The influence of cigarette smoking on endothelial function

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Abstract
Cigarette smoking is well recognized as a major risk factor for atherosclerosis in men and women. Brachial flow-mediated dilation (FMD) is a physiologic measure and carotid intima-media thickness (IMT) is an anatomic structural measure of subclinical atherosclerosis. The purpose of this study was to observe the effects of cigarette smoking expressed in package-years on endothelial function evaluated by ultrasonographic measurement of brachial FMD and carotid IMT in four groups of subjects: arterial hypertension [HTN] group, atherogenic dyslipidemia [DYS] group, coronary artery disease, angiographically confirmed [BCV] group and [CON] group. Cigarette smoking, measured in package-years was significantly higher in [BCV] group compared to the [DYS] group (p<0.001), the [HTN] group (p<0.001) and the [CON] group (p<0.001). The forearm reactive hyperemic response (FMD) was significantly blunted in [BCV] group (p<0.001) compared to [HTN] group (p=0.001), [DYS] group (p<0.001) and to [CON] group (p=0.001). Patients from [BCV] group had highly significant greater IMT compared to patients from [HTN] group, [DYS] group and [CON] group (p<0.001). In our study, we observed that cigarette smoking induces a significant deterioration of endothelial dysfunction that is dependent of the number of package-years.

Key words: Intima-media thickness, flow mediated vasodilatation, cigarette smoking, endothelial function.

Introduction
Cigarette smoking may cause an imbalance between antioxidant nutrient status and free-radical load incurred by smoking which may initiate the deterioration process associated with cardiovascular disease, thereby increasing the risk of atherosclerosis and other diseases 1.

The main site of toxic effects of cigarette smoking on the vascular system is the endothelium, and, specifically with regards to the coronary system, the coronary endothelium. Cigarette smoking exerts atherogenic effects through chronic activation of pro-inflammatory cytokines and increased platelet adhesion and aggregation, as well as by promoting monocyte adhesion and transendothelial migration into the subintimal space 2. Smoking of two cigarettes per day doubles the number of nuclear damaged endothelial cells in the circulating blood 3.

Subclinical cardiovascular disease has both physiologic and anatomic components. Non-invasive measurement techniques allow for the characterization of both physiologic as well as anatomic structural changes in the arterial wall 4.

Early atherosclerotic lesions due to cigarette smoking or other cardiovascular risk factors may be detected in individuals noninvasively by using ultrasound to measure the intima-media thickness (IMT) of the affected vessel 5.

Flow-mediated dilation (FMD) is a simple and noninvasive measure of the capacity of the endothelium, when stimulated by a sudden increase in shear stress, to cause smooth muscle cell relaxation and vasodilation and is a validated measure that quantifies endothelial function 5.

Materials and Methods
The study was conducted in 68 smoker subjects from the IVth Medical Clinic of the University of Medicine and Pharmacy Victor Babes Timisoara after informed consent: 32 patients with coronary artery disease [BCV] (mean age 50 ± 3.15 years, 71% males and 29% females), 12 hypertensive patients [HTN] (mean age 55 ± 4.75 years, 60% males and 40% females) and 12 patients with atherogenic dyslipidemia [DYS] (mean age 53 ± 5.68 years, 68% males and 32% females). The control group [CON] consisted of 12 healthy subjects (mean age 57 ± 4.25 years, 68% males and 32% females).

The smoking subjects refrained from smoking cigarettes for at least 12 h prior to testing to rule out the influence of acute cigarette smoking. Antihypertensive medication was interrupted for at least two weeks before the study.

Smoking burden was evaluated by package-years (1 package-year was equivalent to 20 cigarettes smoked per day for 1 year).

Blood pressure was taken on the left arm after five minutes of relaxation, using a standard mercury sphygmomanometer. Hypertension was diagnosed according to 2007 European Guidelines for the Management of Arterial Hypertension 6.

