SAGE-Hindawi Access to Research
Cardiology Research and Practice
Volume 2011, Article ID 541467, 8 pages
doi:10.4061/2011/541467

Research Article

The Association of the Metabolic Syndrome with PAI-1 and t-PA Levels

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Received 16 September 2010; Revised 12 January 2011; Accepted 9 February 2011

Academic Editor: Ken Ichi Aihara

Background. We used a random sample ($n = 2,495$) from the population-based Prevention of Renal and Vascular End-stage Disease (PREVEND) study population to examine the association of the metabolic syndrome (Met S) with plasminogen activator inhibitor type 1 (PAI-1) and tissue plasminogen activator (t-PA) antigen levels. *Results*. The overall prevalence of the Met S was 18%, was dependent on age and gender, and was positively associated with higher antigen levels of both PAI-1 and t-PA. These significant effects were maintained after adjustment for age, gender, BMI, elevated C-reactive protein, smoking status, urinary albumin excretion, and insulin levels. We found no significant interactions between the Met S and other covariates on PAI-1 and t-PA levels. *Conclusions*. Our study demonstrates that those with the Met S have significantly higher levels of PAI-1 and t-PA antigen, factors known to increase the risk of cardiovascular disease.

1. Introduction

The metabolic syndrome (Met S)—characterized by insulin resistance, abdominal obesity, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, hypertension, and hyperglycemia—is becoming increasingly common. The Met S has been demonstrated to be a risk factor for cardiovascular disease [1] and type 2 diabetes [2]. Reported differences in the prevalence of the Met S among various ethnic groups [3] highlight the need to use population-based studies to examine descriptive epidemiology of the syndrome. Further, limited information is available on its association with plasminogen activator inhibitor type 1 (PAI-1) and tissue-type plasminogen activator (t-PA) antigen levels, both of which are biomarkers of fibrinolysis associated with an increased risk of cardiovascular disease [4–6].

We were also interested in how two more recently identified risk factors for cardiovascular disease—high-sensitivity (hs) C-reactive protein and microalbuminuria—might affect associations observed between the Met S and PAI-1 and t-PA antigen levels. High sensitivity C-reactive protein is a sensitive indicator of low-grade inflammation. C-reactive protein has been demonstrated to be an independent risk factor for cardiovascular disease [7, 8], and it is positively associated with both the Met S [9] and PAI-1 antigen levels [10]. Microalbuminuria, generally defined as a urinary albumin excretion of 30–300 mg per 24 hours [11], has been demonstrated to be an independent risk factor for

cardiovascular disease and mortality [12, 13]. Its appearance in the urine, in addition to renal pathology, indicates early vascular and endothelial damage in the vascular tree in general [14], and it is positively associated with both the Met S [15] and PAI-1 levels [16, 17].

While previous studies have generally reported positive associations between the Met S and PAI-1 and t-PA antigen levels [3, 18], few, if any, large population-based studies have controlled for C-reactive protein levels or albuminuria status or considered possible interactions between the Met S and other key variables on PAI-1 and t-PA antigen levels. The availability of a large population-based sample of subjects with detailed clinical data provided an opportunity to describe the Met S in a Dutch population and to examine the association of the Met S with PAI-1 and t-PA antigen levels, taking into account potential confounders and/or effect modifiers.

