ORIGINAL ARTICLE

Tobacco smoke exposure and endothelial dysfunction in patients with advanced coronary artery disease

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KEY WORDS

atherosclerosis, cigarette smoking, coronary artery disease, cotinine, endothelial dysfunction

ABSTRACT

INTRODUCTION Exposure to tobacco smoke is associated with a higher cardiovascular risk, especially in patients with coronary artery disease (CAD).

OBJECTIVES The aim of the study was to evaluate the effect of active and passive tobacco smoking on the activity of endothelial markers in advanced atherosclerosis.

PATIENTS AND METHODS We studied 181 consecutive patients with advanced CAD (53 women and 128 men) aged 60 ±8 years, including 102 active self-declared smokers (56.3%). We determined plasma asymmetric dimethylarginine (ADMA), thrombomodulin (TM), and plasminogen activator inhibitor-1 (PAI-1) levels, along with serum cotinine concentrations as a marker of tobacco smoking.

RESULTS Plasma ADMA levels were higher in active smokers compared with nonsmokers (0.60 ±0.09 μmol/l vs. 0.49 ±0.08 μmol/l, P <0.001). There were similar intergroup differences in TM (4.60 ±2.11 ng/ml vs. 3.0 ±1.7 ng/ml, P <0.0001) and PAI-1 levels (30.3 ±12.4 ng/ml vs. 23.6 ±11.3 ng/ml, P <0.0001). We observed positive correlations between cotinine and ADMA (r = 0.71, P <0.0001), TM (r = 0.53, P <0.0001), and PAI-1 (r = 0.58, P <0.0001). In 21 patients (26.6%) who declared to be nonsmokers, cotinine levels (mean, 6.30 ±22.5 ng/ml) significantly correlated with ADMA, TM, and PAI-1 (all P <0.001). A multivariate regression analysis showed that cotinine was an independent predictor of ADMA, TM, and PAI-1 in the whole patient group.

CONCLUSIONS Despite long-lasting endothelial injury in advanced CAD, continued cigarette smoking is able to further enhance endothelial damage by increasing ADMA levels and resultant inhibition of fibrinolysis.

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INTRODUCTION Tobacco smoking is a major risk factor for cardiovascular diseases.[1] The Global Adult Tobacco Survey showed that 48.6% of men and 11.3% of women in the participating countries were tobacco users. In addition, 40.7% of men (ranging from 21.6% in Brazil to 60.2% in Russia) and 5.0% of women (24.4% in Poland) participating in the survey smoked a tobacco product.[2] Smoking cessation is of paramount importance as secondary prevention of myocardial infarction (MI) but approximately 30% to 45% of the patients continue to smoke, while 59% of the patients refrain from smoking only for a year following MI.[3] Several studies have demonstrated that cessation of smoking reduces the incidence of recurrent MI and associated mortality rates.[4,5] Proatherogenic and prothrombotic effects of cigarette smoking are related to toxicity of tobacco smoke.[6-8] It is estimated that over 4300 chemical compounds are present in tobacco smoke.[9] The most toxic vapor phase is carbon monoxide. The particulate phase is a solid aerosol, of which nicotine constitutes about 85% to 90% of total alkaloid weight.[9] Cotinine, the metabolite

of nicotine, is a specific biomarker of smoking and allows to distinguish active smokers from non-smokers or second-hand smokers.[10-12]

Endothelial dysfunction is typical of atherosclerotic vascular disease in its early stage.[13,14] Reduced bioavailability of nitric oxide (NO) produced by endothelial synthase (eNOS) from L-arginine is partially associated with an increased formation of asymmetric dimethylarginine (ADMA), an eNOS inhibitor involved in endothelial damage and atherosclerosis.[15,16]

Thrombomodulin (TM), an integral transmembrane glycoprotein, is a sensitive marker of endothelial damage. TM exhibits prothrombotic effects including activation of protein C and inactivation of plasminogen activator inhibitor type-1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor. TM deficiency may lead to increased activation of endothelial cells and progression of atherosclerosis.[17] Owing to its anticoagulant properties, TM plays an important role in the regulation of intravascular coagulation. It is also recognized as an early marker of atherosclerotic vascular disease.[18] Elevated plasma PAI-1 levels are a marker of endothelial damage associated with higher cardiovascular risk.[19]

The aim of this study was to evaluate whether active and passive smoking may affect the markers of endothelial dysfunction in advanced atherosclerosis.

