Association between Plasma Levels of Plasminogen Activator Inhibitor-1 and Colorectal Neoplasms

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Background/Aims: Plasminogen activator inhibitor-1 (PAI-1) is important for tumor growth, Invasion, and metastasis. In this study, we investigated the relationship between plasma levels of PAI-1 and colorectal adenomas. **Methods:** We reviewed the medical records of 3,136 subjects who underwent colonoscopy as a screening exam. The subjects were classified into a case group with adenomas (n=990) and a control group (n=2,146). Plasma PAI-1 levels were categorized into three groups based on tertile. **Results:** The plasma levels of PAI-1 were significantly higher in adenoma cases than in controls (p=0.023). The prevalence of colorectal adenomas increased significantly with increasing levels of PAI-1 (p=0.038). In the adenoma group, advanced pathologic features, size, and number of adenomas did not differ among the three groups based on tertiles for plasma PAI-1 levels. Using multivariate analysis, we found that plasma level of PAI-1 was not associated with the risk of colorectal adenomas (p=0.675). Adjusted odds ratios for colorectal adenomas according to increasing plasma levels of PAI-1 were 0.980 (95% confidence interval [CI], 0.768 to 1.251) for the second-highest plasma level and 1.091 (95% CI, 0.898 to 1.326) for the highest level, compared with the lowest levels. **Conclusions:** These results suggest that elevated plasma PAI-1 levels are not associated with the risk of colorectal neoplasms. **(Gut Liver 2013;7:519-523)**

Key Words: Plasminogen activator inhibitor 1; Colorectal neoplasms

INTRODUCTION

Colon cancer is one of the most common solid cancers in developed countries. Most colorectal cancers arise from pre-existing benign adenomatous polyps, and early detection and removal of adenomatous polyps could prevent the development of colorectal cancer. Therefore, to find molecular markers that could be used to better identify patients with a higher risk for adenomatous polyps would be valuable.

The fibrinolytic system, more appropriately referred to as the plasminogen activator system (PAS), controls not only the intravascular fibrin deposition, but also participates in a wide variety of physiologic and pathologic processes in cancer.[1] Plasminogen activator inhibitor-1 (PAI-1) is a factor in the urokinase-type plasminogen activator proteolytic system, which is important for tumor growth, invasion, and metastasis. In one experiment using PAI-1-deficient mice, PAI-1 deficiency abolished cancer invasion and angiogenesis.[2] Some studies showed that PAI-1 was significantly upregulated in the neoplastic tissue of the human colon.[3,4] Also, *in vivo* studies have demonstrated a strong positive correlation between plasma levels of very low density lipoprotein, which is a triglyceride-rich protein, and plasma PAI-1 activity levels.[5,6] Previous studies have implicated that metabolic syndrome, including dyslipidemia, is an independent risk factor for colorectal adenoma.[7,8] These results suggest that PAI-1 levels may be correlated with the development of colorectal adenoma.

However, previous studies have only shown the correlation between PAI-1 and colorectal cancer.[4,9] Some studies on colorectal adenomas were based on tissue extracts.[10,11] According to these studies, before the tissues were taken by colonoscopic biopsy or surgery, it was difficult to predict the presence of colorectal adenomas. Therefore, in this study, we analyzed the plasma levels of PAI-1 according to the presence of adenomas in colonoscopy and investigated the relationship between

plasma levels of PAI-1 and the presence of colorectal adenomas.

MATERIALS AND METHODS

1. Patients

We retrospectively evaluated all asymptomatic adults who underwent screening colonoscopic examinations and who had blood samples taken to test for levels of PAI-1 at the Center for Health Promotion of Samsung Medical Center from January 2006 to September 2008.

We excluded patients with a history of cancer, including colorectal cancer, polyposis, and prior resection of any part of the colorectum and inflammatory bowel disease. Patients unable to undergo complete colonoscopy were also excluded. A total of 3,136 subjects were enrolled. The subjects were classified into the case group with colorectal adenomas (n=990) or the control group (n=2,146). Information about medical history, nonsteroidal anti-inflammatory drug or aspirin use, smoking, and alcohol consumption was collected from a standardized questionnaire. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared. Advanced adenomas were defined as tubular adenomas with high-grade dysplasia, large size ($\geq$10 mm), or villous features.

