IMMUNOLOGICAL CHANGES IN ELASTIN DEGRADATION INDICIES IN PREHYPERTENSIVE PATIENTS

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ABSTRACT
Survey target is to investigate and compare the elastin degradation immunological indices in prehypertension patients and in control group comprised by healthy persons, and to analyze the relations between systolic, diastolic and pulse arterial tension with the elastin degradation immunological indices in prehypertensive persons and in control group comprised by healthy persons. A group of 30 persons was formed whose average age was 42.7 years; the control group comprised 20 persons whose average age was the same as the one in the tested group. In both groups by means of immunological and biochemical methods were tested carbohydrate and lipid turnover, as well as the immunological indices of elastin degradation. Conclusions were drawn for changes in elastin turnover in prehypertensive condition.

Introduction
Introduction of high normal tension concept is rational in the practice due to the fact that persons with such arterial Pressure (AP) should be under control more frequently (8, 13, 16). According Framingham study results, 26 years after the initial survey 23.6% of men and 36.2% of women with normal AP were hypertensive (11, 12). Among these who had high normal AT, after 26 years arterial tension had 54.2% from men and 36.2% from women.

Pathogenetic mechanisms starting the process which leads to Arterial Hypertension (AH) could be predicted if the patients are in the so called prehypertensive phase (17). The new understanding (idea) for AP optimal values ensuring the vessels’ and aimed organs’ protection and the necessity of accessible daily clinic work classification of AP are presented in Seventh Report of Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (18), where for the concept prehypertension are taken SAT values of 120-139 mm Hg or DAT values 80-89 mm Hg.

Early detection of arterial lesions and the relation between these changes and the ones of AP values is very important for using them as an early marker for cardiovascular risk.

To study and compare elastin degradation immunological indices in prehypertensive patients and control group comprised by healthy persons.

To study and analyze the relations between systolic, diastolic and pulse arterial tension with elastin degradation immunological indices in prehypertensive persons and control group comprised by healthy persons.

Materials and Methods
Clinical groups
In 2002 the prehypertensive group was formed after preventive examination of healthy persons. The selection criteria in
TABLE 1

Features of the persons from prehypertensive group and their control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Prehypertensive</th>
<th>Control</th>
<th>Dif.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>X</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>26</td>
<td>53</td>
<td>42.7</td>
</tr>
<tr>
<td>Blood Sugar (mmol/l)</td>
<td>4.1</td>
<td>6.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>3.7</td>
<td>5.25</td>
<td>4.9</td>
</tr>
<tr>
<td>3-glycerides (mmol/l)</td>
<td>0.8</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td>SAT (mmHg)</td>
<td>121</td>
<td>135</td>
<td>130.2</td>
</tr>
<tr>
<td>DAT(mmHg)</td>
<td>82</td>
<td>90</td>
<td>83.1</td>
</tr>
<tr>
<td>PAT(mmHg)</td>
<td>26</td>
<td>47</td>
<td>47.1</td>
</tr>
<tr>
<td>BMI (%)</td>
<td>24</td>
<td>31</td>
<td>27.3</td>
</tr>
</tbody>
</table>

The comprised group was tested:
- every months in the course of a whole year was carried control ambulatory check-up of the tested persons, including also a triple measurement of AP in sitting position.
- elastin degradation – elastin degrada
tional peptides and anti-elastin antibo
dies, antibodies for glicated elastin
- blood sugar antibodies, total cholesterol,
  HDL, threeglycerides.

Control Group – healthy persons – 10 men and 10 women with an average age of 41.7±2.4 yrs. comprise the prehypertensive group. Twelve of them are smokers and eight are nonsmokers.