PATIENTS AND METHODS

Patients The study included 181 consecutive patients with stable coronary artery disease (CAD). In all patients, stenosis of more than 50% in at least 1 epicardial coronary artery was documented within the previous year. The exclusion criteria were as follows: acute infections, acute cardiovascular events within the preceding 6 months, end-stage kidney disease, cancer, severe chronic obstructive pulmonary disease, and anticoagulant therapy. All subjects either declared cessation of cigarette smoking within at least 3 months prior to examination or were current smokers who had smoked for at least 10 years. Diabetes mellitus was defined as the use of insulin or oral hypoglycemic agents. Renal failure was defined as serum creatinine exceeding 120 μmol/l. Family history of CAD was defined as having a parent or sibling with MI or coronary revascularization at the age of 50 years or younger.

The Bioethics Committee of the Jagiellonian University approved the study and all participants provided their written informed consent.

Laboratory tests Blood samples were obtained by venipuncture from the antecubital vein using minimal stasis after 12 hours of fasting. Lipid profiles, glucose, creatinine, blood cell and platelet counts were assayed by routine laboratory techniques. Plasma fibrinogen was determined using the von Clauss method. High-sensitivity C-reactive protein was measured by latex nephelometry (Siemens, Marburg, Germany). Plasma samples were mixed with 3.2% sodium citrate (9:1), centrifuged for 60 minutes, and stored at –80°C. Plasma PAI-1 antigen and soluble TM levels were measured by immunoenzymatic assays (American Diagnostica, United States, and Diagnostica Stago, Asniéres, France, respectively). All measurements were performed by technicians blinded to the sample status. The coefficients of intra- and interassay variations were less than 7%.

Plasma ADMA concentrations were measured in EDTA plasma using high-performance liquid chromatography (HPLC) with precolumn derivatization as previously described.[20] In brief, equilibrated CBA columns (BountElut, Varian Inc., California, United States) were used for 3-fold flushing with 1 ml of plasma samples and then washed with methanol and distilled water. Then, the samples were eluted with 10% ammonia and dried. The sediment obtained was dissolved in 1 ml of water, the solution centrifuged, and the supernatant was subjected to HPLC using the ODS column (Fisher Scientific, St. Louis, Missouri, United States). The inter- and intraassay coefficients of variation for ADMA were below 7%.

Serum cotinine was extracted by the liquid––liquid extraction method as an internal standard and a deuterated cotinine was used. Cotinine levels were determined using gas chromatography tandem mass spectrometry. Chromatographic separation was performed with Thermo Scientific ITQ 1100 (Thermo Scientific, Woltham, Massachusetts United States) on a DB5MS column (30×0.25×0.25; Agilent Technology, United States). The temperature program of the column was applied as follows: 70°C at baseline, then increased to 290°C over the next 20 minutes, and maintained at 290°C for 20 minutes. The calibration curve was linear between 2 and 500 ng/ml. The limit of the quantification of cotinine was 2 ng/ml. The inter- and intraassay coefficients of variation for low- and high-concentration of cotinine were below 10%.

Patients that declared to be nonsmokers and had cotinine levels exceeded 2 ng/ml were considered to be passive smokers and were included into the group with cotinine concentrations of <12 ng/ml. Patients with cotinine concentrations exceeding 12 ng/ml were considered to be smokers and were classified accordingly despite self-declared nonsmoking.

Statistical analysis Statistical analysis was performed with the SAS® 9.3 software (SAS Institute, Cary, North Carolina, United States).

Most of the variables in the study interval were not normally distributed, as indicated by the Shapiro-Wilk test. The statistical distributions of biochemical parameters between patient groups were compared using the Wilcoxon rank-sum test or Kruskal-Wallis test. Categorical variables were compared by the χ^2 exact test. The SAS MULTTEST procedure was used to control significance level for multiple comparisons. Spearman rank correlation coefficients between

TABLE 1 Clinical characteristics of the study population

	Smokers, n = 74	Nonsmokers, n = 93	*P* value
age, y	61.5 (12)	60.0 (13)	0.80
male, n (%)	52 (70)	63 (68)	0.74
BMI, kg/m²	27.0 (6.5)	27.0 (3.2)	0.57
risk factors			
myocardial infarction, n (%)	37 (50)	43 (46)	0.64
heart failure, n (%)	17 (23)	17 (18)	0.29
hypertension, n (%)	46 (62)	54 (58)	0.64
diabetes, n (%)	25 (34)	35 (38)	0.89
dyslipidemia, n (%)	33 (79)	40 (91)	0.14
renal failure, n (%)	1 (2)	1 (2)	1.00
stroke, n (%)	4 (10)	3 (7)	0.71
STEMI/NSTEMI, n (%)	22 (30)	15 (16)	0.04
family history, n (%)	17 (40)	41 (32)	0.50
medications			
acetylsalicylic acid, n (%)	52 (70)	56 (67)	0.73
clopidogrel, n (%)	26 (35)	31 (37)	0.87
statin, n (%)	52 (70)	59 (70)	1.00
laboratory tests			
TC, mmol/l	5.08 (1.22)	5.10 (1.41)	0.89
LDL-C, mmol/l	3.00 (1.18)	2.93 (1.15)	0.73
HDL-C, mmol/l	1.17 (0.40)	1.30 (0.43)	0.12
triglycerides, mmol/l	1.34 (0.65)	1.38 (0.59)	0.76
fibrinogen, g/l	3.95 (1.53)	3.72 (1.48)	0.63
hsCRP, mg/l	2.90 (2.59)	2.80 (2.65)	0.39
glucose, mmol/l	5.50 (0.89)	5.40 (1.18)	0.31
creatinine, µmol/l	80.0 (21.1)	75 (21.8)	0.10
platelets, × 1000/µl	229 (72)	240 (89)	0.22
cotinine, ng/ml	15.7 (57.1)	0 (0)	<0.0001
ADMA, µmol/l	0.60 (0.14)	0.485 (0.115)	<0.0001
TM, ng/ml	3.96 (2.84)	2.77 (2.07)	<0.0001
PAI-1, ng/ml	30.0 (10.8)	20.1 (6.5)	<0.0001

Data shown as median (interquartile range) or number (%) as appropriate.

Abbreviations: ADMA – asymmetric dimethylarginine, BMI – body mass index, HDL-C – high-density lipoprotein cholesterol, hsCRP – high-sensitivity C-reactive protein, LDL-C – low-density lipoprotein cholesterol, NSTEMI – non-ST-segment elevation myocardial infarction, STEMI – PAI-1 – plasminogen activator inhibitor-1, ST-elevation myocardial infarction, TC – total cholesterol, TM – thrombomodulin

individual parameters were also computed along with their significance tests.

A linear multiple regression analysis was performed for both groups in addition to the combined data to identify factors that affect ADMA, TM, and PAI-1. To that end, a forward selection method was applied using a *P* value of less than 0.5 as the inclusion criterion. For the full model, the square of the multiple correlation coefficients was calculated, while for the model covariates the regression coefficients and squared partial correlation coefficients (using type II sum of squares) were computed. A *P* value of less than 0.05 was considered significant.

RESULTS The study population comprised 181 patients with CAD, including 53 women and 128 men. There were 102 current smokers (56.4%). As shown in **TABLE 1**, current smokers and nonsmokers did not differ with regard to the majority of the variables. Only creatinine levels were slightly higher in smokers.

Cotinine levels were higher in the group of current smokers. Eight patients (7.8%) in the group of self-declared nonsmokers had cotinine levels above 12 ng/ml, suggesting active smoking. These patients were regarded as smokers and included in the smoking group.

