Association between Inflammatory Markers, Renal Damage Parameters and Carotid Intima-Media Thickness in Hypertensive Patients

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Abstract: - In recent years, high sensitive C-reactive protein (hsCRP) and fibrinogen were recognized as valuable vascular markers of low-grade inflammation and microalbuminuria and serum uric acid levels as renal damage markers in cardiovascular disease (CVD). Carotid intima-media thickness (carotid IMT) is considered a subclinical marker of generalised atherosclerosis. In hypertensive patients, the presence of target organ damage is important for an adequate treatment.

Objective: The aims of this study were to determine inflammatory and renal damage parameters in hypertensive patients with or without target organ damage and to evaluate the relationship between these parameters and carotid IMT.

Methods: The study included 44 patients with arterial hypertension without TOD (33% men and 67% women, mean age 54 ± 4.80 years) and 48 patients with arterial hypertension and TOD (39% men and 61% women, mean age 59 ± 4.73 years). Target organ damage was defined by the presence of left ventricular hypertrophy, increased carotid IMT, or kidney damage assessed by microalbuminuria. In all patients, serum lipid profile, hsCRP, fibrinogen, serum uric acid levels and microalbuminuria were evaluated. B-mode carotid ultrasound was performed in all subjects.

Results: Hypertensive patients with TOD had higher levels of hsCRP and fibrinogen than hypertensive patients without TOD (5.02 ±0.33 g/l vs. 2.65±0.23 g/l, \(p<0.001\), and 6.83±0.23 g/l vs. 2.73±0.34 g/l, \(p<0.001\)), and an increased value of carotid IMT (1.30±0.04 mm vs. 0.84 ±0.03 mm, \(p<0.001\)), respectively. Hypertensive patients with TOD had higher levels of serum uric acid levels and microalbuminuria than hypertensive patients without TOD (8.38 ± 0.31 vs. 6.04 ± 0.41mg/l, \(p<0.001\), and 111 ± 20.70 vs. 13 ± 593 mg/l, \(p<0.001\)). We found a strong positive independent correlation between fibrinogen and carotid IMT (\(r = 0.87\), \(p < 0.001\)) and between hsCRP and carotid IMT (\(r = 0.86\), \(p<0.001\)). A positive correlation was observed also between carotid IMT and microalbuminuria (\(r = 0.74\), \(p < 0.001\)) and between carotid IMT and serum uric acid levels (\(r = 0.86\), \(p < 0.001\)).

Conclusion: inflammatory markers (hsCRP, fibrinogen) and renal damage parameters (serum uric acid, microalbuminuria) are significantly elevated in hypertensive patients with TOD. Measurement of carotid IMT and determination of inflammatory and renal damage markers seems to be valuable tools to monitor and treat hypertensive subjects with target organ damage.

Key-Words: - microalbuminuria, serum uric acid levels, C-reactive protein, fibrinogen, intima-media thickness, target organ damage

1 Introduction

Hypertension is considered a multifactorial disease and the product of dynamic interactions between genetic, physiological, and environmental elements (1). Recently, European Guidelines on cardiovascular disease prevention in clinical practice (version 2012) reappraised the importance of target organ damage as an intermediate stage in the continuum of vascular disease and as a determinant of overall cardiovascular risk, therefore signs of organ involvement should be sought carefully (2). An early detection and adequate management of hypertensive target organ damage can slow or prevent damage, or even allow disease regression where organ damage is still at reversible stage (3).

Vascular inflammation might increase oxidative stress and endothelial dysfunction and has an important role in all stages of the atherosclerotic process including the development of endothelial dysfunction, plaque development, plaque instability and rupture (4, 5). Produced mainly in the liver in response to IL-6 secretion, CRP may stimulate the...
expression of adhesion molecules, tissue factor, MCP-1, and PAI-1 and may activate leukocytes and the complement system and also reduce the bioavailability of NO (6). Increased levels of fibrinogen can produce changes of blood rheological properties such as increases in plasma viscosity, erythrocyte aggregation, platelet thrombogenesis, and alterations in vascular reactivity and compromises the endothelial layer integrity (7). Recently, inflammation and more particularly tumour necrosis factor-α (TNF-α), has been implicated in the cascade leading to TOD in patients with essential hypertension (8).

