Microalbuminuria and markers of the atherosclerotic process: the D.E.S.I.R. study

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Abstract

The relationship between microalbuminuria and tissue-type plasminogen activator antigen (tPA-ag) and fibrinogen was evaluated in non-diabetic subjects. Subjects were participants of the D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance syndrome) Study. Analyses were carried out on 2248 women and 2402 men for fibrinogen and on 272 women and 284 men for tPA-ag. Microalbuminuria was defined as urinary albumin concentration greater than 20 mg/l. Men with microalbuminuria had a 6% higher fibrinogen concentration than those without (3.07 g/l (95% confidence interval: 2.99,3.15) vs. 2.89 g/l (2.87,2.91), adjusted for age and smoking). This relationship existed in hypertensive as well as non-hypertensive subjects. The association between microalbuminuria and tPA-ag existed only in hypertensive men, those with microalbuminuria having a 21% higher tPA-ag than those without (4.39 ng/ml (3.70,5.08) vs. 3.63 ng/ml (3.32,3.94), adjusted for age and smoking). Adjustment for other risk markers for cardiovascular disease did not change the results. There was no relationship between microalbuminuria and these haemostatic factors in women. The results of this study suggest that in non-diabetic men, microalbuminuria is associated with fibrinogen, but with tPA-ag only when concomitant with hypertension. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Microalbuminuria; Tissue-type plasminogen activator antigen (tPA-ag); Fibrinogen; Atherosclerosis; Epidemiology

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1. Introduction

In several prospective studies microalbuminuria was shown to be associated with an increased risk of mortality and cardiovascular events in patients with type 2 diabetes mellitus [1–5]. Classical risk factors for cardiovascular disease, but also haemostatic factors, are related to microalbuminuria in diabetic patients [6,7], and it is suggested that microalbuminuria and atherosclerosis result from the same phenomenon [8]. Biochemical alterations in the extracellular matrix may lead to glomerulosclerosis and premature atherosclerosis [8]. A similar mechanism may lead to microalbuminuria and atherosclerosis in non-diabetic subjects if they are susceptible to alterations in the extracellular matrix [8]. This suggests that in healthy subjects microalbuminuria could be a risk marker for cardiovascular disease and be associated not only with the classical risk factors but also with markers of endothelial cell damage and atherosclerosis. Indeed it has been found that microalbuminuria predicted cardiovascular disease in non-diabetic subjects [9,10]. In other studies a positive association of microalbuminuria with blood pressure, fasting insulin and serum-triglycerides, and a negative one with HDL-cholesterol has been observed [11–13]. Whether microalbuminuria is also associated with markers of endothelial damage and atherosclerosis in healthy subjects is, however, not completely clear. The results of studies which evaluated the association of microalbuminuria with fibrinogen, a marker of atherosclerosis [14], and tissue-type plasminogen activator antigen (tPA-ag, a marker of endothelial cell damage [15]) are contradictory [16–18]. We investigated whether microalbuminuria was associated with fibrinogen and tPA-ag in a relatively large population of non-diabetic men and women.

2. Subjects and methods

2.1. Subjects

The study population consisted of 5214 men and women, aged 30–64 years, who participated in the D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance syndrome) study. D.E.S.I.R. is a 9-year follow-up study which aims to clarify the development of insulin resistance syndrome [19]. Participants were recruited from volunteers insured by the French Social Security system, which offers periodic health examinations free of charge. Subjects came from ten Health Examination Centres in the western central part of France. The study was approved by the CCPPRB (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale) of Bicêtre, Hôpital Bicêtre, by the CNIL (Commission Nationale de l’Informatique et des Libertés) and INSERM (Institut National de la Santé et de la Recherche Médicale) was the promoter. Participants signed a statement of informed consent.

For the present study, subjects with diabetes (fasting glucose = 7.0 mmol/l or on hypoglycemic treatment [20], n = 211), subjects with infection, and subjects with macroalbuminuria or hematuria (n = 370) were excluded. Data on tPA-ag were only available for a subsample of the total study population (the first 556 subjects who entered the study). Analyses were performed using data of 2248 women and 2402 men for fibrinogen and on 272 women and 284 men for tPA-ag.