Atherogenic dyslipidemia was defined as low plasma high-density lipoprotein cholesterol and elevated triglycerides.

Clinical evaluation included blood pressure measurement, physical examination, chest radiograph, 12-lead electrocardiogram. While on their usual diet, a venous blood sample was drawn from an antecubital vein in all subjects after an overnight fast to determine TC, HDL-C and TG using standard enzymatic methods.
LDL cholesterol was calculated as described by Friedewald and collaborators.  

Endothelial function was assessed by means of flow-mediated vasodilatation on brachial artery, using B-mode ultrasonography (ALOKA ProSound 4000, with linear transducer of 7.5 MHz). Before the FMD determination, the patients were relaxed in a stable room temperature of 20–25ºC and the smoking was prohibited.  

The diameter of brachial artery was measured incident with the R wave of the electrocardiograph trace (Df). Then, ischemia was induced by inflated the pneumatic cuff to a pressure 50 mmHg above systolic one, in order to obliterate the brachial artery and induce ischemia. After 5 min, the cuff was deflated and the diameter was measured after 60 s post–deflation (Df). FMD was calculated with the formula:

$$FMD = \left\{\frac{Df - D_i}{D_i}\right\} \times 100.$$  

Carotid IMT was measured by high-resolution B-mode ultrasonography with an ultrasonographic apparatus (ALOKA ProSound 4000, with linear transducer of 7.5 MHz). The image was focused on the posterior (far) of the left carotid artery. A minimum of 4 measurements of the common carotid far wall were taken 10 mm proximal to the bifurcation to derive mean carotid IMT. We analyzed the maximum thickness of intima-media complex, as carotid IMT (normal values < 0.9 mm).

Continuous variables were expressed as means ± SD. Means were compared using analysis of variance or the Student’s t-test. Pearson’s correlation was used to test bivariate correlations and results were verified using the non-parametric Spearman’s rank correlation test. Statistical significance was defined as two-sided p<0.05. All statistical analyses were performed using Excel Microsoft Office 2003.

The procedures followed were in accordance with the ethical standards of the Hospital Ethics Committee and with the Helsinki Declaration of 1975, as revised in 2000.

### Results and Discussion

The baseline characteristics of the subjects, the mean values of package-years, brachial FMD and carotid IMT are summarized in Table 1. Cigarette smoking, measured in package-years, was significantly higher in [BCV] group compared to the [DYS] group (p<0.001), the [HTN] group (p<0.001) and the [CON] group (p<0.001). Another finding of the present study was that the forearm reactive hyperemic response (FMD) was significantly blunted in [BCV] group (p<0.001) compared to [HTN] group (p<0.001), [DYS] group (p<0.001) and to [CON] group (p<0.001). The patients from [BCV] group had significantly greater IMT compared to patients from [HTN] group, [DYS] group and [CON] group (p<0.001).

The correlation between cigarette smoking and FMD was negative, strong and significant for [BCV] group (r = -0.72, p<0.001) (Fig. 1), for [HTN] group (r = -0.76, p<0.001) (Fig. 2) and for [DYS] group (r = -0.82, p<0.001) (Fig. 3). For [CON] group was obtained only a negative correlation, weak but significant (r = -0.29, p<0.001) between the cigarette smoking measured in package-years and the value of FMD (Fig. 4).

For the entire group of subjects included in the study, the correlation between cigarette smoking and the value of FMD was negative, strong and significant (r = -0.78, p<0.001) (Fig. 5), demonstrating that smoking is a significant risk factor involved in the modification of endothelial-dependent vasodilator response.

The correlation between cigarette smoking and carotid IMT was positive, weak but significant only for [BCV] group (r = 0.30, p<0.001) (Fig. 6). The correlation was not significant for the [CON] group (r = 0.10, p<0.001), the [HTN] group (r = 0.12, p<0.001), the [DYS] group (r = 0.12, p<0.001) and for the entire group of subjects included in the study (r = 0.10, p<0.001).