Essential hypertension is frequently associated with renal damage, for example, renal arteriolar thickening, fibrinoid deposition in the glomeruli, and proteinuria (9). Serum uric acid levels were proposed as marker of subclinical target damage in hypertensive patients (10).

The clinical value of carotid IMT in the assessment of cardiovascular risk is well established (11). Furthermore, in 2007, the ESC/ESH hypertension guide proposed the carotid artery IMT as an indicator of target organ damage (12). The presence of target organ damage was defined as carotid IMT>0.9 mm, and the presence of atherosclerotic plaque was defined as carotid IMT>1.3 mm.

The aims of this study were to assess the relationship between inflammatory markers (hsCRP and fibrinogen), renal damage markers and carotid IMT in hypertensive patients and to compare these markers between hypertensive patients with or without TOD.

2 Material and methods
The study comprised 92 hypertensive patients that were divided into those with and without TOD, according to the presence of one or more of the following: left ventricular hypertrophy by ECG, increased carotid IMT by ultrasound, or kidney damage assessed by microalbuminuria.

We obtained by interview information regarding age, sex, hypertension, presence of diabetes, history of smoking and medication use. Exclusion criteria from the study were: treatment with anti-inflammatory or hypolipidemic agents, estrogen therapy, antiplatelet drugs; hsCRP greater than 10 mg/L; serum creatinine more than 1.4 mg/dL for women and than 1.5 mg/dL for men; proteinuria greater than 300 mg/24-hour; secondary hypertension; chronic heart failure (New York Heart Association class III and IV); positive history or clinical signs of ischemic heart disease; diabetes mellitus; neoplastic or hepatic disease.

We performed a 12-lead electrocardiogram (ECG) in participants lying supine in an air-conditioned room at a room temperature of 25°C. Left ventricular hypertrophy (LVH) on ECG was diagnosed based on the voltage criteria of Cornell (13), and of Sokolow-Lyon (14). The presence of ECG-LVH was accepted if the patient met: a) the voltage criterion of Cornell (sum of the R-wave on lead aVL + S-wave on V3 > 20 mm in women, or >28 mm in males); or b) the voltage criterion of Sokolow-Lyon (sum of the S-wave on V1+ R-wave on leads V5 or V6 >38 mm).

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). A value higher of 30 Kg/m² was considered obesity.

Blood pressure was measured using three measurements of systolic and diastolic blood pressure, using the average of the last two measurements, with a standard sphygmomanometer (Riester, Germany). The subject was placed in a seated position, with arm raised to the heart, physically and mentally after a rest of 10 to 15 min.

Blood pressure values used for statistical calculations were the average of six determinations in two moments of study: 3 BP determinations every 5 min at inclusion in the study group, and 3 measurements of BP every 5 min before the carotid ultrasound examination. The values obtained were interpreted following the recommendations of the European Society of Hypertension in 2007 (12).

Blood samples were collected in the morning, after patient fasting 8 h. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Low density lipoprotein cholesterol was calculated using Friedewald’s formula (15).

Fibrinogen was measured by Dimension RxL Max (Dade Behring Inc., USA), using a nephelometric research assay. Serum high-sensitivity CRP (hsCRP) levels were measured using a wide-range latex-enhanced immunoturbidimetric assay.

Serum creatinine was assessed by the modified Jaffe method. Microalbuminuria was measured by immunonephelometry. Serum uric acid levels (reference range, 3-7 mg/dL) were measured by enzymatic method (uricaseperoxidase).

Estimated glomerular filtration rate (estimated GFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (16).
The carotid IMT was measured by high resolution B-mode ultrasound imaging with a 7.5-MHz linear array transducer (Model ProSound SSD-4000, Aloka Co., Ltd., Tokyo, Japan) according to the Mannheim Consensus (17). Determination of carotid IMT was performed by average of three measurements in each of the three successive segments of the vessel: 3 measurements in the distal 2 cm of common carotid artery (right or left), 3 measurements in the carotid bulb (right or left), and 3 measurements in the top 2 cm of internal carotid artery (right or left). In the same point was measured also the vessel diameter. The individuals performing the different tests were blinded to the clinical data of the patient. The study was conducted according to the Declaration of Helsinki, and the written informed consent was obtained from each subject.

