Oxidative Stress and Vascular Inflammation in Post-menopausal Woman with Metabolic Syndrome

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Abstract— In the past decade, many clinicians have suggested that essential hypertension must be related to the renin-angiotensin system and to undefined renal dysfunction. These assumptions are motivated first by the efficacy of angiotensin converting-enzyme inhibitors (ACEI) or angiotensin II receptor blockers to reduce blood pressure in essential hypertension, even when plasma levels of angiotensin (Ang) are normal or slightly elevated. Angiotensin II exerts multiple effects on the cardiovascular system, including free radical production proposed as a mechanism participating in Ang II induced cardiovascular alterations. There is increasing evidence that inflammation and endothelial dysfunction are the most important pathogenic pathways explaining the propensity to atherosclerosis and its complications in metabolic syndrome. Most adipocytokines and proinflammatory biomarkers (adiponectin, cell adhesion molecules, TNF-α, IL, CRP) are elevated in the serum and vessel walls of patients with metabolic syndrome, being positive predictors for cardiovascular events. Aims: To evaluate the relationship between oxidative stress, hs C-reactive protein, apo A1/apo B and classical cardiovascular risk factors in newly diagnosed, never treated, non-smoking, post-menopausal women (age: 57.18±6.68 years, TA: 158.8±9/ 91.75±7.75 mmHg) with uncomplicated essential hypertension with/without MetS and age-, sex-matched control group. Methods: The concentration of serum and erythrocyte superoxiddismutase (SOD), catalase (CAT) and malonaldialdehyde (MDA) were analysed by spectrofotometry. All the other risk factors (uric acid, fasting glucose, lipid profile) were assessed by validated standard procedures. C-reactive protein high sensitive (hs CRP), A-1 and B-100 apolipoproteins have been performed by a sandwich ELISA method. Results: Plasma levels of oxidative stress parameters determined and CRP are significantly higher than the control group (p<0.0001). Oxidative stress markers are strongly correlated (r>0.7) with the number of criteria for MetS and CRP, they have an average correlation with the age, weight, BMI, waist, fasting glucose, triglyceride, HDL-C and are not correlated with the BP values. The coefficient of determination is significantly increased between the number of criteria for the MetS and the oxidative stress parameters.

Keywords—apoA-1, apoB-100, cardiovascular risk factors, C-reactive protein high sensitive, metabolic syndrome, oxidative stress

I. INTRODUCTION

Hypertension and diabetes are major cardiovascular risk factors greatly responsible for mortality and cardiovascular morbidity. Prevalence of hypertension in the diabetic population is two times higher than in the non-diabetic population. On the other hand hypertension (HTA) is a strong predictor for developing diabetes (DM). Up to 75% heart disease conditions in diabetics can be attributed to hypertension. The most common pathogenic element in hypertension and diabetes is the endothelial malfunction which may severely disrupt the body’s homeostasis by generating pro-aggregation, pro-coagulation and pro-inflammatory statuses, respectively. One of the pathogenic mechanisms that can explain this increased risk in diabetes is the imbalance between the pro-oxidants and the antioxidants, which results in oxidative stress. Hyperglicemia results in glucose auto-oxidation, non-enzymatic glycation and monocyte dysfunction, which lead to increased production of free radicals. This is
further aggravated by the decreased levels of antioxidants and leads to oxidative damage [1], [2]. Strong experimental evidence indicates that increased oxidative stress and associated oxidative damage are mediators of renovascular injury in cardiovascular pathologies. An increase in the production of superoxide anion and hydrogen peroxide, the reduced nitric oxide synthesis and the decreased bioavailability of antioxidants have been demonstrated in experimental and human hypertension.

Vascular oxidative stress has been demonstrated in spontaneous (genetic) and experimental hypertension. Spontaneously hypertensive rats (SHR) and stroke-prone SHR, two genetic models that develop hypertension spontaneously, exhibit increased NAD(P)H driven •O2 generation in resistance (mesenteric) and conduit (aortic) vessels. [6], [8], [13], [14]. This is associated with an over expression of the NAD(P)H oxidase subunit and enhanced oxidase activity [4], [7], [13], [15]. Oxidative stress in genetic hypertension involves enhanced NAD(P)H oxidase activity and dysfunctional endothelial nitric oxide synthase (uncoupled NOS) and is partly regulated by AT1 receptors.

