The impact of TFPI on coronary atherosclerotic burden

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ABSTRACT
OBJECTIVES: We aimed to associate a coronary artery disease (CAD) presence and severity with endothelial dysfunction (ED), carotid intima media thickness (CIMT) and Tissue Factor Pathway Inhibitor (TFPI).

BACKGROUND: ED has a central role in atherosclerosis. CIMT and TFPI activity are also related with atherosclerosis and CAD.

METHODS: In our prospective observational study, 50 patients had CAD and 30 had normal coronary arteries. Endothelial function was evaluated by endothelium-dependent flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) measurements. CIMT and Serum TFPI levels were also measured.

RESULTS: TFPI was a statistically significant determinant between the two groups with an increased level in CAD (+) group (84.9 ± 19.3 vs 70.2 ± 14.7, p = 0.001). There was a positive correlation between CIMT and Gensini (r = 0.34, p = 0.014). There was a strong negative correlation between Gensini and FMD-NMD, statistically significant (FMD: r = −0.715, p < 0.001; NMD: r = −0.718, p < 0.001).

CONCLUSION: We observed that ED, increased CIMT and TFPI levels were associated with CAD. Additionally, increased CIMT measurements and decreased FMD and NMD values had a positive correlation with GSS (Tab. 4, Fig. 6, Ref. 50). Text in PDF www.eils.sk.

KEY WORDS: coronary artery disease, endothelial dysfunction, tissue factor pathway inhibitor.

Introduction

The vascular endothelium is a large paracrine, autocrine and endocrine organ that plays an important role in vascular tone, thrombogenesis and inflammation. Endothelium regulates these systems by producing some mediators; vonoconstrictor and vasodilator factors such as nitric oxide (NO), and also some others regulating platelet activity, clotting cascade and fibrinolysis (1–4). Vasomotor disturbances, abnormal coagulation and fibrinolysis can be seen in endothelial dysfunction (ED) due to impairment of these mediator balances (3,5–6). Under chronic inflammatory process, endothelium loses its ability to protect the vascular system and it leads to atherosclerosis. Decreasing NO activity in vascular system has a central role in endothelial dysfunction dependent vascular atherosclerosis. In addition to this, vascular endothelium is the production site of some pro- and anti-coagulation factors (2–3). In case of ED, disbalance of this ratio in favor of pro-thrombotic properties results in thrombosis. Tissue factor pathway inhibitor (TFPI), which is one of these factors, has an important role in clotting cascade and thrombosis. Tissue factor (TF) is an endothelium cell surface glycoprotein, which is not normally present on the surface of endothelium and does not support coagulation (7). If it contacts the coagulation factor VII, TF and FVII complex initiates coagulation (8). TFPI is produced by endothelium and it is related to endothelial dysfunction as well (9). TFPI inhibits factor VII-TF complex, which starts the clotting cascade (10). TFPI activity is related to atherosclerotic process and coronary artery disease (CAD) (11–15) (Figs 1–3).

Carotid artery changes due to atherosclerosis are related to increased cardiovascular morbidity and mortality (16). Carotid intima media thickness (CIMT) is of importance for atherosclerotic process and is used to evaluate future cardiovascular events and mortality (17–18). The previous studies showed that there was a strong relationship between ED and atherosclerosis (19–20). Some noninvasive tests, which are endothelium dependent flow-mediated dilatation (FMD) and endothelium independent nitroglycerin-mediated dilatation (NMD) in the brachial artery (BA), are used to evaluate endothelial function (21–24). In our study, we aimed to associate CAD presence and severity with endothelial dysfunction, carotid intima media thickness and TFPI as an endothelial derived factor.

Methods

Study design and population

Eighty patients were included in this study between January 2010 – January 2013 with suspected CAD owing to typical chest pain or positive noninvasive cardiovascular stress testing, who
underwent cardiac catheterization. Patients with acute coronary syndrome, history of coronary angioplasty or coronary artery bypass surgery, chronic renal failure, chronic hepatic failure, rheumatological disorder, history of malignant disease, usage of antithrombotic or anticoagulant treatment were excluded.

The study protocol was approved by local ethics committee and a written informed consent was obtained from all subjects.

**Study protocol**

The study was designed as a prospective observational study. Basic demographic data of the enrolled patients included age, gender, body mass index (BMI), glomerular filtration rate (GFR), presence of traditional major cardiovascular risk factors (hypertension (HT), diabetes mellitus (DM), dyslipidemia, family history of premature cardiovascular disease (CVD), and current smoking) and a usage of cardiovascular treatment. Endothelial dysfunction was evaluated before coronary angiography via left brachial artery using brachial artery Doppler ultrasonography (USG) in two different methods; FMD and NMD. CIMT was measured by using carotid artery Doppler USG. All patients’ TFPI levels were analyzed. Coronary angiography was performed, and presence and severity of atherosclerosis was evaluated by using Gensini scoring system (GSS) (25).