2.1 Statistical methods
Means were calculated for continuous variables and proportions for categorical variables. Unpaired Student t tests or analyses of variance were conducted to assess statistical significance of differences between groups using Epi 6 program. Simple and multiple linear regressions were used to analyze the association between fibrinogen and carotid IMT, hsCRP and carotid IMT, microalbuminuria and carotid IMT and serum uric acid levels and carotid IMT before and after adjusting for potential confounding demographic variables.

3 Results
The demographic, clinical and laboratory parameters of the participants are shown in table 1. 44 patients were with arterial hypertension without TOD (33% men and 67% women, mean age 54 ± 4.80 years) and 48 patients with arterial hypertension and TOD (39% men and 61% women, mean age 59 ± 4.73 years). As expected, between hypertensive patients with TOD and hypertensive without TOD there was a significant difference in age distribution (p<0.001). Positive history for CVD, sex, and smoker number did not differ among studied groups, but significant trends for increased systolic blood pressure, diastolic blood pressure, body mass index, lipid profile values and fasting glycemia were seen in hypertensive patients with TOD, whereas HDL-cholesterol, decreased significantly in hypertensive patients with TOD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HTA-TOD (n=44)</th>
<th>HTA+TOD (n=48)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54 ± 4.80</td>
<td>59 ± 4.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex M/F (%)</td>
<td>33/67</td>
<td>39/61</td>
<td>0.96</td>
</tr>
<tr>
<td>Positive history of CVD (%)</td>
<td>20</td>
<td>41</td>
<td>0.30</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>25</td>
<td>45.8</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23.24 ± 0.93</td>
<td>27.83 ± 2.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>148 ± 5.07</td>
<td>175 ± 6.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>88 ± 2.52</td>
<td>94 ± 3.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glycemia (mg/dL)</td>
<td>78 ± 6.21</td>
<td>126± 37.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>181 ± 7.15</td>
<td>209±19.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>138 ±10.38</td>
<td>169 ±13.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>107 ± 6.68</td>
<td>135 ± 19.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>47 ± 4.30</td>
<td>40 ± 5.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum uric acid (mg/dL)</td>
<td>6.04 ± 0.41</td>
<td>8.38 ± 0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.81 ± 0.13</td>
<td>0.97 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1.73 m²)</td>
<td>94 ± 2.04</td>
<td>71 ± 1.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria (mg/L)</td>
<td>13 ± 593</td>
<td>111 ± 20.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.65 ± 0.23</td>
<td>5.02±0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>2.73 ± 0.34</td>
<td>6.83 ± 0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carotid IMT (mm)</td>
<td>0.84 ± 0.03</td>
<td>1.30 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Hypertensive patients with TOD exhibit significantly higher levels of both fibrinogen and hsCRP, indicative of inflammatory process compared to hypertensive patients without TOD.

Hypertensive patients with TOD had significantly higher levels of serum uric acid, serum creatinine and microalbuminuria levels and significantly lower values of estimated GFR, as indicators of renal damage, compared to hypertensive patients without TOD.

After adjusting for established cardiovascular risk factors (ie, age, smoking habit, BMI, systolic and diastolic blood pressures, fasting glucose, total cholesterol, triglycerides, and LDL-cholesterol, hsCRP and fibrinogen remained strong correlated with target organ damage, defined by a carotid IMT greater than 0.9 mm.

Therefore, a positive correlation was obtained between carotid IMT and fibrinogen (r = 0.87, p <0.001) (fig. 1).
Fig. 1. Correlation between carotid IMT and fibrinogen

It was observed a positive correlation between carotid IMT and hsCRP ($r = 0.86$, $p < 0.001$) (fig.2).

Fig. 2. Correlation between carotid IMT and hsCRP

A positive correlation was observed between carotid IMT and microalbuminuria ($r = 0.74$, $p < 0.001$) (Fig. 3).