Criteria for test inclusion are:
- ECG without pathologic changes
- lung X-ray without fibrosis and emphysema
- normal values of immunoglobulins in the serum
- to be no hypertensive (AP under 120/80 mmHg)
- to have no active inflammation process
  (according clinic and paraclinic data)
- to have taken no medicines 2 weeks before
carrying out the test
- to have no illness (disease) two weeks after the test
- to have no sugar diabetes (blood sugar under 6.5 mmol/l when hungry)
- to have oncologic or haematologic dis
gese (anamnestic data)
- to have no family record for early heart vascular disease or brain-vessel disease
- to have no dislipidimies (total holesterol under 5.25 mmol/l; 3-gl <2.3 mmol/m ).

Immunologic methods for Elastin and Collagen testing
The elastin and collagen immunologic tests are carried out in “General Biology” Sec
tion in Medical University – city of Pleven. To determine the specific antibodies and
degradation products in a serum (1, 2, 3, 7, 9) were applied test methods based on en
yme-linking immunosorbent analysis (ELISA). Blood test (10 ml) was taken...
from the tested persons in the morning hours (8 – 9.30 a.m.) twelve hours after their last food intake. After one hour stay the blood was centrifuged at slow turnover and the extracted serum was divided for triple test in chemically clean glasses, well-closed and kept at -18°C.

We used a method for elastin-degradation peptides (EDP) determination developed in “General Biology” Section (4, 15). Every well of the used polystereene plates is flushed with antihuman monoclonal antibodies against elastin / SIGMA, USA/ (10µg/ml) with 0.05 M carbonate buffer (pH–9.6) and was incubated for three hours up to 37° C and twenty four hours at 4° C.

There followed a wash out with phosphate buffer 0.015 mol/l PBS which contains 0.05% Tween-20 and 0.1% bovine serum albumen /BSA/, SIGMA, USA/.100µl of the human serum (diluted 1: 5) is incubated at 37° C for one hour on the polysterol well walls within one plate.

After that follows a wash out with PBS and Tween-20.

A dilution of 1:1000 is made after incubation with antimice IgG –peroxy-dasis immunoconnugates (SIGMA, USA). Reaction is terminated with 50 µmol 8N sulphur acid. The absorption value is calculated as such via MICROELISA READER 210 (Organon Teknika, Belgium) at wave length 492 nm.

Anti-elastin antibodies are detected by ensyme-linked immunosorbsent sample /ELISA/ which includes the following stages:

Polysterene plate is covered with human aort α-elastin prepared after the description of Baydanov et al. (4, 5, 14)-1 mg elastin in 100 ml 0.05 M carbonate buffer, pH-9.6.

The remaining “active” polystereene wells’ centers are blocked by polystereene plate incubation for 24 hours with 1% solution of bovine serum albumen / BSD-SIGMA, USA/ in 0.05 carbonate buffer having pH 9.6, at room temperature;

The tested human serums are incubated, in diluted condition 1:10, with phosphate buffer physiological solution (PBS), pH 7.4 for one hour at 37° C;

Mice anihuman monoclonal antibodies specific for IgG, IgM and IgA are incubated with respective dilutions 1:1000 (52);

One hour incubation at 37° C /anihuman immunoglobulin peroxydase connugate of SIGMA to the heavy Ig chain and antimi immunoglobulin pero-xydase connugate of SIGMA to the mice monoclonal antibody/. The connugates are diluted 1:1000 with PBS containing 1% serum albumen and 0.05 Tween-20.

Incubation with substrate dilution / o-phenilenediamin, 4 mg/ml in 10 ml 0.05 citrate buffer, pH = 5.0 + 0.01% H2O2/ for one hour at room temperature in dark room. Reaction is discontinued via addition of 4M sulphur acid to every polysterene well. All samples are tested three times and the peripheral wells are not used in order to avoid the so called “peripheral effect” (18). Variation within the samples is less than 6%, and the one between the samples – 10%. All extinctive values are given with MICROELISA READER 210 (Organon Teknika, Belgium) at wave length of 492 nm.

The following laboratory methods are applied also:

• Method for total cholesterol estimation – CHOD-PAP –method, ensymolitic method carried out in a routine way in Central Clinic Laboratory (CCL) of Multi-profile Hospital for Active Treatment – city of Pleven.