**Statistical analysis**

Data were expressed as the “mean (standard deviation; SD), “n (%))” and “median (minimum–maximum)” values, where appropriate. Fisher’s exact test and Pearson chi-square analysis was performed for categorical variables. Fitness to normal distribution was analyzed with the Kolmogorov–Smirnov test. Mann–Whitney U test was used for comparing quantitative variables with abnormal distribution, while Student t-test was used for comparing the means between two groups with normal distribution. Spearman correlation test was performed for correlations between ordinal variables or continuous variables with abnormal distribution. Simple linear regression analyses were applied for CIMT, FMD and NMD to assess the association with Gensini. Multiple logistic regression analysis was used for predicting presence of CAD. Three separate models were conducted for CIMT, FMD and NMD adjusting for age, smoking status and gender. Statistical analysis was made using the computer software Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 21.0. released 2012, IBM Corp., Armonk, New York, USA). The p value < 0.05 was considered statistically significant.

**Results**

Clinical and demographic characteristics of the CAD and the control groups are presented in Table 1. In these two groups, there were 50 subjects having CAD and 30 of them had normal coronary arteries. The number of male patients and nonsmokers in the CAD (+) group and the mean age of this group were significantly higher.

Endothelial dependent and independent vasodilatory responses and CIMT were compared between the CAD (+) and control groups in Table 2.

TFPI was a statistically significant determinant between the two groups with an increased level in CAD (+) group (84.9 ± 19.3 vs 70.2 ± 14.7, p = 0.001) (Tab. 3).

For patients with CAD, simple linear regression analyses were performed for CIMT, FMD and NMD to predict GSS. These analy-
ses revealed that CIMT ($\beta$ = 0.282; $p$ = 0.047), FMD ($\beta$ = –0.581; $p$ < 0.001) and NMD ($\beta$ = –0.593; $p$ < 0.001) were independent risk factors for GSS. There was a positive correlation between CIMT and Gensini ($r$ = 0.34, $p$ = 0.014) (Fig. 4). There was a strong negative correlation between Gensini and FMD and NMD with statistical significance (FMD: $r$ = –0.715, $p$ < 0.001) (Fig. 5); NMD: $r$ = –0.718, $p$ < 0.001) (Fig. 6).

Three regression models were conducted to evaluate the association between CIMT, FMD, NMD with CAD adjusting for gender, age and smoking status. The patient’s smoking status was not significant in three models. Model 1 showed that CIMT was positively associated with CAD ($p$ = 0.002). TFPI was significantly associated with CAD in Model 1, 2 and 3 ($p_1$ = 0.017; $p_2$ = 0.036; $p_3$ = 0.031 respectively). In model 1 and 3, both gender (male) and age were significantly related with CAD. In Model 2, the association between gender and CAD was not statistically significant, but the association between age and CAD remained significant ($p$ = 0.024). In Model 2 and 3, it was found that both FMD and NMD values of patients were negatively related to CAD ($p_{\text{FMD}}$ = 0.004; $p_{\text{NMD}}$ = 0.009) (Tab. 4).