2. PAI-1 assay

Peripheral blood samples were collected at the time of colonoscopy. Venous blood was collected in 0.109 M trisodium citrate anticoagulant in a nonwettable tube and was then centrifuged for 15 minutes at 3,000 g and a temperature of about 4°C. PAI-1 (CTAD plasma) was analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Diagnostica Stago, Asnieres, France). The intra-assay and interassay coefficients of variation were 5.48% to 6.53% and 6.52% to 8.69%, respectively, with a lower detection limit of 4 ng/mL.

3. Statistical analysis

Plasma PAI-1 levels were categorized into three groups according to tertile (<25 percentile, 25 to 75 percentile, and $\geq$75 percentile) to evaluate the differences in adenoma characteristics relative to plasma levels of PAI-1 in the adenoma groups. Logistic regression was used to evaluate associations between colorectal adenoma and plasma levels of PAI-1. Statistical analysis was performed using PASW Statistics 17.0 (SPSS Inc., Chicago, IL, USA). Two-sided p-values <0.05 were considered statistically significant.

RESULTS

The median ages for the case and control group were 51 years (range, 34 to 78 years) and 50 years (range, 23 to 77 years), respectively. As shown in Table 1, compared with the control group, the case group had more males, smokers, and alcohol consumers (p<0.05). A higher BMI was observed in the case group, as compared with the control group. As for the laboratory tests, the case group had a higher triglyceride concentration and fasting glucose level, and lower high density lipoprotein cholesterol level, as compared with the control group (p<0.05). The plasma levels of PAI-1 were significantly higher in the case group (mean, 32.96$\pm$20.15 ng/mL), as compared to the control group (mean, 31.22$\pm$19.90 ng/mL; p=0.023).

When Plasma PAI-1 levels were divided by tertiles, the case group ranged from 22.0% in the first tertile, to 51.4% in the second tertile, and 26.6% in the third tertile. The control group ranged from 26.2% in the first tertile, to 49.4% in the second tertile, and 24.4% in the third tertile. The case group had a higher proportion of patients in the second and third tertiles for levels of PAI-1, as compared to the proportions of patients in the control group for each tertile (p=0.038) (Table 2). In the case group, advanced pathologic features, size, and number of adenomas did not differ among the plasma level tertiles (p=0.222, p=0.116, and p=0.525, respectively) (Table 3). Using multiple logistic regression, we found that plasma levels of PAI-1 were not associated with risk of colorectal adenomas (odds ratio [OR], 0.999; 95% confidence interval [CI], 0.995 to 1.003; p=0.675). The adjusted OR for colorectal adenomas according to increasing plasma levels of PAI-1 were 0.980 (95% CI, 0.768 to 1.251) for the second-highest plasma levels and 1.091 (95% CI, 0.898 to 1.326) for the highest level, as compared with the lowest levels (Table 4).

DISCUSSION

In the present study, we investigated the association between plasma levels of PAI-1 and colorectal adenomas. We observed that a high plasma PAI-1 level in univariate analysis was associated with the prevalence of colorectal adenoma. However, in multivariate analysis, elevated plasma PAI-1 levels were not associated with the risk of colorectal neoplasm.

With malignancy, alterations in the fibrinolytic system could lead to a variety of clinical outcomes. Thus, plasminogen activators and their inhibitors play a key role in the proteolytic cascade that is responsible for the breakdown of several components of the extracellular matrix that surrounds tumor cells. This process results in invasion and subsequent metastasis formation.[4] PAI-1 is perhaps the most important component of the PAS in the regulation of both physiologic processes and pathogenesis of many disorders, including cancer.[1,12,13] High tumor expression of PAI-1 and resultant inhibition of fibrinolysis could potentially exacerbate the hypercoagulability associated with malignancy. Furthermore, high PAI-1 levels have been shown to be adverse prognostic markers in several types of cancer, such as breast, esophageal, gastric, ovarian, prostate, renal, and endometrial cancers.[1,14,15]