Vascular oxidative stress has also been demonstrated in experimentally-induced hypertension, such as Ang II-mediated hypertension, Dahl salt-sensitive hypertension, lead-induced hypertension, obesity-associated hypertension, mineralocorticoid hypertension, and aldosterone-provoked hypertension [16], [17]. Activation of vascular NAD(P)H oxidase and xanthine oxidase and endothelial nitric oxide synthase uncoupling [9], [10], [14], [18], [19] have been implicated in amplification of •O2 generation in experimental hypertension.

Clinical studies demonstrated increased ROS production in patients with essential hypertension, renovascular hypertension, malignant hypertension, and pre-eclampsia [20]–[22]. These findings are generally based on increased levels of plasma thiobarbituric acid-reactive substances and 8-epi isoprostanes, biomarkers of lipid peroxidation and oxidative stress [5], [23]. Accumulation of ROS byproducts from oxidized genomic and mitochondrial DNA have also been found in hypertensive individuals [5]. Polymorphonuclear leukocytes and platelets, rich in •O2 sources, also participate in vascular oxidative stress and inflammation in hypertensive patients [24], [25]. Decreased antioxidant activity (SOD, catalase) and reduced levels of ROS scavengers (vitamin E, glutathione) may contribute to oxidative stress [5], [23]. Activation of the renin-angiotensin system has been proposed as a mediator of NAD(P)H oxidase activation and ROS production [3], [9]–[13]. In fact, some of the therapeutic BP-lowering actions of AT1-receptor blockers and angiotensin-converting enzyme inhibitors (ACEI) have been attributed to NAD(P)H oxidase inhibition and decreased ROS production [26], [27].

Obesity is associated with diabetes and elevated blood pressure [28]. This chronic condition reflects over-nutrition and is associated with important oxidative stress, as shown by the increased indexes of lipid peroxidation, protein carbonylation, and oxidative damage of amino acids, respectively [29]. High caloric intake of glucose, lipid, or protein causes an increase in the generation of reactive oxygen species (ROS) by the leukocytes and the p47phox protein (a key protein in the enzyme NADPH oxidase), along with the activation of the nuclear factor-β and the inflammation [30]–[33]. Different macronutrients can induce a distinctive pattern of the increase in ROS generation [34]. Glucose induces a peak at 2 h in ROS generation both by mononuclear cells and polymorphonuclear leukocytes, whereas the lipid produces a peak at 1 h. The peak increase is the highest for the glucose and the lowest for the protein. Lipid intake causes a prolonged increase in lipid peroxidation.

Obese subjects, caloric restriction and weight loss over a short period of 4 weeks lead to a decrease in the ROS generation by the leukocytes and the oxidative damage to lipids, proteins, and amino acids [29]. In normal subjects, only a 48-h fast can reduce the ROS generation, the total oxidative load and the oxidative damage to amino acids [35]. Ang-receptor blockade with valsartan or irbesartan exerted a profound and rapid ROS and inflammation suppressive effect that may be explained by the beneficial result of these compounds [36], [37]. These kinds of benefits were not observed with the ACE inhibitors and the HMG-CoA reductase inhibitors. An association was observed between the glucose and the macronutrient intake, obesity, Ang II, and the oxidative stress, which may also suggest that the oxidative stress plays a role in the pathogenesis of obesity-hypertension. In normal individuals, insulin has been shown to suppress several pro-inflammatory transcription factors, such as the NF-kB and the activating protein-1 (AP-1) [39]. In the metabolic syndrome, the insulin-resistant state will determine a pro-inflammatory condition and, therefore, the inflammation could be the most important link between the pathogenesis of atherosclerosis and the intervention in some important cardiovascular risk factors, such as the obesity or the diabetes mellitus. CRP, an important pro-inflammatory marker, has recently been introduced as a new factor of the metabolic syndrome [40]. In obesity and metabolic syndrome, the adipose tissue produces adipokines, some of them with an important influence on inflammation: TNF-α, IL-6, IL-1β, leptin, adiponectin and resistin [41]. The insulin action resistance on the lipid metabolism is associated with the increase in the free fatty acid (FFA) concentrations in plasma, resulting in the induction of oxidative stress and inflammation [42]. Nowadays, atherosclerosis, the main cause of coronary artery disease, is equally considered an inflammatory and a metabolic disease influenced both by the hereditary and the environmental factors. The atheromatous lesions contain immune cells (mast cells, T cells, macrophages) that when activated produce inflammatory cytokines. The hemodynamic profile, the retention of LDL in the arterial wall, and the oxidation of LDL may initiate an inflammatory response in the arterial wall [43]. The cytokines present in the atherosclerotic lesions promote a type 1 helper T (Th1) response, similar to delayed hypersensitivity, rather than a helper T type 2 (Th2) one. As a result, the most powerful pro-inflammatory cytokines are CRP and IL-6, and from the 2 cytokines with the most demonstrated anti-inflammatory properties are the interleukin-10 (IL-10) and the transforming growth factor β (TGF-β), respectively [44], [45].
In the metabolic syndrome, elevated levels of free fatty acids associated with insulin resistance, obesity, hypertension, impaired glucose tolerance or diabetes mellitus will activate NF-κB that regulates transcription of vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 and promotes synthesis and release of pro-inflammatory cytokines [38], [46]. IL-1β, IL-6 and TNF-α are released from macrophages and will mediate the hepatic synthesis of fibrinogen, CRP, and serum amyloid A. CRP determines the activation of some tissue factors and the extrinsic coagulation cascade, establishing a link between inflammation and thrombosis. Many of these pro-inflammatory biomarkers could be used as indicators of increased cardiovascular risk and may become a target of therapeutic intervention.