2. Materials and Methods

2.1. PREVEND Sample. This study is part of the ongoing Prevention of Renal and Vascular ENd-stage Disease (PREVEND) study, a large prospective study among inhabitants of the city of Groningen, the Netherlands. Details of the study protocol have been described elsewhere [12]. In brief, all inhabitants of the city between the ages of 28 and 75 (85,421 subjects) were asked to send in a morning urine sample and to fill out a short questionnaire on demographics and cardiovascular history. A total of 40,856 (47.8%) responded. From this group, 30,890 subjects had a urinary albumin concentration <10 mg/L and 9,996 subjects had a urinary albumin concentration of ≥10 mg/L in their morning urine sample. After exclusion of subjects with type 1 diabetes mellitus and women who were possibly pregnant, all subjects with a urinary albumin concentration ≥10 mg/L ($n = 7,768$) and a randomly selected control group with a urinary albumin concentration <10 mg/L ($n = 3,395$) were invited for further investigations in an outpatient clinic (total $n = 11,163$). The screening program in the outpatient clinic consisted of two visits. At the first visit, participants completed a self-administered questionnaire regarding demographics, cardiovascular and renal history, and drug use. At the second visit, blood was drawn after an overnight fast for determination of plasma glucose and serum creatinine. Finally, 8592 subjects completed the total screening program and form the baseline PREVEND cohort. From this cohort, we selected a random sample of 2,527 subjects representative of the entire PREVEND cohort. Of these, 32 subjects were missing a sufficient number of components of the Met S such that it could not be determined whether or not they had the Met S. Hence, this analysis is based on the subset of 2,495 subjects in the random sample with complete information regarding the Met S. The PREVEND study was approved by the local medical ethics committee and conducted in accordance with the guidelines of the declaration of Helsinki. All participants who attended the outpatient clinic gave written informed consent.

2.2. Measurements. Stored plasma samples collected at baseline were thawed and assayed by the Haemoprobe laboratory (Groningen, The Netherlands) using ELISA kits from Technoclone GmbH (Vienna, Austria). These assays measured both free and bound PAI-1 and t-PA antigen levels and have been described in detail elsewhere [19].

The metabolic syndrome was the primary independent variable of interest. For the purposes of the described analyses, the Met S was defined using the modified criteria of the National Cholesterol Education Program, Third Adult Treatment Panel [20]. Individuals with 3 or more of the following components were categorized as having the overall syndrome: (1) abdominal obesity (waist measurement >102 cm for men and >88 cm for women); (2) hypertriglyceridemia (>1.69 mmol/L (150 mg/dL)); (3) low HDL cholesterol (≤1.03 mmol/L (40 mg/dL) in men and ≤1.29 mmol/L (50 mg/dL) in women); (4) high blood pressure (≥130 mm Hg systolic and/or ≥85 mm Hg diastolic BP); (5) high fasting glucose (>5.5 mmol/L (100 mg/dL)). Waist and hip measurements were performed by several research nurses and medical students at the outpatient clinic for the PREVEND study. Waist circumference was measured on bare skin at the natural indentation between the 10th rib and iliac crest. Systolic and diastolic BPs were calculated as the average of the last two measurements taken at two clinic visits. After removal of shoes and heavy clothing, weight was measured to the nearest 0.5 kg using a Seca balance scale (Seca Vogel & Halke GmbH & Co, Hamburg, Germany). Height was measured to the nearest 0.5 cm. Blood samples were drawn after an overnight fast. Glucose was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY, USA), an automatic enzymatic method. Triglycerides were measured enzymatically. A commercially available assay system was used to assess HDL cholesterol (Abbott Inc., Abbott Park, IL, USA). High-sensitivity C-reactive protein was determined by nephelometry with a threshold of 0.175 mg/L and intra- and interassay coefficients of less than 4.4% and 5.7%, respectively (BNIN, Dade Behring, Marburg, Germany).

Age was subdivided into categories of <40, 40–60, and >60 years of age. Smoking was defined as a current smoker or cessation of smoking less than a year before the study. BMI was subdivided into categories of <25, 25–30 (overweight), and >30 (obese) kg/m^2. Elevated C-reactive protein was defined as a level >3 mg/L. Urinary albumin excretion (UAE) was measured as the mean of two 24-hour urine collections. Participants were categorized as having normoalbuminuria (<30 mg/24 h), microalbuminuria (30–300 mg/24 h), or macroalbuminuria (>300 mg/24 hr). Insulin levels were subdivided into categories of <8, 8–12, and >12 μU/mL. Diabetes was defined as a fasting plasma glucose level >7.0 mmol/L, a nonfasting plasma glucose level >11.1 mmol/L, or the use of glucose-lowering medication.

2.3. Statistical Analyses. We initially described the prevalence of the Met S by gender and age. Logarithmic transformations were used to normalize the distributions of PAI-1 and t-PA levels. Although reported P values are for comparisons of the log-transformed means, we report medians and interquartile

Table 1: Characteristics of the study population by presence or absence of the metabolic syndrome.