Fig. 3. Correlation between carotid IMT and microalbuminuria

It was noticed a positive correlation between carotid IMT and serum uric acid levels ($r = 0.86$, $p < 0.001$) (fig.4).

Fig. 4. Correlation between carotid IMT and serum uric acid levels

4 Discussions

We investigated biochemical data and identified a number of significant factors associated with carotid IMT. The present study shows that the measures of inflammatory (fibrinogen and hsCRP) and renal damage parameters (microalbuminuria, serum uric acid levels) are correlated with subclinical atherosclerosis evaluated by carotid IMT. Through recruitment of inflammatory cells, the increased adhesion molecules, secretion of chemoattractant and proinflammatory cytokines from the endothelial cells and the migration and proliferation of smooth muscle cells from media is initiated inflammation in hypertensive patients (18, 19). Furthermore, atherosclerosis is regarded as an inflammatory disease (20).

A number of studies analysed the association of carotid IMT with inflammatory markers of atherosclerosis. Furthermore, a study found that carotid IMT, as well as high levels of TNF-α and hsCRP stratify CV events likelihood, independently to classic risk factors (21). Recent findings of the Multi-Ethnic Study of Atherosclerosis indicate further that increased carotid IMT predicted CVD events in individuals without coronary calcification (22). A study of 1422 stroke-free participants from the NOMAS cohort showed that in Hispanics there is an association between leukocyte count and carotid atheroma (23).

On the contrary, other studies have failed to find consistent and independent associations of CRP or other inflammatory markers with carotid wall thickness (24, 25). It seems like that hsCRP and fibrinogen may provide a better measure of later processes of atherosclerosis and the likelihood of a clinical event.

Microalbuminuria might indicate the presence of generalized microvascular damage in patients with essential hypertension (26). Our study indicated that carotid IMT is associated with microalbuminuria in hypertensive patients, suggesting that microalbuminuria can be considered a sensitive marker of vascular remodelling in hypertensive patients. Our findings are in agreement with other studies that showed an association of microalbuminuria with carotid intima-media thickness and coronary artery disease (27).

Evaluation of hypertensive patient should not be restricted only in measurement of blood pressure. Other risk factors such as diabetes or metabolic syndrome, and secondary target organ damage
should be considered. Evaluation of target organ damage in hypertensive patients is important in order to determine total cardiovascular risk. All possible affected organs should be screened. Our study may contribute to better understanding of the relation between inflammation and hypertension by showing that both fibrinogen and hsCRP are strongly associated with carotid IMT as a marker of target organ damage, highlighting the role of chronic low grade inflammation as a pathogenic factor of target organ damage in essential hypertension.

Different evidences suggested that even mild renal impairment may be considered a risk factor for cardiovascular events (28). Higher levels of SUA may have effect on atherosclerotic process by several ways including deleterious effects on endothelial dysfunction, oxidative metabolism, platelet adhesiveness, hemorheology, and aggregation (29). In our study impaired renal function, expressed as microalbuminuria and increased serum levels of uric acid, was positively and significantly correlated with carotid IMT. The results of our study are in concordance with other studies that showed that hyperuricemia is an independent predictor for early atherosclerosis in hypertensive subjects with normal renal function or an independent risk factor for carotid atherosclerosis in patients without metabolic syndrome (30, 31). In summary, the present study suggests that TOD in hypertensive patients is mainly associated with increased value of inflammatory and renal damage parameters.

Strengths of the current study include the detailed clinical and laboratory characterisation of a cohort of hypertensive patients. Limitations of this study include the small number of patients and carotid IMT measurement: reproducibility of single measurement, and questionable accuracy of repeated measurements in our study.

4 Conclusion

Inflammatory markers (hsCRP, fibrinogen) and renal damage parameters (microalbuminuria, serum uric acid levels) are significantly elevated in hypertensive patients with TOD. Measurement of carotid IMT and evaluation of inflammatory and renal damage markers can be valuable tools to monitor and treat hypertensive subjects with TOD.

References:


