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The study to measure the level of serum annexin V in patients with renal hypertension.

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ABSTRACT : *Renovascular hypertension reflects the causal relation between anatomically evident arterial occlusive disease and elevated blood pressure. The coexistence of renal arterial vascular disease and hypertension roughly defines this type of nonessential hypertension.*
The ***aim*** *of this study was to measure the level of serum Anti-Annexin V antibodies in patients with renal hypertension.*
Methods. *This study was conducted on 115 patients, diagnosed with renal hypertension and hypertension. Informed consents were obtained from the patients and the study was approved by the Kharkiv National Medical University ethics committee. Ten healthy age and sex matched volunteers were included as a control group. All patients and controls were subjected to the following full history taking and thorough clinical examination. Routine laboratory testing included a complete blood count, and erythrocyte sedimentation rate (ESR) and kidney function tests (blood urea nitrogen and serum creatinine). Immunological tests for antinuclear antibody (ANA) and anticentromere antibodies (ACA) was performed by the indirect immunofluorescence technique. AntiScl-70 (anti-topoisomerase antibodies) and anticardiolipin antibodies (ACA: IgG and IgM) were tested using the ELISA technique. The anti-annexin V antibodies titre used the ZYMUTEST anti-Annexin IgG ELISA kit. [Hyphen-BioMed, France.]: to measure the IgG isotype of auto-antibodies to annexin V in human serum.*
Results. *Anti-annexin V antibodies were present in 75% of patients (mean 83.46 ± 22.44 AU/mL) vs. 0% in the controls (mean 3.94 ± 4.5 AU/mL). Comparison between patients and controls as regards levels of anti-annexin V showed a highly significant difference (*$P < 0.001$*). Furthermore, correlation of anti-annexin V titres with the disease activity score in the patient group showed a statistically significant positive correlation ($r = 0.51$, $P < 0.05$).In addition, the anti-annexin V antibody titres in this study showed a highly significant positive correlation with ACL antibodies ($r = 0.74$, $P < 0.001$). Patients with antiphospholipid syndrome (APS) have been known to have a higher frequency of anti-annexin V antibodies, and thrombotic events have been reported more frequently in patients with positive anti-annexin V antibodies. Furthermore, inhibition of annexin V binding to negatively charged phospholipids may be an additional pathogenic mechanism of APS.*

I. INTRODUCTION.

Renovascular hypertension (RVHT) reflects the causal relation between anatomically evident arterial occlusive disease and elevated blood pressure. The coexistence of renal arterial vascular (renovascular) disease and hypertension roughly defines this type of nonessential hypertension. More specific diagnoses are made retrospectively when hypertension is improved after intravascular intervention [1-7].It has been recognized that declining renal function, estimated by the glomerular filtration rate (GFR), is an independent risk factor for all-cause mortality [8-12] and poorer outcomes in cardiovascular disease (CVD) [13-18]. Clinical studies in hypertensive patients and patients at high cardiovascular risk have shown that classic risk factors such as diabetes mellitus, hyperlipidemia, and smoking, are major correlates of renal dysfunction [19-21]. Since clinical evidence indicates that declining renal function is associated with an increased risk for cardiovascular and all-cause mortality, identification of hypertensive individuals at high risk for developing chronic kidney disease (CKD) is an important issue for primary and secondary prevention of CKD in the community.

Annexin V, a member of the lipocortin family, is expressed within various cells, including nucleoli. Many of these proteins are target molecules for autoantibodies generated in connective tissue diseases, especially in scleroderma and overlap syndromes. Annexin V inhibits prothrombin activation and is able to prevent thrombus formation under normal venous and arterial blood flow conditions. Antibodies to Annexin V have been identified in association with several pathological conditions, such as fetal loss, and venous and/or arterial thrombosis in Systemic lupus erythematosus patients, as well as digital ischemia and gangrene in Systemic sclerosis; however, their true pathogenic role remains to be proven [22-26].

Aim of the Study : The aim of this study was to measure the level of serum Anti-Annexin V antibodies in patients with renal hypertension.

Patients and Methods : This study was conducted on 115 patients, diagnosed with renal hypertension and hypertension. Informed consents were obtained from the patients and the study was approved by the Kharkiv National Medical University ethics committee. Ten healthy age and sex matched volunteers were included as a control group. All patients and controls were subjected to the following full history taking and thorough clinical examination.

Laboratory investigations : Routine laboratory testing included a complete blood count, and erythrocyte sedimentation rate (ESR) and kidney function tests (blood urea nitrogen and serum creatinine). Immunological tests for antinuclear antibody (ANA) and anticentromere antibodies (ACA) was performed by the indirect immunofluorescence technique. AntiScl-70 (anti-topoisomerase antibodies) and anticardiolipin antibodies (ACA: IgG and IgM) were tested using the ELISA technique. The anti-annexin V antibodies titre used the ZYMUTEST anti-Annexin IgG ELISA kit. [Hyphen-BioMed, France.]: to measure the IgG isotype of auto-antibodies to annexin V in human serum.