	Metabolic syndrome		P value
	Present ($N = 440$)	Absent ($N = 2055$)	
Age at baseline			<.0001[1]
<40 yrs (%)	8.9	91.1	
40–60 yrs (%)	17.5	82.5	
>60 yrs (%)	26.9	70.1	
Gender			<.0001[1]
Male (%)	20.9	79.1	
Female (%)	14.8	85.2	
BMI			<.0001[1]
<25 kg/m^2 (%)	3.7	96.4	
25–30 kg/m^2 (%)	22.7	77.3	
>30 kg/m^2 (%)	53.1	47.0	
Smoker			.506[1]
Yes (%)	18.4	81.6	
No (%)	17.3	82.7	
C-reactive protein			<.0001[1]
≥3.0 mg/L (%)	33.0	67.0	
<3.0 mg/L (%)	13.4	86.6	
Urinary albumin excretion			<.0001[1]
<30 mg/24 h (%)	15.7	84.3	
30–300 mg/24 h (%)	44.9	55.2	
>300 mg/24 h (%)	80.0	20.0	
Insulin levels			<.0001[1]
<8 μU/mL (%)	5.1	94.9	
8–12 μU/mL (%)	18.6	81.4	
>12 μU/mL (%)	45.3	54.7	
PAI-1 antigen (ng/mL) (median (IQR))	126.7 (81.4, 194.2)	57.3 (35.5, 99.7)	<.0001[2]
t-PA antigen (ng/mL) (median (IQR))	3.9 (2.8, 6.0)	2.9 (2.2, 4.2)	<.0001[2]

BMI: body mass index; PAI-1: plasminogen activator inhibitor type 1; t-PA: tissue-type plasminogen activator; IQR: interquartile range.
[1]Chi-square test, [2]T-test based on log values.

ranges (IQR) for summary statistics since such comparisons are more similar to a comparison of medians than means in the original metric. Univariate linear models were used to examine the individual effects of the Met S, age at baseline, gender, BMI, smoking status, elevated C-reactive protein, albuminuria status, and insulin levels with PAI-1 and t-PA levels. We also used multivariable linear regression models with backwards selection and split-sample validation to explore whether the association of the Met S with PAI-1 and t-PA levels held up after adjusting for the other variables. The full model of interest included all factors as well as all possible two-way interactions between each factor and the Met S. At each step, the interaction term with the highest P value (provided it was >.10) was removed from the model and the model was refit with all remaining terms. This process was continued until either no interaction terms remained or all remaining interactions were significant.

Since it is well known that models obtained using such data-driven methods are prone to increased type 1 errors [21], proper validation of such models is crucial. For this analysis, model validation was performed using the split-sample validation techniques as described in Muller and Fetterman [22]. Essentially, this involves splitting the analysis dataset into a training and hold-out sample. The shrinkage statistic is defined as the difference between the R^2 value in the final model obtained using the training sample and the cross-validity coefficient calculated from the holdout sample. Small values of relative shrinkage imply better generalizability of the final model. All statistical analyses were performed using SAS version 9.1.

3. Results and Discussion

3.1. Description of Met S in the Population. The average age of the study population was 48 years and 53% were women. The overall prevalence of the Met S in the population was 18% and rose with age in both genders. Although the overall prevalence was significantly higher in men than in women (21% versus 15%, $P < .01$), the prevalence was dependent on age and gender. As shown in Figure 1, in subjects less than 40 years, the prevalence was significantly higher in men (13% versus 6%, $P < .01$). In contrast, there was no significant

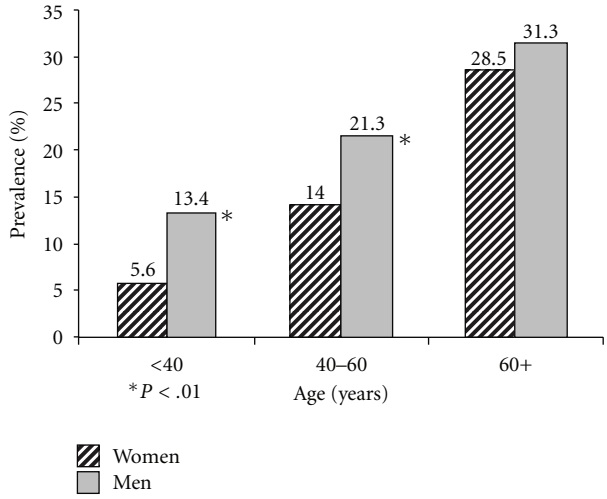

Figure 1: Prevalence of the metabolic syndrome by gender and age.

difference in the prevalence for men and women over 60 years (31% versus 29%, $P = .46$).

