significant difference between hypertensive patients and normotensive controls with respect to plasma total Hcy levels, patients in our study had lower plasma total Hcy levels than those of the controls.

The relationship between microalbuminuria and hyperhomocysteinemia is still controversial. These two cardiovascular risk factors have been investigated in different patient populations to elucidate whether they have an association or not. In the Hoorn Study (16), it was suggested that hyperhomocysteinemia may partly explain the link between microalbuminuria and increased risk of cardiovascular disease since the odds ratio for microalbuminuria per 5 µmol/L total Hcy increment was 1.33 after adjusting for age, sex, glucose tolerance category, hypertension, dyslipidemia and smoking in this general population-based study. The presence of an association between microalbuminuria and Hcy has also been investigated intensively in diabetic patients. Increased plasma total Hcy levels have been reported in some of the studies comparing the microalbuminuric diabetic patients with non-microalbuminuric ones (7,17,18). However, two studies reported that no difference was established between diabetic patients with normoalbuminuria and those with microalbuminuria in regard to plasma Hcy levels (19,20). In the present study performed in patients with EH, microalbuminuric (12.82 ± 6.01 µmol/L) patients had higher plasma total Hcy levels than those of the non-microalbuminuric (12.06 ± 7.45 µmol/L) patients, although the difference did not achieve a statistical significance (p=0.460). These data from our study suggest that Hcy seems not to be involved in development of the microalbuminuria in essentially hypertensive patients. When the data from our study and the results of the two stu-

dies mentioned above (19,20) conducted in diabetic patients are considered together, hyperhomocysteinemia can not be easily associated with microalbuminuria, at least in these patient populations. As recommended (14), much more work through clinical and laboratory studies still needs to be done to establish an association between these two investigative themes.

In the pathogenesis of microalbuminuria seen in patients with EH, several different mechanisms have been implicated. These include increased glomerular hydrostatic pressure (the hemodynamic paradigm), permselectivity changes of the glomerular filter and structural damage to the glomeruli and arterioles (9,21). Among nondiabetic patients with EH, microalbuminuria has also been associated with increased serum total cholesterol, triglycerides and reduced serum HDL cholesterol (22,23). The results of two previous studies which include patients with EH (3,24) yielded that serum levels of LDL cholesterol were greater in microalbuminuric essentially hypertensive patients when compared with normoalbuminuric ones. Similarly, only serum LDL cholesterol levels among the studied parameters in our study were significantly ($p=0.037$) higher in microalbuminuric patients (111.76 ± 30.10 µmol/L) than non-microalbuminuric (87.43±30.39 µmol/L) patients with EH. Lipid content of the diet, urinary loss of protein, which may cause the increase in serum lipoprotein levels and renal damage caused by hyperlipidemia are the suggested mechanisms to explain the association of microalbuminuria with hyperlipidemia (9). Dyslipidemia may be the determinant contributor to the development of microalbuminuria in essentially hypertensive patients who have damaged renal vasculature caused by the direct transmission of increased systemic pressure to the glomeruli (1,21) since the microalbuminuria can not only be attributable to renal hemodynamic changes in EH (9). The fact that microalbuminuric essentially hypertensive patients had significantly higher serum LDL cholesterol levels than non-microalbuminuric patients in the presence of similar blood pressures in our study suggests that increased serum LDL cholesterol levels, an established atherosclerotic risk factor, may cause to alterations in the permselectivity properties of the glomerular filtration barrier.

Although some authors (2,25) suggested that hyperhomocysteinemia may be a risk factor for retinopathy in patients with diabetes, others (26-29) did not agree with them. In our study performed in the patients with EH, plasma total Hcy levels of the patients with retinopathy were not different from those of the patients without retinopathy in both the main group and in the subgroups. Since the study group was too small to obtain the clear-cut conclusions, other detailed studies with larger number of patients are needed to bring out the subject clearly.

In conclusion, serum LDL cholesterol levels, but not plasma total Hcy levels, seem to have a contribution to the development of microalbuminuria in patients with EH and retinopathy in microalbuminuric essentially hypertensive patients is probably not associated with plasma total Hcy levels. Therefore, close monitoring of the patients with EH for dyslipidemia and treating the ones with high LDL cholesterol levels and/or any kind of dyslipidemia vigorously, in addition to the control of hypertension, may be expected to have a beneficial effect in preventing microalbuminuria in these patients.

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