• Method for estimation of 3-glycerids – ensymecolorimetric method which eliminates free glycerene, it is carried out in a routine way in CCL of Multi-profile Hospital for Active Treatment – city of Pleven.

• Method for blood glucose estimation – glucosooxydase method GOD-PAP (Boeringer Manheim, Germany). Measurements are done with bio-chemical analyser “Hitachi”704.
TABLE 2

Changes in systolic arterial tension (SAT), diastolic arterial tension (DAT) and pulse arterial tension (PAT) in pre-hypertensive patients’ group and in control group for one year

<table>
<thead>
<tr>
<th>Indices /mmHg/</th>
<th>Start n</th>
<th>X±SD</th>
<th>3rd m. X±SD</th>
<th>6th m. X±SD</th>
<th>9th m. X±SD</th>
<th>12th m. X±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP</td>
<td>36</td>
<td>130.2±5.1</td>
<td>132.4±6.2</td>
<td>132.2±7.2</td>
<td>131.5±6.3</td>
<td>131.2±4.3</td>
</tr>
<tr>
<td>SAP</td>
<td>20</td>
<td>120.4±4.2</td>
<td>122.3±5.1</td>
<td>122.5±7.1</td>
<td>121.8±5.2</td>
<td>123.1±4.1</td>
</tr>
<tr>
<td>DAP /mmHg/</td>
<td>36</td>
<td>83.2±6.4</td>
<td>84.2±4.4</td>
<td>82.2±6.4</td>
<td>84.4±4.4</td>
<td>86.6±2.4</td>
</tr>
<tr>
<td>DAP /mmHg/</td>
<td>20</td>
<td>78.8±6.2</td>
<td>82.2±6.4</td>
<td>80.2±7.1</td>
<td>83.1±5.1</td>
<td>81.2±6.3</td>
</tr>
<tr>
<td>PAP /mmHg/</td>
<td>36</td>
<td>47.1±4.1</td>
<td>38.2±1.8</td>
<td>50.0±1.8</td>
<td>47.1±1.9</td>
<td>45.6±2.1</td>
</tr>
<tr>
<td>PAP /mmHg/</td>
<td>20</td>
<td>31.6±6.2</td>
<td>40.1±4.5</td>
<td>42.3±6.2</td>
<td>38.7±5.1</td>
<td>40.8±4.9</td>
</tr>
</tbody>
</table>

TABLE 3

Elastin degradation peptides in prehypertensive persons and control persons at the start and the end of the first year

<table>
<thead>
<tr>
<th>Start</th>
<th>Groups</th>
<th>n</th>
<th>EDP(ng/ml)X±SD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prehypertensive Persons</td>
<td>36</td>
<td>0.43±0.061</td>
<td>7.31</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Control Persons</td>
<td>20</td>
<td>0.23±0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After one year</td>
<td>Prehypertensive Persons</td>
<td>36</td>
<td>0.619±0.043</td>
<td>9.47</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Control Persons</td>
<td>20</td>
<td>0.215±0.037</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The method is applied in routine way in CCL of Multi-profile Hospital for Active Treatment – city of Pleven.

AT measurement is executed according the WHO regulations after 10 minutes’ rest in sitting position with arm-band at every patient’s visit. A standard mercury manometer was used. The average value of the second and third measurement is taken as reliable.

Test data are processed via a package of statistical computer programme Statgraphics Plus for Windows and EXCELL. The following statistical tests are applied: basic indices calculation for central tendency and variation; Student-Fisher parametric t-test – in case of normal and close to the normal distribution; parametric Fisher F-test in case of normal distribution; non-parametric KW-Kruskal-Wallis test in distributions which are different from the normal one. Correlation coefficients are calculated to determine correlations and dependencies: Pearson (in case of normal distribution) and Spirman (in case of distribution differing from the normal one).

All correlations, dependencies and differences between the tested groups are stated at significant level of importance – p<0.05.