Table 1 shows the characteristics of the study population by the presence or absence of the Met S. The Met S was positively associated with all covariates except smoking. We also examined the frequency of type 2 diabetes by the presence or absence of the Met S. The presence of diabetes was highly correlated with the Met S (75% of diabetics had the Met S versus 16% of nondiabetics, $P < .001$).

3.2. Association of Met S and Other Covariates with PAI-1 Antigen Levels. The results from the univariate models indicate that an older age at baseline, male gender, higher BMI, smoking, elevated hs C-reactive protein, elevated UAE, and elevated insulin levels were positively associated with PAI-1 antigen levels (Table 2). In addition, PAI-1 levels were significantly higher among those with the Met S as opposed to those without the Met S (median (IQR): 126 (81, 194) ng/ml versus 59 (36, 103 ng/ml), $P < .001$).

From the backwards selection algorithm using the training sample, there were no significant interactions. Cross-validation produced a negative shrinkage statistic which implies a reasonable fit. The results from the final multivariable model applied to the combined datasets are shown in Table 2. The previously observed associations between PAI-1 levels and both UAE and hsCRP went away after adjustment for the other variables. All other variables, including the Met S, continued to have a significant relationship with PAI-1 levels after adjustment.

3.3. Association of Met S and Other Covariates with t-PA Antigen Levels. The results from the univariate models (Table 3) indicate that increasing age at baseline, male gender, higher BMI, not smoking, elevated C-reactive protein, and elevated insulin levels were positively associated with t-PA levels. Further, median t-PA levels were significantly higher among those with the Met S as opposed to those without the Met S (median (IQR): 3.9 (2.8, 6.0) ng/ml versus 2.9 (2.2, 4.2) ng/ml, $P < .001$).

From the backwards selection algorithm using the training sample, as before, there were no significant interactions. Furthermore, cross-validation produced a near zero shrinkage statistic which implies a reasonable fit. The results from the final multivariable model applied to the combined datasets are shown in Table 3. The previously observed relationships between t-PA levels and BMI, smoking status, and C-reactive protein disappear after adjustment. After adjustment for all other covariates, the Met S, increasing age at baseline, male gender, and elevated insulin levels continued to have a significant effect on t-PA levels.

3.4. Prevalence of Met S. The overall prevalence rate of 18% in our Dutch population and the greater prevalence in men as compared to women are similar to findings reported for other adult European populations. Hu et al. [23] reported an overall prevalence of the Met S of 15% in nondiabetic adult Europeans based on 11 study cohorts. If those with type 2 diabetes are removed from our Dutch population, the prevalence of the Met S is reduced to 16%, similar to that found in the study by Hu and others.

3.5. Association of Met S with PAI-1 and t-PA Antigen Levels. There remained a significant positive association between the Met S and both PAI-1 and t-PA antigen levels after adjustment for the covariates of interest. These associations also remained when participants with type 2 diabetes were excluded from the models (results not shown). Further, after adjustment all other covariates with the exception of UAE continued to have a significant effect on PAI-1 levels, whereas, in addition to the Met S, only age, gender, and insulin levels had significant effects on t-PA levels.

Our findings of an association between the Met S and PAI-1 and t-PA antigen levels are generally consistent with the findings of previously conducted studies [3, 18]. Anand et al. [3] also demonstrated a reduction in clot lysis time after induced ischemia by venous cuff occlusion, further supporting impaired fibrinolytic function in those with the Met S. Our study extends previous findings of an association between the Met S and PAI-1 and t-PA antigen levels by adjusting for a number of covariates and by exploring potential interactions between the Met S and these covariates.