Plasma ADMA levels were 18% lower in nonsmokers compared with current smokers ($P < 0.0001$). Similarly, plasma PAI-1 and TM levels were lower in nonsmokers ($P < 0.0001$). Patients, without measurable serum cotinine had lower concentrations of ADMA, TM, and PAI-1 ($P < 0.0001$) than the remaining patients. The highest levels of these parameters were observed in

TABLE 2 Multiple linear regression models for all patients, smokers, and nonsmokers (classified on the basis of the questionnaire)

Parameter		Regression coefficient (95% confidence interval)	Multiple R^2 / partial R^2	*P* value
all patients (n = 163)[a]	ADMA as dependent variable			
	full model		0.477	<0.0001
	cotinine	0.001 (0.001, 0.002)	0.407	<0.0001
	creatinine	0.002 (0.001, 0.002)	0.099	<0.001
	TM as dependent variable			
	full model		0.183	<0.001
	cotinine	0.015 (0.009, 0.021)	0.133	<0.0001
	PAI-1 as dependent variable			
	full model		0.159	<0.001
	cotinine	0.059 (0.033, 0.084)	0.115	<0.001
nonsmokers (n = 90)	ADMA as dependent variable			
	full model		0.463	<.0001
	age	–0.002 (–0,004, –0.0004)	0.068	<0.05
	creatinine	0.001 (0.001, 0.002)	0.183	<0.05
	TM as dependent variable			
	full model		0.115	<0.05
	BMI	0.020 (–0.272, –0.023)	0.061	<0.01
	PAI-1 as dependent variable			
	full model		0.107	<0.05
	LDL	1.536 (0.145, 2.927)	0.054	<0.05
smokers (n = 73)	ADMA as dependent variable			
	full model		0.458	<0.001
	cotinine	0.0001 (0.0006, 0.001)	0.404	<0.0001
	creatinine	0.002 (0.0006, 0.003)	0.120	0.01
	TM as dependent variable			
	full model		0.3425	<0.01
	cotinine	0.011 (0.004, 0.018)	0.1393	<0.01
	PAI-1 as dependent variable			
	full model		0.282	<0.01
	LDL-C	–3.145 (–5.689, –0.611)	0.088	<0.05
	BMI	–0.543 (–1.060, –0.026)	0.064	<0.05

a includes patients with complete laboratory data

Other parameters were nonsignificant for the model.

Abbreviations: see TABLE 1.

the group with cotinine levels exceeding 12 ng/ml (P <0.05). The highest ADMA levels were observed in patients with cotinine levels exceeding 12 ng/ml (P <0.0001), while there were no differences between patients with undetectable cotinine and those with its levels of less than 12 ng/ml. The lowest TM and PAI-1 levels were observed in the group with undetectable cotinine levels, higher in the group with cotinine levels of less than 12 ng/ml, and the highest in the group with cotinine levels exceeding 12 ng/ml (TABLE 2).

There was no association between endothelial dysfunction, clinical parameters, and treatment of CAD. In the whole patient group, there were significant correlations (as indicated by Spearman correlation coefficient) between cotinine and ADMA (r = 0.71, P <0.0001), TM (r = 0.53, P <0.0001), and PAI (r = 0.58, P <0.0001). The positive correlations between ADMA and TM (r = 0.33, P <0.0001) and ADMA and PAI-1 (r = 0.40, P <0.0001) were also observed. In the group of current smokers (n = 72), similar positive correlations between cotinine and ADMA (r = 0.72, P <0.0001), TM (r = 0.43, P = 0.0002), and PAI-1 (r = 0.37, P = 0.0003) were noted. Apart from the markers of endothelial dysfunction, cotinine concentratiosn correlated with blood platelet count (P = 0.031). PAI-1 was also found to correlate with ADMA (r = 0.35, P = 0.003), TM (r = 0.27, P = 0.023), and low-density lipoprotein cholesterol (r = 0.27, P = 0.022). Of all the variables studied in the regression analysis, correlations were observed between ADMA and cotinine (r = 0.64, P <0.001) and ADMA and creatinine (r =