Results and Discussion

Arterial Pressure at prehypertensive persons and control ones

According the test results in 30 out of 36 tested persons AP – DAP till the end of the first year was permanently above 90 (Table 2).

Elastin turnover changes in prehypertensive persons and control

At the end of the first year of the test the elastin degradation immunological indices were changed for patients having high arterial tension.

The levels of both elastin degradation indices – elastin degradation peptides and anti-elastin antibodies are represented in Table 3.

Statistically higher quantity of elastin-degradation peptides in prehypertensive persons was stated in comparison with the control persons/ F =7.31; p=0.01/. 

TABLE 4

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IgG X±SD</th>
<th>IgM X±SD</th>
<th>IgA X±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prehypertensive persons</td>
<td>36</td>
<td>0.597±0.056</td>
<td>0.695±0.051</td>
<td>1.016±0.129</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>0.377±0.093</td>
<td>0.085±0.177</td>
<td>0.856±0.157</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>1.19</td>
<td>2.67</td>
<td>2.29</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

TABLE 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>AGE X±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prehypertensive persons</td>
<td>36</td>
<td>0.30±0.035</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>0.33±0.023</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

At the end of the first year of the study patients having DAP more than 90, elastin degradation peptides level is statistically and reliably higher than the one in the control group.

The tests executed in accordance with ELISA do not demonstrate statistically reliable differencies /P>0.05/ in prehypertensive persons and controls for anti-elastin antibodies from class IgA, IgG and IgM.

Antibodies levels against glicated elastin in prehypertensive persons and control are not significantly different (Table 5).

While surveying the fat turnover indices there were not stated reliable statistic differences.

Our tests in hypertensive group demonstrate significant increase (F=7.31; p=0.01) in elastin peptides (EDP) compared to those of the control group, as well as significant EDP increase in this group at the end of the first year of the study (Table 3).

Antielastin antibodies (Table 4) in prehypertensive group do no prove the difference compared to the control (0>0.05).

Our study demonstrate that before to reach the border arterial hipertension values the future hypertensive persons have increased leastin degradation peptides quantities. At the end of the first year AT and elastin degradation peptides’ levels are significantly higher in the group of hypertensive persons having family record (history). Elastin degradation peptides (EDP) quantities is an index which could be discussed as a predictor of future hypertensive condition.

Some authors (10) consider that antibodies increase compared to glicated proteins is a marker for speeded age process of vessel wall. It is known that glicated proteins have common epitops (14) and antibodies which react cross-like to different glicated proteins. In our studies we did not state statistically reliable difference in antibodies’ levels present in both tested groups (F=0.54; p >0.05). According our data, changes in antibodies’ levels compared to glicated proteins do not occur in prehypertensive phase of AP.

In order to eliminate age impact we have selected control persons of same age group. The tested persons and controls respond to the whole constellation of excluding criteria in order to eliminate other diseases’ impact on vessels or on other organs where these collagens and elastin are widely produced.

Confirming our group selection, the cholesterol, three-glycerides and blood sugar do not demonstrate statistically reliable differences between control group and prehypertensive persons. This fact makes the tested groups comparable as the influence...
of hypercholesterolomy, hyper-3-glyceridemy and hyperglycemia on serum elastases activity is well known.

In Framingham study was stated that high normal blood pressure was a reliable predictor for arterial tension (AH). In our study of prehypertensive persons, while stating changes in elastin degradation indices before AT to be determined, we can accept that these changes are “predictor of the predictor”.

The following conclusions could be drawn from the results cited above:

Conclusions
1. In arterial hypertension evolution happen immunological indices changes which are typical for degradation of basic fibril components in vessel wall ECM, i.e. elastin.
2. In prehypertensive patients compared to healthy persons we register significant increase in elastin-degradation peptides quantities –this is a sign of accelerated elastolysis where the changes precede hypertension fixation.
3. In prehypertensive patients we did not state significant changes in antielastin antibodies’ levels and of those compared to glicated elastins.

REFERENCES