2.2. Measurements

Weight, height, and waist and hip circumferences were measured by trained personnel, and BMI (kg/m²) and waist-hip ratio were calculated. Smoking status was assessed by means of an auto-questionnaire. Blood pressure was measured after at least 5 min of rest, with subjects in a supine position. Venous blood samples were collected in the morning after subjects had fasted for 12 h. Fibrinogen was measured in EDTA-tripotassium salt plasma with the nephelometric method (BNA Behring, Germany). All the manufacturers’ recommendations were strictly followed and the inter-assay coefficient of variation was 3.3%. The plasma concentration (ng/ml) of tPA was measured by ELISA (Technoclone, ImmunoFrance, SENIA, Orly, France) in EDTA-tripotassium salt plasma that had been stored at −80°C [21]. The intra and inter series coefficients of variation were 8.1 and 12.1%, respectively. Serum-triglycerides, total cholesterol, HDL-cholesterol and glucose concentrations were assayed with a DAX24 (Bayer, Puteaux, France) or with a KONE (Every, France). LDL-cholesterol concentration was estimated using Dahlen’s equation [22]. Fasting insulin was measured by an enzymoimmunoassay (MEIA) with IMX (Abbott, Rungis, France). Urinary albumin concentration (UAC) was measured in a morning urine sample by nephelometry (BNA Behring) and expressed in mg/l. Microalbuminuria was defined as an UAC greater than 20 mg/l [23].

2.3. Data analysis

Spearman correlation coefficients were calculated between UAC, haemostatic factors and other risk markers for cardiovascular disease. Analysis of covariance was used to investigate the association of microalbuminuria with fibrinogen and tPA-ag. Adjustments were made for smoking status, age, BMI, serum-triglycerides, waist-hip ratio, blood pressure and LDL-cholesterol when appropriate. All analyses were carried out for men and women separately.
Table 1
General characteristics of the study population by microalbuminuria (means (S.D.)) (the D.E.S.I.R. Study)

<table>
<thead>
<tr>
<th></th>
<th>Women, albuminuria</th>
<th></th>
<th>Men, albuminuria</th>
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<tbody>
<tr>
<td></td>
<td>Normo</td>
<td>Micro</td>
<td>Normo</td>
<td>Micro</td>
</tr>
<tr>
<td>nI (%)</td>
<td></td>
<td>190 (8)</td>
<td></td>
<td>188 (11)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47 (10)</td>
<td></td>
<td>47 (10)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.9 (4.1)</td>
<td>23.7 (4.5)</td>
<td>25.3 (3.23)</td>
<td>26.1 (3.57)</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.79 (0.07)</td>
<td>0.80 (0.07)</td>
<td>0.92 (0.06)</td>
<td>0.93 (0.07)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127 (15)</td>
<td>130 (16)</td>
<td>134 (14)</td>
<td>141 (18)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 (9)</td>
<td>79 (10)</td>
<td>82 (9)</td>
<td>86 (11)</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>6.08 (3.61)</td>
<td>6.63 (4.60)</td>
<td>6.63 (4.47)</td>
<td>7.33 (4.29)</td>
</tr>
<tr>
<td>Serum-triglycerides (mmol/l)</td>
<td>0.94 (0.52)</td>
<td>1.03 (0.55)</td>
<td>1.31 (0.94)</td>
<td>1.63 (1.26)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.62 (0.98)</td>
<td>5.47 (1.07)</td>
<td>5.80 (0.99)</td>
<td>6.13 (1.16)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>1.79 (0.42)</td>
<td>1.70 (0.40)</td>
<td>1.49 (0.38)</td>
<td>1.50 (0.43)</td>
</tr>
<tr>
<td>Urinary albumin (mg/l)</td>
<td>8.0 (0.5–19)</td>
<td>28.3 (20–200)</td>
<td>8.0 (1.8–19)</td>
<td>30.3 (20–200)</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3.05 (0.64)</td>
<td>3.10 (0.68)</td>
<td>2.89 (0.62)</td>
<td>3.11 (0.74)</td>
</tr>
<tr>
<td>tPA-ag (ng/ml)</td>
<td>2.48 (1.19)</td>
<td>2.50 (1.00)</td>
<td>3.15 (1.14)</td>
<td>3.70 (1.31)</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td></td>
<td>16</td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>

* Median (range).