Statistical methods : The clinical and laboratory data were transferred to IBM cards using an IBM personal computer with the statistical software package "Microstat Version 2" to obtain the following:

- *Descriptive statistics***:** Mean, standard deviation (SD), minimum and maximum values range, number and percentage of qualitative data
- *Analytical statistics***:** Student's t-test to compare between two independent means, Pearson's correlation coefficient to find the relationship between different variables in the same group, and the Chi-square test to compare between different groups as regards qualitative data. For all tests, $P > 0.05$ was insignificant (NS), $P < 0.05$ was significant (S), and $P < 0.001$ was highly significant (HS). All data was graphically represented using Harvard graphics and Power Point programs.

II. RESULTS AND DISCUSSION

The mean age of the 115 hypertensive patients (62 males, 62%) was 62±14 years. All study subjects were evaluated periodically for clinical, biochemical, and cardiovascular measurements. Renal function was determined in all study subjects at baseline and at 34±6 months later (range 24–48 months). The baseline mean eGFR was 82.44±20.92 mL/min/1.73 m2, which decreased to 78.34±23.51 mL/min·1.73 m−2 by the end of the observation period. In the general population, the normal annual mean decline in GFR with age from the peak GFR (120 mL/min·1.73 m−2) attained during the third decade of life is 1 mL/min/1.73 m2 per year, reaching a mean value of 70 mL/min/1.73 m2 by age 70 [7]. In our study cohort, the mean yearly decline in GFR was 1.49±3.26 mL/min/1.73 m2 per year, which was similar with the natural history of CKD and was not statistically different between males and females (p = 0.230).

Table 1.
Baseline characteristics in 2groups of hypertensive patients.

Groups	G1n = 55	G2n = 60	*p* value
Age (yrs)	63±16	62±14	0.988
Men	12 (48%)	17 (68%)	0.439
Diabetes	6 (24%)	5 (20%)	0.112
BMI	27.3±3.3	26.2±3.2	0.025
Current smoker	8 (32%)	8 (32%)	0.612
Lipid profile			
T. Chol	184±44	199±36	0.400
Triglyceride	192±175	163±120	0.412
HDL	44±9	41±9	0.247
LDL	102±42	123±36	0.093
Fasting glucose	118±40	110±28	0.387
Serum Cr	0.98±0.39	1.02±0.41	0.452
Uric acid	6.3±1.9	6.4±1.4	0.848
Initial eGFR	79.3±24.7	80.2±24.3	0.555
Systolic BP	148±22	143±18	0.253
Diastolic BP	82±15	80±13	0.649
FRS	8.7±7.6	11.2±9.1	0.508
MAU (ACR)	0.07±0.14	0.16±0.31	0.028
hsCRP	0.38±0.31	0.29±0.23	0.659
NT-pro-BNP	88.8±40.3	70.6±50.1	0.382

Table 1.

Baseline characteristics in 2groups of hypertensive patients.

Medications			
ACE-I	2 (8%)	3 (12%)	0.227
ARB	20 (80%)	18 (72%)	0.311
CCB	18 (72%)	20 (80%)	0.816
Beta-blocker	6 (24%)	8 (32%)	0.828
Thiazides	11 (44%)	7 (28%)	0.113
Statin	6 (24%)	5 (20%)	0.870
Antihypertensive therapy duration(years)	9.2±6.7	7.8±6.2	0.375

The baseline characteristics of the patients in each group are presented in Table 1. There were no significant differences among the 2 groups with respect to age, sex, diabetes, smoking status, serum levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, baseline serum creatinine, systolic blood pressure, diastolic blood pressure, uric acid, initial eGFR, or Framingham risk score. However, patients in group 1 had a significantly higher body mass index, and there was increasing level of microalbuminuria (ACR, albumin/creatinine ratio) among the 2 groups ($p = 0.028$). There were no significant differences in their antihypertensive therapy duration and medication usage among the 2 groups, including angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, calcium-channel blockers, beta-blockers, statins, and thiazides.

Anti-annexin V antibodies were present in 75% of patients (mean 83.46 ± 22.44 AU/mL) vs. 0% in the controls (mean 3.94 ± 4.5 AU/mL). Comparison between patients and controls as regards levels of anti-annexin V showed a highly significant difference ($P < 0.001$). Furthermore, correlation of anti-annexin V titres with the disease activity score in the patient group showed a statistically significant positive correlation ($r = 0.51$, $P < 0.05$).In addition, the anti-annexin V antibody titres in this study showed a highly significant positive correlation with ACL antibodies ($r = 0.74$, $P < 0.001$). Patients with antiphospholipid syndrome (APS) have been known to have a higher frequency of anti-annexin V antibodies, and thrombotic events have been reported more frequently in patients with positive anti-annexin V antibodies. Furthermore, inhibition of annexin V binding to negatively charged phospholipids may be an additional pathogenic mechanism of APS.

III. CONCLUSIONS

In conclusion, this is the first study to show that an increased ratio of the level of circulating annexin V is associated with subsequent declines in GFR in hypertensive patients. The results suggest that reduced vascular repair capacity and escalating endothelial damage may contribute to deterioration of renal function in hypertensive patients. These findings may explain the pathogenetic processes coupling the balance of endothelial injury and the subsequent pathogenetic progression of hypertensive kidney disease.

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