TABLE 3 Multiple linear regression models based on serum cotinine concentration

		Regression coefficient (95% confidence interval)	Multiple R^2 / partial R^2	*P* value
cotinine concentration, ≤2 ng/ml (n = 80)				
ADMA as dependent variable	full model		0.238	<0.01
	age	–0.002 (–0.004, 0.0004)	0.076	<0.05
	creatinine	0.001 (0.0001, 0.002)	0.067	<0.05
	HDL-C	–0.040 (–0.079, –0.001)	0.056	<0.05
TM as dependent variable	full model		0.204	<0.01
	BMI	–0.116 (–0.214, –0.018)	0.071	<0.01
	platelets	0.005 (0.000003, 0.001)	0.052	<0.05
PAI-1 as dependent variable	full model		0.1219	NS
cotinine concentration, <2 and ≥12 ng/ml (n = 34)				
ADMA as dependent variable	full model		0.4973	<0.01
	cotinine	0.009 (0.002, 0.020)	0.206	<0.05
	triglycerides	0.050 (0.008, 0.093)	0.191	<0.05
	creatinine	0.002 (0.0002, 0.003)	0.168	<0.05
TM as dependent variable	full model		0.134	NS
PAI-1 as dependent variable	full model		0.197	NS
cotinine concentration, >12 ng/ml (n = 50)				
ADMA as dependent variable	full model		0.4983	<0.0001
	creatinine	0.003 (0.001, 0.005)	0.307	<0.0001
	cotinine	0.0005 (0.0003, 0.0008)	0.289	<0.0001
	fibrinogen	–0.02257 (–0.039, –0.006)	0.156	<0.001
TM as dependent variable	full model		0.263	NS
PAI-1 as dependent variable	full model		0.298	<0.05

Abbreviations: NS – nonsignificant, others – see TABLE 1

0.32, $P < 0.001$). A correlation between cotinine and ADMA was also present in the group with cotinine levels exceeding 2 ng/ml ($r = 0.45$, $P < 0.05$) as well as in the group with cotinine exceeding 12 ng/ml ($r = 0.54$, $P < 0.0001$). TM was found to correlate with cotinine in the whole study group ($r = 0.34$, $P < 0.001$) and, separately, in smokers ($r = 0.38$, $P < 0.01$). In the group with cotinine levels of less than 2 ng/ml, TM also correlated with the body mass index ($r = 0.27$, $P < 0.01$). PAI-1 correlated with cotinine levels in all study subjects ($r = 0.34$, $P < 0.001$; TABLE 3).

In the group with cotinine exceeding 12 ng/ml, the incidence of MI was higher than in the other groups (46.91% vs. 56.06%, $P = 0.002$; TABLE 4).

For the whole study group, a multivariate analysis showed that cotinine was an independent predictor of ADMA, TM, and PAI-1 levels.

DISCUSSION Our study demonstrated that current smoking in patients with advanced atherosclerosis is associated with worsening of endothelial damage, which is reflected by elevated ADMA, TM, and PAI-1 concentrations in plasma. To our knowledge, this study is the first to show that despite long-lasting endothelial injury in advanced CAD, continued cigarette smoking is still able to enhance endothelial damage via elevated ADMA associated with increased inhibition of fibrinolysis.

A number of studies have shown that components of tobacco smoke exert adverse effects on the cardiovascular system.[1,3,11] Our study conducted in older patients with severe CAD demonstrates that exposure to tobacco smoke is potent enough to enhance endothelial cell damage despite the presence of several strong cardiovascular risk factors. Furthermore, cotinine has been identified as an independent predictor of ADMA, TM, and PAI-1 levels in advanced CAD. Our findings provide additional evidence for the harmful effects of passive and active smoking in individuals with atherosclerotic vascular disease.

Based on the available data, we used serum cotinine levels to distinguish active and passive smokers from nonsmokers not exposed to tobacco smoke.[6,12] In our study, we used a cut-off value of 12 ng/ml to distinguish between smokers and exposed individuals, and those with no cotinine in serum (levels below 2 ng/ml) were classified as nonsmokers. The presence of cotinine in the group of nonsmokers indicates that the measurement of serum cotinine levels is more reliable than self-reported nonsmoking.