3. Results

In the total population, 8% of women and 11% of men were classified as having microalbuminuria (Table 1). Urinary albumin was not normally distributed and ranged from 0.5 to 200 in women and from 1.8 to 200 in men. The general characteristics in the subpopulation in which tPA-ag was measured did not differ from those in the total population. The mean age, BMI, systolic and diastolic blood pressure and percentage of smokers were: 47.0 years (S.D.: 10.1) versus 46.8 years (10.0), 24.6 kg/m² (3.7) versus 24.7 kg/m² (3.8), 131 mmHg (17) versus 131 mmHg (16), 80 mmHg (10) versus 80 mmHg (10) and 77 versus 77% for the sub-sample and the total population, respectively.

In women, only fasting insulin, total and HDL-cholesterol, fasting glucose and body mass index were statistically significantly, but weakly correlated with microalbuminuria, while in men all risk markers except HDL-cholesterol and fasting glucose were correlated with microalbuminuria (Table 2). Although in men the correlation coefficients were low, the strongest correlation of 0.24 was observed between microalbuminuria and tPA-ag.

After adjustment for current smoking and age, microalbuminuria was positively associated with tPA-ag in men, but not in women (Fig. 1). Men with microalbuminuria had a 13% higher tPA-ag concentration than those without microalbuminuria. After multiple adjustment for serum-triglycerides, waist-hip ratio and blood pressure, however, the difference in tPA-ag concentration in men with and without microalbuminuria was reduced to 5%, which was no longer statistically significant.

In men, microalbuminuria was also positively associated with fibrinogen, the difference in fibrinogen concentration between men with and without microalbuminuria being 6% after adjustment for age and smoking (Fig. 2). Multiple adjustment for other risk markers of cardiovascular disease did not change these results. No association was observed between microalbuminuria and fibrinogen in women.

Table 2
Spearman correlation coefficients* between urinary albumin concentration, fibrinogen and tissue-type plasminogen activator antigen and other risk markers for cardiovascular disease (the D.E.S.I.R. Study)

<table>
<thead>
<tr>
<th></th>
<th>Urinary albumin concentration (mg/l)</th>
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<tbody>
<tr>
<td></td>
<td>Women (n = 1632)</td>
<td>Men (n = 1745)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-0.046</td>
<td>0.103</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.006</td>
<td>0.095</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.016</td>
<td>0.160</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>-0.014</td>
<td>0.110</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>0.052</td>
<td>0.079</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>-0.059</td>
<td>-0.025</td>
</tr>
<tr>
<td>Serum-triglycerides (mmol/l)</td>
<td>0.035</td>
<td>0.097</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.063</td>
<td>0.066</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>-0.112</td>
<td>-0.033</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>-0.007</td>
<td>0.054</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>-0.023</td>
<td>0.096</td>
</tr>
<tr>
<td>tPA-ag (ng/ml)</td>
<td>0.0004</td>
<td>0.240</td>
</tr>
</tbody>
</table>

* Where coefficient is larger than 0.04 P<0.05.
tPA-ag, however, was not related to microalbuminuria in non-hypertensive men while in hypertensive men the association remained strong (Fig. 4). Multiple adjustment did not change the results and there was no effect modification by hypertension in women.