For colorectal cancer, there have also been reports demon-

Table 1. Baseline Characteristics of Colorectal Adenoma Cases and Controls

Characteristic	Control (n=2,146)	Case (n=990)	p-value
Age, yr			<0.001
Median age (range)	50 (23-77)	51 (34-78)	
23-49	1,046 (48.7)	388 (39.2)	
50-64	1,036 (48.3)	534 (53.9)	
≥65	64 (3.0)	68 (6.9)	
Male sex	1,629 (75.9)	848 (85.7)	<0.001
BMI, kg/m^2			<0.001
Median BMI (range)	24.13 (17.0-36.4)	24.61 (18.2-35.9)	
<25	1,351 (63.0)	550 (55.6)	
25-29.9	751 (35.0)	413 (41.7)	
≥30	44 (2.1)	27 (2.7)	
Smoking			<0.001
None	839 (39.1)	316 (31.9)	
Former	699 (32.6)	311 (31.4)	
Current	608 (28.3)	363 (36.7)	
Alcohol consumption			0.037
None	473 (22.0)	179 (18.1)	
Former	97 (4.5)	44 (4.4)	
Current	1,576 (73.4)	767 (77.5)	
Aspirin or other NSAIDs use	301 (14.0)	133 (13.4)	0.656
Total cholesterol, mg/dL	195.22±32.97	195.99±34.21	0.550
LDL-C, mg/dL	125.34±28.39	125.94±29.77	0.587
HDL-C, mg/dL	55.09±13.42	53.09±13.19	<0.001
Triglyceride, mg/dL	131.83±75.27	148.45±90.38	<0.001
Fasting glucose, mg/dL	93.73±16.80	95.23±18.30	0.025
CRP, ng/dL	131.6±471.46	126.7±255.66	0.808
PAI-1, ng/mL	31.22±19.90	32.96±20.15	0.023

Data are presented as number (%) or mean±SD.
BMI, body mass index; NSAIDs, nonsteroidal anti-inflammatory drugs; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor-1.

Table 2. Associations between Colorectal Adenoma and Plasma Levels of Plasminogen Activator Inhibitor-1

	Control (n=2,146)	Case (n=990)	p-value
PAI-1			0.038
<17.60 (lowest)*	562 (26.2)	218 (22.0)	
17.60-40.69	1,061 (49.4)	509 (51.4)	
≥40.70 (highest)†	523 (24.4)	263 (26.6)	

Data are presented as number (%).
PAI-1, plasminogen activator inhibitor-1.
*Lowest, <25th percentile; †Highest, ≥75th percentile.

strating a correlation between high PAI-1 tumor levels and poor prognosis. Seetoo et al.[16] reported that overexpression of PAI-1 in the tumor tissue was significantly associated with liver metastasis in colorectal cancer. Baker and Leaper[9] showed that, in colorectal cancer, the expression of both matrix metalloproteinase-1 (MMP-1) and PAI-1 was correlated with pathology, i.e., Dukes' stage, differentiation, lymphatic or vascular invasion, and tumor depth. In 308 colorectal cancer patients followed for up to 16 years, patients with PAI-1 –675 5G/5G genotype showed better survival than patients with 4G/4G or 4G/5G genotypes when they had Dukes' stage A or B tumors.[17] Another study of PAI-1 gene polymorphism demonstrated that the 4G/4G genotype was associated with more advanced tumors (Dukes' C&D), whereas the 5G/5G homozygosity was associated with the Dukes' A&B tumors.[18] In addition, colorectal cancer is one of the few cancers for which data on the correlation between plasma levels of PAI-1 and prognosis are available. In a recent study, Langenskiöld et al.[19] found that high plasma and tumor tissue expression of PAI-1 protein were associated with survival in rectal cancer, and PAI-1 levels in plasma correlated with metastatic disease. This finding regarding PAI-1 expression

Table 3. Plasma Levels of Plasminogen Activator Inhibitor-1 according to Variables in Colorectal Adenoma Cases