The link between PAI-1 and Met S is complex, and several mechanisms could contribute to the elevated PAI-1 concentrations in Met S patients [24]. One of the proposed mechanisms is the contribution of the rennin-angiotensin system on PAI-1 synthesis. Angiotensin-converting enzyme inhibition has been shown to reduce PAI-1 levels in obese patients [25], and angiotensin II promotes PAI-1 excretion in human adipocytes [26]. In addition, the expression of rennin-angiotensin system genes is regulated differently in obese subjects [27], and a combination of variants in rennin-angiotensin system genes was associated with the Met S. Interestingly, our group previously found that polymorphisms in genes from the rennin, angiotensin, and

Table 2: PAI-1 antigen levels (ng/mL) for the association of the metabolic syndrome and other covariates.

	Median (IQR)	Univariate models	Multivariable model
Age at baseline		<0.001	<0.001
<40 yrs	51 (32, 89)		
40–60 yrs	72 (42, 123)		
>60 yrs	80 (47, 138)		
Gender		<0.001	<0.001
Male	75 (46, 132)		
Female	59 (34, 107)		
BMI		<0.001	<0.001
<25 kg/m^2 (%)	49 (31, 79)		
25–30 kg/m^2 (%)	80 (49, 143)		
>30 kg/m^2 (%)	115 (71, 189)		
Smoking status		<0.001	<0.001
Current/former	70 (43, 126)		
Nonsmokers	64 (37, 114)		
C-reactive protein		<0.001	0.633
≥3.0 mg/L	84 (45, 152)		
<3.0 mg/L	63 (38, 109)		
Urinary albumin excretion		<0.001	0.303
<30 mg/24 h (%)	66 (39, 116)		
30–300 mg/24 h (%)	80 (48, 154)		
>300 mg/24 h (%)	104 (68, 134)		
Insulin levels		<0.001	<0.001
<8 μU/mL (%)	52 (34, 82)		
8–12 μU/mL (%)	77 (44, 128)		
>12 μU/mL (%)	114 (71, 181)		
Metabolic syndrome		<0.001	<0.001
Present	126 (81, 194)		
Absent	59 (36, 103)		

PAI-1 levels were missing in 29 (1.2%) of subjects.

bradykinin system were related to PAI-1 and t-PA levels and this relationship was dependent on body size as assessed by body mass index and waist-to-hip ratio [28, 29]. Further research is needed to further elucidate the underlying mechanisms responsible for the observed relation between PAI-1, t-PA, and the Met S.

The positive associations of PAI-1 with age, male gender, BMI, smoking, and insulin levels as well as the positive associations of t-PA with age, male gender, and insulin levels have been reported elsewhere [10, 30–35]. The lack of association between C-reactive protein with PAI-1 and t-PA levels is interesting and may suggest that the elevation of PAI-1 may be due to production of PAI-1 by adipose tissue and less as a response to changes in inflammatory status. This lack of association has been reported previously [36]. The lack of association of UAE with PAI-1 levels and of smoking and obesity with t-PA levels was unanticipated since associations have been previously reported [16, 17, 31, 37].

3.6. Strengths and Limitations. Strengths of our research include the fact that the sample used for analysis was population-based and therefore more representative of the general adult population than would be other population groups. Further, our sample size was relatively large and we had data available on a large number of variables including hs C-reactive protein and UAE, novel risk factors for cardiovascular disease associated with both the Met S and PAI-1 antigen levels.

There were also several limitations of our study. As our study was cross-sectional, although the associations between the Met S and PAI-1 and t-PA antigen levels remained after control for other covariates, we are unable to evaluate causality. One possibility, consistent with the hypothesis that the increase in cardiovascular disease among individuals with the Met S occurs at least in part through thrombotic mechanisms, is that the Met S causes elevations of PAI-1 and t-PA levels. Alternatively, it has been hypothesized that PAI-1 levels may play a causal role in the development of obesity and the Met S [24]. This is a promising hypothesis as PAI-1 was recently demonstrated to predict the development of type 2 diabetes in the prospective Insulinpagebreak Resistance Atherosclerosis Study (IRAS) [38]. Hence, the choice of PAI-1 and t-PA antigen levels as the endpoints for the study was somewhat arbitrary since, although the

Table 3: t-PA antigen levels (ng/mL) for the association of the metabolic syndrome and other covariates.