CRP was the most studied marker of inflammation in cardiovascular diseases and it was revealed to be an independent predictor of risk for myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death [47]. These effects of CRP were attenuated by lifestyle interventions (Mediterranean-diet, aerobic exercises) and administration of statins (simvastatin, atorvastatin), ramipril, losartan or rosiglitazone [38].

II. MATERIALS and METHODS

A prospective study of 20 newly diagnosed, never treated, non-smoking patients (women, at least 2 years menopausal) with metabolic syndrome were recruited for this study. Anthropometrical, biochemical and hormonal parameters were determined. Blood pressure was recorded. The anthropometrical measurement included waist circumference (WC) and body mass index (BMI). BMI was computed as a ratio of weight to the square of height (kg/m2). Waist circumference was taken at the midpoint between the lowest rib and the iliac crest. Blood pressure was measured with a mercury sphygmomanometer. The protocol included three measurements; the mean of all 3 measurements was used to fast for 12 h before the blood sampling that was collected around 7:00 a.m. Fasting plasma glucose, serum triglycerides, serum HDL and LDL, total cholesterol, uric acid, fibrinogen, were measured enzymatically. C-reactive protein high sensitive (hs CRP), A-1 and B-100 apolipoproteins have been performed by a sandwich ELISA method (IBL International GMBH and Cayman Chemical kits). The concentration of serum and erythrocyte superoxidismutase, catalase and malonaldehyde were analysed by spectrofotometry. According to the International Diabetes Federation the metabolic syndrome is diagnosed for a person with central obesity plus at least two of the following criteria: raised TG level ≥ 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality, reduced HDL cholesterol < 50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality, raised blood pressure ≥ 130 / 85 mm Hg, or treatment of previously diagnosed hypertension, raised fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes. The results were compared with measurements from the control group, consisting of 15 healthy persons matched for age and sex (free from the metabolic syndrome, hypertension or dislipidemia). Rest and stress test electrocardiograms were performed to exclude coronary artery disease.

Statistical analysis: data are given as mean ± standard deviations. Statistical analysis has been performed using the Microsoft Office Excel 2007+ Analyse-it software, applying parametric and non-parametric tests (one-way breakdown ANOVA, Mann-Whitney U test, Spearman correlation). The results are considered statistical significant when p < 0.05, α=95%.

III. RESULTS and DISCUSSION

There were significant differences for the recorded parameters between patients with metabolic syndrome and control group. The group characteristics (age, weight, waist, systolic and diastolic blood pressure, number of criteria for MetS) and the routine blood parameters (fasting plasma glucose, cholesterol, HDL, LDL, TG) for the two studied groups are presented in table I.