Atherosclerosis is a chronic disease resulting from past and persistent exposures to risk factors, such as cigarette smoking, over a long period of time. Our data reinforce the known association of smoking with atherosclerosis and evidence the relationship between cigarette smoking and vascular dysfunction evaluated by B-mode ultrasonography.

In this study, we demonstrated that carotid IMT is most increased in patients with coronary artery disease, angiographically confirmed. A well-established cardiovascular risk factor, cigarette smoking, expressed in package-years, was correlated to carotid IMT in BCV group, in our study. Our findings that cigarette smoking was associated with carotid IMT indicate that cigarette smoking played a fundamental role in the initiation and progression of atherosclerosis in these patients. These results significantly extend previous findings that have shown that vascular dysfunction is associated with long-term chronic cigarette smoking. Carotid IMT was significantly increased in [BCV] group compared to the [DYS] group, the [HTN] group and the [CON] group. An explanation could be that these patients had significantly increased the cigarette smoking, measured in package-years.

Impaired endothelial-dependent FMD of conduit arteries is a characteristic feature of subjects with known vascular disease risk factors. Several previous studies have demonstrated that cigarette smoking alone is sufficient to impair flow-mediated brachial artery dilation. Furthermore, a study showed that, even in long-term smokers, acute impairment of endothelial function can be provoked by the smoking of one single cigarette. These previous results compares closely with the present study, where hyperemia induced dilation of the brachial artery was significantly reduced in [BCV] group.

### Table 1. Subjects characteristics (values are presented as mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>[CON] (n = 12)</th>
<th>[BCV] (n = 32)</th>
<th>[HTN] (n = 12)</th>
<th>[DYS] (n = 12)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57 ± 4.25</td>
<td>50 ± 3.15</td>
<td>55 ± 4.75</td>
<td>53 ± 5.68</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cigarette smoking (pack–years)</td>
<td>30 ± 4.11</td>
<td>52 ± 4.50</td>
<td>43 ± 4.72</td>
<td>45 ± 6.30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114 ± 6.08</td>
<td>149±15.96</td>
<td>147 ± 10.10</td>
<td>120 ± 11.17</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>68 ± 5.84</td>
<td>90 ± 10.23</td>
<td>88 ± 7.78</td>
<td>72 ± 8.88</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>13 ± 4.74</td>
<td>5.44 ±2.50</td>
<td>7.67 ±2.53</td>
<td>10 ± 4.19</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.7 ± 0.24</td>
<td>1.6 ± 0.18</td>
<td>0.8 ± 0.23</td>
<td>0.7 ± 0.21</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

NOTE: SBP = systolic blood pressure, DBP = diastolic blood pressure, FMD = flow-mediated vasodilatation, IMT = carotid intima – media thickness.
compared with the [HTN] group, [DYS] group and [CON] group. The fact that brachial FMD was most reduced in [BCV] group compared to the [DYS] group, the [HTN] group and the [CON] group could be explained by the fact that these patients had significantly increased cigarette smoking, measured in package – years.

One interpretation of these findings is that smoking-related endothelial dysfunction in the brachial artery verified by loss of FMD or by increased of IMT in carotid artery is dependent of the number of package-years of cigarette smoking.

Smoking cessation, in addition to control of other cardiovascular risk factors, might have effect on delaying or stopping atherosclerosis process, which means that new plaques might be prevented, old plaques might be stabilized after stopping smoking.

**Study Limitations**

The duration of the nicotine-induced endothelial dysfunction and the order and exact time course of changes in FMD is currently unclear and remains to be studied. Acute effects of cigarette smoking on the vascular system with observance of time course of functional changes were not investigated.

**Conclusions**

Cigarette smoking caused a significant impairment in endothelial function. An increase in number of package-years would also result in greater structural and functional damage of vessels.

**References**

6. Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R.,