	Median (IQR)	Univariate models	Multivariable model
Age at baseline		<0.001	<0.001
<40 yrs	2.7 (1.9, 3.5)		
40–60 yrs	3.1 (2.3, 4.4)		
>60 yrs	3.7 (2.7, 5.7)		
Gender		<0.001	<0.001
Male	3.3 (2.4, 4.9)		
Female	2.8 (2.1, 4.0)		
BMI		<0.001	0.153
<25 kg/m^2 (%)	2.7 (2.1, 3.7)		
25–30 kg/m^2 (%)	3.4 (2.4, 5.1)		
>30 kg/m^2 (%)	3.6 (2.6, 4.9)		
Smoking status		0.042	0.391
Current/former	3.0 (2.2, 4.4)		
Nonsmokers	3.1 (2.3, 4.5)		
C-reactive protein		0.001	0.418
≥3.0 mg/L	3.3 (2.3, 4.9)		
<3.0 mg/L	3.0 (2.2, 4.3)		
Urinary albumin excretion		0.10	0.383
<30 mg/24 h (%)	3.0 (2.2, 4.4)		
30–300 mg/24 h (%)	3.3 (2.4, 5.0)		
>300 mg/24 h (%)	4.6 (4.2, 6.3)		
Insulin levels		<0.001	<0.001
<8 μU/mL (%)	2.8 (2.1, 3.8)		
8–12 μU/mL (%)	3.2 (2.3, 4.7)		
>12 μU/mL (%)	3.6 (2.6, 5.7)		
Metabolic syndrome		<0.001	0.002
Present	3.9 (2.8, 6.0)		
Absent	2.9 (2.2, 4.2)		

t-PA levels were missing in 26 (1.0%) of subjects.

Met S may influence PAI-1 and t-PA levels, PAI-1 has also been hypothesized to causally influence obesity and the Met S. It would also be of interest to know whether the subjects were on statin therapy, receiving other lipid lowering drugs, or receiving ACE inhibitors or ARBs. Unfortunately, the PREVEND database does not include detailed information regarding the use of statins or ARBs. Only a small percentage of subjects were found to be receiving any ACE inhibitor (4.4%) or any lipid lowering medication (5.0%), which suggests that this would have a minimal impact on the overall conclusions of the study. Finally, due to the size of the study and the curse of dimensionality, we did not explore interactions among the covariates of interest nor did we explore interactions higher than second order among the covariates and the Met S. Hence, we may have missed an important higher-order interaction among the variables of interest.

4. Conclusions

In conclusion, the present study demonstrates that those with the Met S have higher levels of PAI-1 and t-PA antigen, factors known to increase the risk of cardiovascular disease. However, prospective studies are needed to establish causality. To demonstrate that the Met S causes increases in PAI-1 and t-PA antigen levels that result in cardiovascular disease requires monitoring changes in these fibrinolytic variables, in addition to the development of clinical events,

in subjects with and without the Met S. Alternatively, to determine if PAI-1 is an underlying cause of the Met S would require examining the associations of baseline levels of PAI-1 with incident cases of the Met S. After causality is established, clinical trials will be needed to investigate whether lifestyle or pharmacologic interventions targeted to improve fibrinolytic function reduce cardiovascular events in subjects with the Met S or, alternatively, are effective in preventing the Met S.

Acknowledgments

This work was supported by National Institutes of Health (NIH) Grant HL65234, HL67466, RR018787, and LM010098 and by Grant E.013 of Netherlands Kidney Foundation. Dr. F. W. Asselbergs is supported by a ZonMW Clinical Fellowship (Grant 90700342). A portion of the work was described in a paper submitted by Q. Li in partial fulfillment of the requirements for the M.S. degree in biostatistics at the University of Alabama at Birmingham. The authors would like to thank the Associate Editor and two anonymous reviewers for helpful comments on an earlier version of the paper. The authors have no conflict of interests to report.

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