<table>
<thead>
<tr>
<th>Tab. 1. Clinical and demographic characteristics.</th>
<th>Control (n=30)</th>
<th>CAD (n=50)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male), n(%)</td>
<td>12(40)</td>
<td>33(66)</td>
<td>0.023*</td>
</tr>
<tr>
<td>Age*</td>
<td>53.7±9.1</td>
<td>61.8±7.2</td>
<td>0.001***</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27(22–40)</td>
<td>28(21.2–43)</td>
<td>0.823</td>
</tr>
<tr>
<td>HT, n(%)</td>
<td>16(53.3)</td>
<td>31(62)</td>
<td>0.446</td>
</tr>
<tr>
<td>Smoking, n(%)</td>
<td>3(10)</td>
<td>17(34)</td>
<td>0.016*</td>
</tr>
<tr>
<td>DM, n(%)</td>
<td>6(20)</td>
<td>16(32)</td>
<td>0.245</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>216±34.1</td>
<td>217.6±42</td>
<td>0.854</td>
</tr>
<tr>
<td>HDL* (mg/dl)</td>
<td>44(29–104)</td>
<td>45(25–69)</td>
<td>0.598</td>
</tr>
<tr>
<td>nonHDL* (mg/dl)</td>
<td>167.7±38</td>
<td>171.3±40.5</td>
<td>0.693</td>
</tr>
<tr>
<td>LDL* (mg/dl)</td>
<td>137.6±29.4</td>
<td>142.6±35.8</td>
<td>0.522</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>149.5(63–348)</td>
<td>150(45–313)</td>
<td>0.839</td>
</tr>
<tr>
<td>GFR* (ml/min)</td>
<td>86.1±20.5</td>
<td>83.5±17.5</td>
<td>0.552</td>
</tr>
<tr>
<td>Hgbb (g/dl)</td>
<td>13.3±1.6</td>
<td>13.4±1.9</td>
<td>0.836</td>
</tr>
<tr>
<td>ACEI, n(%)</td>
<td>4(13.3)</td>
<td>10(20)</td>
<td>0.447</td>
</tr>
<tr>
<td>ARB, n(%)</td>
<td>3(10)</td>
<td>10(20)</td>
<td>0.351</td>
</tr>
<tr>
<td>Betablocker, n(%)</td>
<td>6(20)</td>
<td>14(28)</td>
<td>0.424</td>
</tr>
<tr>
<td>CCB, n(%)</td>
<td>1(3.3)</td>
<td>4(8)</td>
<td>0.645</td>
</tr>
<tr>
<td>Nitroglycerin, n(%)</td>
<td>0(0)</td>
<td>4(8)</td>
<td>0.291</td>
</tr>
<tr>
<td>Insulin, n(%)</td>
<td>0(0)</td>
<td>4(8)</td>
<td>0.291</td>
</tr>
<tr>
<td>OAD, n(%)</td>
<td>5(16.7)</td>
<td>6(12)</td>
<td>0.739</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Tab. 2. CIMT, NMD and FMD values of CAD and control groups.</th>
<th>Control</th>
<th>CAD</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIMT* (cm)</td>
<td>0.64 ± 0.1</td>
<td>0.86 ± 0.1</td>
<td>0.001***</td>
</tr>
<tr>
<td>FMD* (%)</td>
<td>11.2(4.1–17.2)</td>
<td>4.9(0.8–16)</td>
<td>0.001***</td>
</tr>
<tr>
<td>NMD* (%)</td>
<td>17.3(7–22.5)</td>
<td>9.1(1.2–21.5)</td>
<td>0.001***</td>
</tr>
</tbody>
</table>

| ***$p$ < 0.001, *Student’s t test, †Mann–Whitney - U test, CAD – coronary artery disease, CIMT – carotid intima media thickness, FMD – flow-mediated dilatation, NMD – nitroglycerin-mediated dilatation |

<table>
<thead>
<tr>
<th>Tab. 3. TFPI levels of CAD and control groups.</th>
<th>Control</th>
<th>CAD</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFPI (mg/dl)</td>
<td>70.2 ± 14.7</td>
<td>84.9 ± 19.3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

| *Student’s t test, CAD – coronary artery disease, TFPI – tissue factor pathway inhibitor |

Fig. 4. The relationship between CIMT and Gensini (CIMT – Carotid intima media thickness).

Fig. 5. The relationship between FMD and Gensini (FMD – flow-mediated vasodilatory response).

Fig. 6. The relationship between NMD and Gensini (NMD – nitroglycerin-mediated vasodilatory response).
Discussion

In the present study, we investigated the relationship of TFPI levels and endothelial dysfunction with coronary artery atherosclerosis. Atherosclerosis is the most common cause of morbidity and mortality all over the world (27). This inflammatory process can be affected and progressed by ED. There is a strong correlation between ED and atherosclerosis. Atherosclerotic lesions can be progressed rapidly in the presence of ED (28). First human study of ED by Ludmer et al. indicated the evidence of impaired endothelium dependent dilatation in the presence of atherosclerosis (29). According to this study, in the mild CAD patients, there was a paradoxical construction in the arteries and it was indicated that ED could be present in the early stage of atherosclerosis. The impairment of endothelial function also predicts cardiovascular clinical outcomes. Cardiovascular events, including percutaneous and surgical revascularization occurred more often in patients with impaired endothelium dependent dilatation in the presence of atherosclerosis (30). In our study, there was a strong relationship between ED and coronary atherosclerosis. Both endothelial dependent flow mediated, and independent nitroglycerin mediated vasodilatory responses were significantly decreased in the CAD (+) group compared to the control group (p < 0.001).

