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Carbonic anhydrase I and II autoantibody levels in primary hypertension: our preliminary results

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Abstract. – OBJECTIVE: The pathogenesis of primary hypertension (HT) is still not completely clear, although autoimmunity has been implicated in recent years. Carbonic anhydrase (CA) is an enzyme involved in a number of important metabolic processes. CA I and II autoantibodies have been linked to various autoimmune diseases. However, CA I and II autoantibody levels in primary HT have not been previously investigated. The purpose of this study was, therefore, to investigate levels of CA I and II autoantibodies in primary HT.

PATIENTS AND METHODS: Fifty-six patients newly diagnosed with primary HT and 33 healthy individuals were included in the study. Twenty-four-hour ambulatory blood pressure monitoring was performed following office controls. Blood specimens were collected under appropriate conditions for CA I and II autoantibody level investigation and biochemical tests. Urine sodium and protein excretion were measured after 24 h. Demographic and biochemical parameters and CA I and II autoantibody levels were then compared between the patient and healthy groups.

RESULTS: CA II autoantibody and uric acid levels were significantly higher in the hypertensive group than in the control group (p=0.005, and p<0.001, respectively). CA II autoantibody (exp ß: 79.06 CI: 4.44-1407.02) (p=0.003) and uric acid elevation (exp ß: 2.10 CI: 1.31- 3.34) (p=0.002) were identified as independent predictors of HT development at logistic regression analysis.

CONCLUSIONS: CA II autoantibody levels were higher in hypertensive patients, and this elevation is an independent predictor of HT development.

Key Words:
Hypertension, Carbonic anhydrase, CA, CA I autoantibody, CA II autoantibody.

Introduction

Hypertension (HT) is a disease with high morbidity and mortality, the prevalence of which is increasing worldwide. Ninety percent of HT is of unknown cause, and is defined as primary or idiopathic HT. The remaining 10% consists of secondary HT. Polygenic inheritance and environmental factors have been implicated in the pathogenesis of primary HT. Genetic and environmental factors are thought to lead to membrane ion channel defects, particularly Na/K ATPase, to activation in the sympathetic nervous system and the renin-angiotensin-aldosterone system, and to a decrease in vasodilator mediators. The activation of these systems then results in primary HT development through increased vascular smooth muscle contraction and decreased salt excretion from the kidneys[1-3]. Recent studies[4] have reported that oxidative damage, inflammation, and autoimmunity also occupy an important place in the pathogenesis of primary HT.

Carbonic anhydrase (CA) is a zinc enzyme responsible for reversible catalyzation of CO_2. It has 16 known isoenzymes in mammals. These isoenzymes are involved in such physiological processes as CO_2 transport, pH regulation, and calcification. CA autoantibodies have recently been reported in various autoimmune or idiopathic diseases and carcinomas[5,6]. However, no previous studies have investigated the role of these in the pathogenesis of primary HT. In the light of the effect mechanism of CA, we

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hypothesized that CA I and II autoantibodies may be involved in the pathogenesis of HT.

The purpose of this study was to investigate CA I and II autoantibody levels in a newly diagnosed primary HT patient group.

Patients and Methods

Patients were selected once the local Ethical Committee had confirmed that the study protocol was compatible with the Second Declaration of Helsinki. Patients were included following receipt of written and verbal consent. Fifty-six primary hypertensive patients aged over 18, presenting to the Namik Kemal University Medical Faculty Nephrology and Hypertension and Internal Medicine Department (Turkey), and diagnosed with HT based on the European Society of Cardiology and the European Society of Hypertension (ESC/ESH) 2018 guideline[7] and with no previous receipt of antihypertensive therapy, were enrolled together with 33 healthy controls. Subjects with secondary HT, renal failure, diabetes mellitus (DM), connective tissue disease, a history of cancer, thyroid disorder, coronary artery disease and liver disease, and smokers, were excluded from the study.