Significant associations between ADMA, TM, and PAI-1 were shown in patients with detectable serum cotinine levels. Of note, regression analysis showed significant correlations between

TABLE 4 Comparison of clinical and biochemical parameters based on serum cotinine levels

Parameter	Cotinine levels			*P* value		
	<LOD (n = 81)	≤12 ng/ml (n = 34)	>12 ng/ml (n = 52)	<LOD – >12	<LOD – <12	<12 – >12
age, y	60 (12)	61.5 (12)	62 (12)	0.43	0.61	0.88
male/female, n	54/67	23/68	38/73	0.56	1.00	0.64
BMI (kg/m²)	27 (4)	27.6 (5)	26 (4)	0.16	0.45	0.08
diseases, n (%)						
myocardial infarction	37 (46)	15 (44)	28 (54)	0.47	1.00	0.51
heart failure	17 (21)	4 (12)	13 (25)	0.67	0.42	0.26
hypertension	49(60)	18 (53)	33 (63)	0.85	0.54	0.38
diabetes	28 (35)	13 (38)	19 (37)	0.85	0.83	1.00
dyslipidemia	35 (95)	11 (85)	39 (75)	0.04	0.28	0.71
renal failure	0	0	2 (6)	0.24	1.00	1.00
stroke	3 (8)	0	4 (11)	0.71	0.56	0.56
STEMI/NSTEMI	12 (15)	4 (12)	21 (40)	0.003	0.77	0.007
family history	9 (24)	4 (31)	18 (50)	0.03	0.73	0.33
medications, n (%)						
aspirin	49 (67)	20 (61)	39 (75)	0.43	0.66	0.23
clopidogrel	24 (33)	15 (45)	18 (35)	1.00	0.28	0.36
statins	53 (73)	19 (58)	39 (75)	0.83	0.18	0.15
laboratory tests						
TC, mmol/l	5.09 (1.23)	5.20 (0.93)	5.14 (1.96)	0.68	0.86	0.61
LDL-C, mmol/l	3.02 (1.09)	2.85 (1.18)	3.13 (1.39)	0.68	0.71	0.49
HDL-C, mmol/l	1.26 (0.46)	1.36 (0.42)	1.15 (0.37)	0.12	0.13	0.005
triglycerides, mmol/l	1.37 (0.60)	1.36 (0.57)	1.36 (0.72)	0.36	0.47	0.92
fibrinogen, g/l	3.83 (1.64)	3.57 (1.79)	4.04 (1.23)	0.23	0.72	0.21
hsCRP, mg/dl	2.92 (2.60)	2.65 (2.29)	2.70 (3.32)	0.84	0.94	0.93
glucose, mmol/l	5.40 (1.10)	5.40 (1.00)	5.45 (1.10)	0.89	0.98	0.92
creatinine, µmol/l	75.8 (22.2)	79.1 (22.9)	79.0 (20.1)	0.35	0.72	0.69
platelets, × 1000/µl	247 (85)	229 (61)	214.5 (70.5)	0.06	0.05	0.89
ADMA, µmol/l	0.48 (0.10)	0.54 (0.11)	0.66 (0.10)	<0.001	0.011	<0.0001
TM, ng/ml	2.51 (1.62)	3.96 (2.63)	4.71 (3.56)	<0.0001	<0.0001	0.17
PAI-1, ng/ml	18.9 (4.60)	27.8 (11.7)	30.5 (7.8)	<0.0001	<0.0001	0.14

Data are presented as number (percentage) or mean ± standard deviation.
Abbreviations: LOD – limit of detection, others – see **TABLE 1**

ADMA, TM, and PAI-1 and cotinine in all analyzed subgroups.

Elevated levels of endothelial dysfunction markers in patients with advanced atherosclerosis exposed to tobacco smoke indicate progressive endothelial damage associated with smoking. Sustained activation of the endothelium leads to decreased bioavailability of NO by increasing the production of ADMA and the activity of free radicals. Endothelial dysfunction that accompanies atherosclerosis, in addition to the reduced NO availability, is also characterized by an increased synthesis of prothrombotic factors and the impairment of fibrinolytic activity.[15,21-23] The latter observation based on increased circulating PAI-1 levels is particularly important given evidence for the links between impaired fibrinolysis, largely determined by PAI-1 levels, and the risk of MI.[17,19,24,25] Several studies have demonstrated poor fibrinolytic capacity in smokers and significant negative effect of active smoking on fibrin clot properties.[21,26,27] The present study supports the concept that despite the presence of several cardiovascular risk factors known to impair fibrinolysis, including hypertension, diabetes, and previous cardiovascular events,[26,27] current smoking, reflected by elevated cotinine levels, can still increase the release of PAI-1 and, consequently, further attenuate plasmin generation in patients with CAD.