When analyses were performed separately for the 546 subjects with hypertension (diastolic blood pressure = 95 mmHg and/or systolic blood pressure = 160 mmHg or use of antihypertensive drugs, (there were 39 hypertensive subjects for the subsample where tPA-ag was available)) and those without, the results were different for fibrinogen and tPA-ag. Fibrinogen was still higher in men with microalbuminuria than in those without in both hypertensive and non-hypertensive men (Fig. 3).
4. Discussion

In this study we observed a positive association of microalbuminuria with fibrinogen in non-diabetic men and a strong relation between microalbuminuria and tPA-ag in non-diabetic hypertensive men only. No association between any of these variables was observed in women.

UAC was measured in one morning urine sample. Although it may be preferable to determine UAC in several morning samples because of high variability in UAC, Cowel et al. [24] have shown that the first morning urinary albumin concentration is a good predictor of 24-h urinary albumin excretion.

One study, which evaluated the association of microalbuminuria with tPA-ag in non-diabetic subjects, did not observe a difference in tPA-ag between subjects with microalbuminuria and those with normoalbuminuria, but hypertensive subjects were excluded [16]. Similar results were found among non-hypertensive subjects with type 2 diabetes mellitus [25]. However, in another study, mean tPA-ag was 12% higher in 12 microalbuminuric diabetic patients than in 12 normoalbuminuric patients [26]. From our results and these previous studies it thus seems that in diabetic patients and hypertensive subjects, tPA-ag may be related to microalbuminuria, while this is not the case in healthy subjects. It is suggested that both microalbuminuria and endothelial cell damage, of which tPA-ag is a marker, result from structural changes in the extracellular matrix [8]. One may speculate that changes in glomeruli extracellular matrix occur before changes in the extracellular matrix of large vessel walls. Another possibility is that more severe changes in the extracellular matrix are needed before there is cell damage of the endothelium. In diabetic patients or in hypertensive subjects with microalbuminuria, the process of a changing extracellular matrix has a longer duration and endothelial cell damage will have developed further, leading to higher tPA-ag concentrations. This is in line with studies which show no relation between von Willebrand factor, another marker of endothelial cell damage, and microalbuminuria in non-diabetic healthy subjects, while it was associated with microalbuminuria in diabetic patients or hypertensive subjects [16,27–29].

The association between microalbuminuria and fibrinogen may be explained by one underlying factor, or by the fact that a high concentration of fibrinogen may lead to a higher blood viscosity, more peripheral vascular resistance and thereby to microalbuminuria. A large study among non-diabetic patients showed an inverse relation between albumin excretion rate and fibrinogen in women [17]. Winocour et al. [18], however, showed a 10% higher fibrinogen concentration in healthy subjects with microalbuminuria than in those without. This was not confirmed in a much smaller study [16]. Four studies among diabetic patients showed inconsistent results [26,30–32]. Two studies in treated and untreated hypertensive non-diabetic men revealed higher fibrinogen concentrations in patients with microalbuminuria than in those without [28,33]. Except for the study of Gould et al. [17], none of the above mentioned studies stratified for sex. Gould et al. showed an inverse association between fibrinogen and microalbuminuria in women only, while we showed a positive association in men. We have tried to seek an explanation for the difference between men and women by the fact that our study contained both pre- and postmenopausal women. As many haemostatic factors change after menopause [34], this could have influenced the relation between fibrinogen and microalbuminuria. Adjustment for menopausal status however, did not change the results. The difference between our study and the one from Gould et al. [17] may be explained by a different method used to measure fibrinogen. Several molecular forms of fibrinogen exist in plasma and different methods may relate to different forms [35–37]. The nephelometric test we used to measure fibrinogen was also used to detect the predictive effect of fibrinogen for ischemic heart disease [38]. More studies in clinically healthy subjects are needed with the same method used to measure fibrinogen, to shed light on the sex-difference in the association of microalbuminuria and fibrinogen.

In conclusion, the results of this study suggest that in men, fibrinogen is positively related to microalbuminuria, but to tPA-ag only when concomitant with hypertension. This does not refute the hypothesis that even in clinically healthy men, microalbuminuria and atherosclerosis come from the same phenomenon, but it may indicate that more physiological stress is needed to damage the vessel wall, than to damage the glomeruli extracellular matrix.

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