Risk factors of coronary atherosclerosis also affect endothelial functions. In the Framingham study, there was a relationship between coronary risk factors and endothelial function (31). It was indicated that ED was related to age, systolic blood pressure, BMI, lipid lowering medication, and smoking and inversely correlated to female gender, heart rate and prior walk test. There is a strong relationship between endothelial function and coronary risk factors with an increased prevalence of endothelial dysfunction in the female gender and advanced age (32–34). In our study, it was revealed that the number of male patients and the mean age of the group were higher in CAD (+) than in the control group, and also the regression analysis indicated that ED was found to be correlated to male gender and advanced age. We did not find any relationship between HT, HL, DM, BMI and ED and CAD. This result maybe explained that almost all of the participants were under an optimal medical therapy for HT, HL and DM.

It is well-known that smoking is one of the most important risk factor for atherosclerosis and smoking causes ED as the result of impairment of endothelial dependent dilatation (35, 36). Additionally, FMD was increased in smokers that did not smoke within six hours before testing than in those who did (31). While the number of smokers was higher in the CAD (+) group in our study, the regression analysis revealed that there was no association between smoking and ED in CAD patients. It could be the result of smoking withheld for at least 12 hours before our study.

The presence and severity of coronary atherosclerosis is of importance for prognosis and treatment. In the previous studies, brachial and coronary artery endothelial functions were found to be related (16). In addition, CIMT, which is an indicator of ED, is regarded as a manifestation of atherosclerosis (37, 38), and is related to an increased cardiovascular morbidity and mortality (16). CIMT is of importance for atherosclerotic process and is used to evaluate future cardiovascular events and mortality (17–18). A strong correlation between ED and the number of diseased coronary vessels and higher cardiovascular event rates were also observed (39–40). We found that an impairment of endothelial function and increased CIMT were observed with a higher incidence in CAD (+) patients. Besides, a strong correlation between ED, which is evaluated with NMD, FMD and CIMT measurements, and coronary atherosclerotic burden, which was evaluated by using GSS, was found in our study.

Healthy endothelium regulates the balance between pro- and anti-inflammatory substances and pro- and anti-coagulant factors. Normally, TF is not present on the surface of the endothelial cells and endothelium does not support coagulation. Chronic endothelial activation results in endothelial dysfunction. It causes an impairment of vasodilatory response, increased thrombogenesis and inflammation (41). ED can be assessed by vasodilatory response
(FMD or NMD) and the measurement of endothelial markers (42). TFPI is one of the most important endothelial markers and is associated with endothelial dysfunction and inflammation. It is the main inhibitor of TF-mediated coagulation and co-localizes with TF in atherosclerotic plaques. It was shown that TFPI shows a positive correlation with ED (9) and TFPI levels increases during ED (43). In the light of foregoing data, increased levels of TFPI are associated with FMD and atherosclerosis (9, 44). The association between increased TFPI levels and asymptomatic or overt atherosclerosis has been studied in several studies. The association between TFPI levels and subclinical atherosclerosis was found to be significant (45). On the other hand, there is a relationship between increased TFPI levels and adverse cardiovascular events. Higher TFPI level was associated with future cardiovascular events including cardiac death, myocardial infarction and a need for revascularization (46) and increased TFPI level was observed in patients with cardiovascular death (47). In our study, an increased TFPI level was shown in patients with CAD. It showed the relationship between TFPI level and atherosclerosis. In addition to this, CAD (+) patients having increased TFPI levels also presented with ED with impairment of FMD and NMD. The association between increased TFPI levels and ED, was revealed. Besides, TFPI activity was associated with subclinical cerebrovascular disease (48) and found to be an independent predictor of CIMT (49,50). In our study, CAD (+) patients had both increased TFPI levels and CIMT. It was not surprising that under inflammatory process, endothelial dysfunction resulted in increased TFPI levels and also caused an increased CIMT as a manifestation of atherosclerosis.

Learning points

It was demonstrated in our study that TFPI had a strong effect on atherosclerotic process including coronary atherosclerosis. Non-coronary atherosclerosis detecting by CIMT, FMD and NMD was also related to coronary atherosclerotic process and burden.

Limitations of the Study

In our study, the major limitation was the small number of subjects in the CAD (+) group. While ultrasonographic evaluation of brachial artery is the accepted method for assessment ED, the role of this method in subclinical disease is not known well.

Conclusion

In the present study, we demonstrated that coronary atherosclerotic burden was associated with impairment of endothelial function as well as increased CIMT and TFPI activity. In addition to these, increased CIMT measurements and decreased FMD and NMD values had a positive correlation to the extend and severity of coronary artery disease. Consequently, we suggested the TFPI as a surrogate marker of coronary atherosclerotic disease as well as ED and increased CIMT.

References