	PAI-1 (n=990)			p-value
	<17.60 (lowest)*	17.60–40.69	≥40.70 (highest)[†]	
Malignant potential				0.222
Nonadvanced adenoma	201 (22.2)	458 (50.6)	246 (27.2)	
Advanced adenoma[‡]	17 (20.0)	51 (60.0)	17 (20.0)	
Polyp size				
<5/≥5 mm	110/108 (19.6/25.1)	295/214 (52.7/49.8)	155/108 (27.7/25.1)	0.116
<10/≥10 mm	202/16 (22.3/19.0)	458/51 (50.6/60.7)	246/17 (27.2/20.2)	0.192
Polyp no.				0.525
<4	208 (22.2)	485 (51.7)	246 (26.2)	
4–6	8 (17.4)	23 (50.0)	15 (32.6)	
>6	2 (40.0)	1 (20.0)	2 (40.0)	

Data are presented as number (%).
PAI-1, plasminogen activator inhibitor-1.
*Lowest, <25th percentile; [†]Highest, ≥75th percentile; [‡]Advanced adenoma was defined as ≥1 cm in estimated diameter, villous features and/or high-grade dysplasia.

Table 4. Multivariate Analysis of Colorectal Adenoma Risk

	OR	95% CI	p-value
Age, yr	1.052	1.040–1.065	<0.001
Male sex	1.695	1.291–2.225	<0.001
BMI, kg/m^2	1.017	0.983–1.051	0.335
Smoking			0.002
None	1	(Reference)	
Former	0.807	0.644–1.011	0.062
Current	0.702	0.579–0.852	<0.001
Alcohol consumption			0.795
None	1	(Reference)	
Former	1.083	0.850–1.380	0.519
Current	0.976	0.669–1.424	0.901
HDL-C, mg/dL	1.000	0.993–1.007	0.985
Triglyceride, mg/dL	1.002	1.001–1.003	0.001
Fasting glucose, mg/dL	1.000	0.995–1.004	0.938
PAI-1, ng/mL			0.461
<17.60 (lowest)*	1		
17.60–40.69	0.980	0.768–1.251	0.872
≥40.70 (highest)[†]	1.091	0.898–1.326	0.379

OR, odds ratio; CI, confidence interval; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor-1.
*Lowest, <25th percentile; [†]Highest, ≥75th percentile.

in plasma confirms previous results that indicate a prognostic value for PAI-1 in plasma. Nielsen and colleagues[20] showed that a high plasma concentration of PAI-1 was associated with increasing severity of disease, according to Duke's stage, which is an established predictor of poor prognosis in patients with colorectal cancer.

Furthermore, several studies have shown the possibility that PAI-1 up-regulation is an early event associated with adenoma formation. Protiva et al.[11] reported that the antigen level of PAI-1 was higher in adenomas and in distant mucosa of subjects with adenomas than in mucosa from normal subjects. Another study demonstrated that the PAI-1 antigen was 10-fold higher in carcinoma samples, as compared with the corresponding normal mucosa samples, and the adenomatous polyps showed an intermediate mean PAI-1 value that was significantly different from that of normal mucosa and carcinoma.[3] Furthermore, in a study of Min (APC-deficit) mice, administration of PAI-1 inhibitors reduced serum PAI-1 levels and total numbers of intestinal polyps to 70% of the untreated group value.[21]

These studies suggest that PAI-1 up-regulation is an early event associated with adenoma formation, and plasma levels of PAI-1 may be useful for the prediction of colorectal adenomas. Our data demonstrated that high levels of plasma PAI-1 are associated with the prevalence of colorectal adenomas, suggesting that plasma levels of PAI-1 can be used as a predictor of colorectal adenomas. But, unfortunately, we did not find an independent correlation between plasma levels of PAI-1 and colorectal adenomas.

The limitations of the present study include the following. First, the plasma level of PAI-1 has a diurnal variation; levels are higher in the early morning hours, due to the up-regulation of the PAI-1 promoter by circardian genes. However, all of the subjects in this study had their blood taken during the morning. Second, because it was not a prospective study, there was a selection bias. The plasma level of PAI-1 was measured only in subjects who chose a program that included a blood test for plasma PAI-1 and not in all of the subjects who underwent

colonoscopy. This study was performed on asymptomatic adults who underwent a health screening.

In summary, the present study demonstrates that the plasma levels of PAI-1 are higher in adenoma patients than in normal control patients. However, elevated plasma PAI-1 levels are not independently associated with the risk of colorectal adenomas. Therefore, well-controlled prospective studies are needed to clarify the relationship between plasma PAI-1 levels and the risk for colorectal adenoma.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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