Another interesting finding is the positive association between cotinine and soluble TM in advanced CAD. In the general population, soluble TM largely synthesized by vascular endothelial cells is weakly associated with cardiovascular risk factors including smoking.[28] To our knowledge, this report is the first to have shown this association in advanced CAD, in which endothelial injury is a typical feature.[29] Plasma TM levels are increased in smokers. Given the robust data on the effects of TM on cellular proliferation, adhesion, and inflammation, all of which

are important processes in atherosclerosis,[17,29] elevated TM might be considered a compensatory mechanism that may attenuate proatherogenic effects produced by continued smoking. However, the potential beneficial properties of TM independent from protein C and thrombin mediated effects are most likely insufficient to overcome deleterious actions of toxic cigarette smoke components. This hypothesis merits further investigation.

Our study has several limitations. First, it was based on an observational retrospective analysis, with a limited number of patients. However, this group represents a real-life consecutive cohort of patients with severe CAD. Moreover, we did not perform functional tests to assess endothelial function, which could provide more information on the links between smoking and endothelial phenotype in severe atherosclerosis. Finally, follow-up studies are needed to investigate whether the parameters studied in smokers versus nonsmokers with CAD may predict recurrent cardiovascular events in a high-risk population and whether smoking cessation can restore normal levels of the studied markers.

In conclusion, our study demonstrated that, in patients with severe CAD, exposure to tobacco smoke, reflected by circulating cotinine levels, can enhance endothelial dysfunction via increased circulating ADMA levels. This phenomenon is associated with elevated TM and PAI-1 levels, which indicates novel links between ADMA and coagulation/fibrinolysis proteins. Moreover, our findings provide additional evidence for the importance of smoking cessation in advanced atherosclerosis.

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Narażenie na dym tytoniowy a uszkodzenie śródbłonka u chorych z zaawansowaną miażdżycą naczyń wieńcowych

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SŁOWA KLUCZOWE

choroba niedokrwienna, kotynina, miażdżyca, palenie tytoniu, uszkodzenie śródbłonka

STRESZCZENIE

WPROWADZENIE Narażenie na dym tytoniowy zwiększa ryzyko sercowo-naczyniowe, zwłaszcza u pacjentów z chorobą wieńcową (*coronary artery disease* – CAD).

CELE Celem badania była ocena wpływu czynnego i biernego palenia tytoniu na aktywność markerów śródbłonka w zaawansowanej miażdżycy.

PACJENCI I METODY Badano 181 pacjentów z zaawansowaną CAD (53 kobiety i 128 mężczyzn) w wieku 60 ±8 lat, w tym 102 osoby (56,3%) deklarujące czynne palenie. W badaniu oznaczano asymetryczną dimetyloargininę (ADMA), trombomodulinę (TM) i inhibitor aktywatora plazminogenu (PAI-1), oraz stężenie kotyniny, jako markera palenia tytoniu.

WYNIKI Stężenie ADMA w osoczu było większe w grupie aktywnie palących niż w grupie niepalących (0,60 ±0,09 *vs* 0,49 ±0,08 µmol/l; p <0,001). Podobne różnice stwierdzono między obiema grupami w zakresie stężeń TM (4,60 ± 2,11 *vs* 3,0 ±1,7 ng/ml; p <0,0001) i PAI-1 (30,3 ±12,4 *vs* 23,6 ±11,3 ng/ml; p <0,0001). Obserwowano dodatnią korelację między stężeniem kotyniny i ADMA (r = 0,71; p <0,0001), TM (r = 0,53; p <0,0001) i PAI-1 (r = 0,58; p <0,0001). U 21 pacjentów deklarujących niepalenie, stężenie kotyniny (6,30 ±22,5 ng/ml) korelowało z wartościami ADMA, TM i PAI-1 (p <0,001). Analiza regresji wykazała, że kotynina jest niezależnym predyktorem ADMA, TM i PAI-1 w całej badanej grupie.

WNIOSKI Pomimo długotrwałego uszkodzenia śródbłonka w zaawansowanej CAD kontynuowanie palenia tytoniu może jeszcze bardziej zwiększyć jego uszkodzenie przez zwiększenie ADMA i związane z tym hamowanie fibrynolizy.

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