### Table I: Anthropometric parameters and blood pressure for the two studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group</th>
<th>Hypertension Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.2±6.33</td>
<td>57.18±6.68</td>
<td>0.616</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.3±6.52</td>
<td>82.4±8.31</td>
<td>0.005</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>88.13±9.87</td>
<td>108.5±12.57</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.56±2.25</td>
<td>31.61±3.96</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>-</td>
<td>158.8±9</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>-</td>
<td>91.75±7.75</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table II: The blood parameters for the two studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group</th>
<th>Hypertension Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>-</td>
<td>144.7±81.3</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>-</td>
<td>223.83±47.49</td>
<td>-</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>-</td>
<td>49.17±15.7</td>
<td>-</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>-</td>
<td>133.79±39.59</td>
<td>-</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>-</td>
<td>207±124.64</td>
<td>-</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>-</td>
<td>2.99±0.65</td>
<td>-</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>-</td>
<td>335.5±44.33</td>
<td>-</td>
</tr>
<tr>
<td>Apo-A1</td>
<td>-</td>
<td>88.5±22.9</td>
<td>-</td>
</tr>
<tr>
<td>Apo-B 100</td>
<td>-</td>
<td>137.7±31.52</td>
<td>-</td>
</tr>
</tbody>
</table>

The biochemical parameters indicating the blood redox status for the two studied groups are listed in table 3.
Age was similar to both groups. For all other parameters there was a statistical significant difference in the MetS group in comparison with the control. Taking into consideration the numbers of the inclusion criteria the MetS group consisted as it follows of: 7 patients with 3, 9 patients with 4, and 4 patients with 5 criteria, respectively. All these patients were diagnosed with HTA that was not previously treated. Patients from the control group had normal values for blood parameters; any noticeable modification would mean exclusion from the group.

Fig. 1 The correlation between waist and serum TG

Fig. 2 The correlation between waist and serum SOD

Fig. 3 The correlation between serum TG and serum SOD

Fig. 4 The correlation between LDL cholesterol and serum SOD

Fig. 5 The correlation between HDL cholesterol and serum SOD

Fig. 6 The correlation between fasting plasma glucose and eritocyte
Fig. 7 The correlation between hs-CRP and eritocyte MDA

Fig. 8 The correlation between hs-CRP and eritrocyte CAT

Fig. 9 The correlation between fibrinogen and serum MDA

Fig. 10 The correlation between fibrinogen and eritrocyte MDA

Fig. 11 The correlation between fibrinogen and eritrocyte SOD

Fig. 12 The correlation between MetS inclusion criteria and serum MDA

Fig. 13 The correlation between MetS inclusion criteria and eritrocyte MDA

Fig. 14 The correlation between MetS inclusion criteria and eritrocyte SOD
Plasma level of oxidative stress parameters analysed for patients with MetS and newly diagnosed HTA are significantly higher than the control group (p<0.0001, α=0.05); they are strong correlated with the number of inclusion criteria in MetS, the parameters of lipid profile and inflammatory status (r>0.7); have a weak correlation with blood pressure values; the determination coefficient (R²) is significantly increased between the number of criteria for MetS and the parameters of oxidative stress.

Level of hs-CRP activity is strongly correlated (p<0.004, r>0.7, R²: 0.49-0.9) with all the lipid profile parameters, vascular inflammatory markers and oxidative stress markers taken into consideration.

IV. CONCLUSIONS

In this study we found significant differences between the parameters recorded for patients with and without metabolic syndrome, respectively; these results support the concept that this group of patients have a higher cardiovascular risk. Increase oxidative stress activity and CRP levels are associated with MetS, but not with the BP values. Applying multiple linear regression adjusted for sex, age, classical cardiovascular risk factors, systolic blood pressure is a powerful and independent determinant factor of oxidative stress parameters. Apolipoproteins are proteic components of lipoproteins that help to evaluate the risk of coronary disease. Apo A-1 is the main component of HDL (90%), while apo B-100 is the main component for LDL, with role in balancing the cholesterol synthesis and metabolism. Lipids and lipoproteins molecules composition and concentration are influenced by normal biological variations, while levels of apo A-1 and apo B are more stable and better correlated with the severity and the degree of expansion of the cardiovascular status. A multimarker strategy may be useful in evaluating the cardiovascular status in this type of patients.

REFERENCES

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