Fifty-six hypertensive patients meeting the inclusion criteria and 33 healthy controls were included in the study. Patients underwent detailed physical examinations, and blood pressure (BP) was measured in line with the ESC/ESH 2018 guideline[7]. Patients with BP of 140/90 mmHg or above were enrolled. Twenty-four-hour ambulatory blood pressure monitoring (24-h ABPM) was then performed in order to confirm diagnosis of HT. Sodium excretion and proteinuria were evaluated by collecting 24-h urine from all patients. Twenty-four-hour protein excretion was measured using the turbidimetric method on a Roche Cobas C 501 autoanalyzer (Roche Diagnostics GmbH, D-68305, Mannheim, Germany). Twenty-four-hour sodium excretion was measured using the ion-selective electrode method. These 24-h sodium excretion values (mmol/d) were calculated as the concentration of sodium in urine (mmol/L) × urinary volume (L/d).

Patients' demographic data were first recorded. Next, 10-ml blood specimens were collected between 08.00 and 10.00 a.m. from a large vein in the antecubital region without tourniquet application after 12-h fasting. Hemoglobin, glucose, urea, creatinine, sodium (Na), potassium, calcium, uric acid (UA), total cholesterol, LDL-cholesterol and HDL-cholesterol were measured on the same day. Blood specimens collected for CA I and II autoantibody investigation were immediately centrifuged for 10 min at 1800 g. Serum samples were stored at -80°C until being used for measurements.

Carbonic Anhydrase I and II Autoantibody Level Determination

CA I and II levels in serum specimens were determined with a modification of the previously developed ELISA method[8]. Measurements were performed twice for each specimen. CA I or II solutions diluted in coating buffer and readied to concentrations of 10 μg/mL were transferred into 50 μL ELISA plate wells and left to incubate for 18 h at 4°C. During coating, each serum specimen was placed into four wells, two coated with CA I or II and two uncoated. The absorbance values of the uncoated wells were subtracted from those of the coated wells during analysis of the results. The plate was washed three times at 5-min intervals with phosphate buffer (first washing solution). Following the washing procedure, 200 μL blocking buffer was added to the wells and left to incubate in a shaker for 2 h at room temperature. The plate was washed three times with phosphate buffer containing Tween-20. Patient and control sera were diluted to 1/200 with dilution buffer. Next, 100 μL was taken from each diluted serum specimen, pipetted into four wells, two coated and two uncoated, and incubated in a shaker for 2 h at room temperature. Following incubation, the plate was washed three times with phosphate buffer containing Tween-20. Next, 100 μL of antibody solution (horseradish peroxidase-conjugated rabbit anti-human IgG antibody) diluted to 1/2000 with dilution buffer containing 1% milk power was added to each well and kept in a shaker in the dark for 90 min. Following incubation, the plate was washed three times with phosphate buffer containing Tween-20. Next, 100 μL substrate solution was added to each plate and left in the dark for 30 min. At the end of that period, the color reaction was halted with the addition by pipette of 100 μL 2 M sulfuric acid to each well. Absorbance at 485 nm was read on an ELISA reader. Results were obtained by subtracting the mean absorbance values of the non-CA-coated wells from the mean value of the CA-coated wells. This value was adopted as the CA autoantibody titer in serum.

Statistical Analysis

Normal distribution of data was examined using the Kolmogorov-Smirnov test. Normally distributed data were compared using the *t*-test, and non-normally distributed data using the Mann-Whitney U test. The chi-square test was used in the evaluation of demographic data, and Pearson's correlation analysis was used to determine correlations. Binary logistic regression analysis was used to identify possible independent predictors of HT. The parameters CA I and II autoantibodies, UA, and body mass index (BMI), which differed significantly between the two groups at univariate analysis or which we predicted might constitute a risk for the development of HT, were included in the logistic regression analysis. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 20 software (IBM Corp., Armonk, NY, USA). *p*-values <0.05 were regarded as statistically significant.

Results

Fifty-six newly diagnosed primary hypertensive patients (mean age: 44.98±12.20 years) and 33 healthy controls (mean age: 46.79±12.77 years) were included in this study. No significant difference was determined between the two groups in terms of mean age or sex. However, BMI was significantly higher in the hypertensive group than in the control group ($p<0.05$). UA levels were also significantly higher in the hypertensive group ($p<0.001$), but no significant difference was observed in other biochemical parameters (Table I). CA II autoantibody levels were significantly higher in the hypertensive group than in the control group ($p=0.005$), but no significant difference was observed between the groups in terms of CA I autoantibody levels (Table I, Figure 1). CA II autoantibody levels were positively correlated with age ($p<0.05$, r=0.308) and urine protein excretion ($p<0.05$, r=0.294) in the HT patient group. CA I autoantibody levels were also positively correlated with urine protein excretion in the patient group ($p<0.05$, r=0.308). However, no significant correlation was determined between CA I and II autoantibody levels and any parameter in the control group. Binary logistic regression analysis identified CA II autoantibody level (exp ß: 79.06 CI: 4.44-1407.02) ($p=0.003$) and UA elevation (exp ß: 2.10 CI: 1.31- 3.34) ($p=0.002$) as independent predictors of development of HT.

Table I. A comparison of the demographic and biochemical parameters of the Hypertensive and Control group.

	Hypertensive group (n: 56)	Control group (n: 33)	*p*-value
Age (year)	44.98 ± 12.20	46.79 ± 12.77	NS
Gender (F) (%)	42.8	60.6	NS
BMI (kg/m^2)	30.23 ± 4.35	28.07 ± 4.83	< 0.05
Office SBP (mmHg)	155.71 ± 13.99	122.21 ± 10.05	< 0.001
Office DBP (mmHg)	97.41 ± 11.03	77.67 ± 6.27	< 0.001
24 h-ABPM all day SBP (mmHg)	138.54 ± 9.71		
24 h-ABPM all day DBP (mmHg)	88.70 ± 10.14		
Glucose (mg/dL)	96.64 ± 11.04	94.76 ± 9.00	NS
Sodium (mEq/L)	140.98 ± 2.31	140.64 ± 2.05	NS
Potassium (mmol/L)	4.54 ± 0.39	4.46 ± 0.31	NS
Urea (mg/dL)	28.65 ± 8.78	26.09 ± 6.30	NS
Creatinine (mg/dL)	0.82 ± 0.17	0.76 ± 0.15	NS
Calcium (mg/dL)	9.61 ± 0.46	9.69 ± 0.36	NS
Uric acid (mg/dL)	5.69 ± 1.59	4.60 ± 1.07	< 0.001
T. Chol (mg/dL)	199.54 ± 35.87	227.59 ± 55.12	NS
HDL (mg/dL)	50.71 ± 17.69	53.08 ± 22.19	NS
LDL (mg/dL)	120.03 ± 41.22	140.51 ± 52.91	NS
Hemoglobin (g/dL)	14.12 ± 1.52	13.50 ± 1.60	NS
24-h urine protein (mg/day)	103 (0-528)	74.40 (4.10-410)	NS
24 h urine sodium (mEq/day)	106.70 (84.70-423)	188.75 (68.60-304)	NS
CA I autoantibody	0.14(0.004-0.99)	0.14(0.004-0.91)	NS
CA II autoantibody	0.72 ± 0.24	0.58 ± 0.19	$p=0.005$

Abbreviations: BMI: body mass index; BUN: blood urea nitrogen; DBP: diastolic blood pressure; HDL: high density lipoprotein, LDL: low density lipoprotein; SBP: systolic blood pressure; T. Chol: total cholesterol, 24-h ABPM; 24-hour ambulatory blood pressure monitoring.

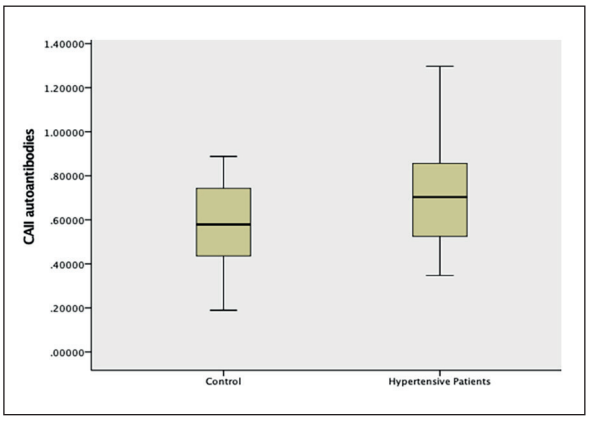

Figure 1. A comparison of CA II autoantibody level of control and hypertensive patient group. p=0.005.

Discussion

We determined significant elevation in CA II autoantibody, UA and BMI values in newly diagnosed primary HT patients compared to healthy individuals. CA II autoantibody levels in the HT patient group exhibited positive correlation with age and 24-h urine protein excretion. In addition, CA II autoantibody and UA values emerged as independent predictors of HT development. To the best of our knowledge, this is the first study to investigate CA I and II autoantibody levels in primary HT.

The pathogenesis of primary HT is still not fully understood. However, since its prevalence and associated morbidity and mortality are continually rising, the pathogenesis is the subject of considerable research. In addition to the mechanisms that have long been implicated in the pathogenesis of primary HT, autoimmunity has also recently become a focus of research. In their analysis of multicenter cohort data for 5578 AIDS patients, Seaberg et al[9] reported that less HT was observed in HIV-negative male patients than in HIV-positive male patients not receiving antiretroviral therapy. Those authors also reported similar higher rates of HT between male patients receiving antiretroviral therapy and with normal CD4+ T cell levels and HIV-negative men. On the basis of these findings, they suggested that the difference in the prevalence of HT might be related to circulating CD4+ T cell levels[9]. Studies have also reported an increase in the incidence of HT in various inflammatory and autoimmune diseases. This increase may be related to drug use, particularly of non-steroidal anti-inflammatory drugs and steroids, although renal parenchymal damage may also contribute to HT. However, changes in the immune system independent of these factors may contribute to the development of HT[10-13]. Studies[13,14] have also suggested that T cell activation is associated with the development of primary HT.

Carbonic anhydrases are enzymes involved in the regulation of a series of physiological processes, particularly PH regulation and ion secretion. CA I and II are cytosolic enzymes. CA I is particularly present in erythrocyte cytoplasm, while CA II has been isolated in several tissues, including various renal tubular epithelium cells. Autosomal recessive CA II deficiency has been linked to diseases such as osteopetrosis, cerebral calcification, and renal tubular acidosis[15]. CA II autoantibodies have also been detected in plasma in various autoimmune diseases, such as systemic lupus erythematosus[16], Sjögren's syndrome[8], Behçet's disease[6] and type I DM[17], various cancers[18-20], and infectious diseases[5]. The purpose of the present study was to investigate levels of CA I and II autoantibodies in primary HT. The place of autoimmunity in the etiopathogenesis of HT is already a subject of considerable research. While no difference was observed in CA I autoantibody levels between the patient and control groups, CA II autoantibody levels were significantly higher in the patient group. We also identified CA II autoantibody levels as an independent predictor of HT development. Based on these findings, we conclude that the CA II autoantibody may be involved in the pathogenesis of primary HT. Additionally, the positive correlation observed between CA II autoantibody levels and proteinuria in the HT patient group suggests that these may be associated with end-organ damage. In that context, CA II autoantibodies may be of potential use as a marker in the investigation of end-organ damage and as a diagnostic tool in hypertensive patients. UA is the end product of purine metabolism. Hyperuricemia is thought to contribute to the development of HT by leading to an increase in angiotensin II production through activation of the tissue renin-angiotensin system, to a decrease in endothelial nitric oxygen release, and to an increase in oxidative damage[21,22]. Studies have shown a positive correlation between serum UA elevation and BP levels[23,24]. There are also studies suggesting that serum UA levels can be used as a biomarker in the diagnosis of HT[25,26]. In agreement with the previous litera-

ture, we determined high UA levels in our HT patient group, and UA elevation emerged as an independent predictor of HT development.

Conclusions

To the best of our knowledge, this is the first study to show potential elevation of CA II autoantibodies in primary HT patients. In addition, the CA II autoantibody in the HT patient group being associated with proteinuria suggests that it may be a potential marker of end-organ damage. However, our study results are preliminary in nature, and are not sufficient to provide any information causing the cause-and-effect relationship in terms of the pathogenesis of HT. Further extensive researches confirming our finding and clarifying the mechanism involved are now needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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