Microalbuminuria in Diabetic Patients: Relationship to Lipid, Glyco-metabolic, Coagulation and Fibrinolysis Parameters

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Summary: One hundred and sixteen insulin treated diabetic patients were evaluated for the relationship between the presence of microalbuminuria and several lipid, glyco-metabolic, coagulation and fibrinolysis factors. A significant correlation existed only between microalbuminuria and HbA_{1c} ($r = 0.23$, $p = 0.008$) and D-dimer ($r = 0.28$, $p = 0.002$). After the subdivision of the patients in a group without ($n = 85$) and a group with microalbuminuria ($n = 31$) significant differences were found between these two groups for the HDL-cholesterol content ($p < 0.05$), the HbA_{1c} level ($p < 0.01$) and for the D-dimer concentration ($p < 0.01$). Comparison of the patient groups without and with microalbuminuria separately with a healthy volunteers group without albuminuria resulted in significant differences for HDL-cholesterol, triacylglycerols, HbA_{1c}, fructosamine, fibrin monomer and D-dimer, whereas fibrinogen also was significantly different between the diabetic group without microalbuminuria and the healthy volunteers group. Several factors predisposing for atherosclerosis (decrease of HDL-cholesterol, increase of triacylglycerols, coagulation activation with relatively insufficient fibrinolysis) were noticed in both diabetic groups without or with microalbuminuria, but more pronounced in the latter group. The appliance of a Receiver Operating Characteristic (ROC) curve for HbA_{1c} against microalbuminuria (cuf-off level 20 µg/min) reconfirmed the value of adequate glycaemic control in diabetics for the prevention of microalbuminuria. In conclusion the results of this study show a significantly poorer glycaemic control in insulin treated diabetics with microalbuminuria than in those without microalbuminuria. The presence of lower HDL-cholesterol, higher triacylglycerols and the elevation of fibrin monomers and D-dimers is more pronounced in the microalbuminuria group. No direct correlation could however be found between the coagulation and fibrinolysis factors and the extent of microalbuminuria in the diabetic patient groups.

Introduction

The occurrence of microalbuminuria (1) is accepted as an early indicator of microangiopathy in diabetes mellitus i.e. diabetic nephropathy. The onset of the clinical symptoms of diabetic nephropathy may be preceded by a silent period of variable duration during which diabetic patients show only microalbuminuria (2). This stage can be defined as the period in which the mean urinary albumin excretion rate is significantly higher (20—200 µg/min) than that in reference subjects (3). The clinical syndrome of diabetic nephropathy is characterised by persistent proteinuria (4), accompanied by increased morbidity and mortality in diabetic patients (5—11). During the stage of

clinical proteinuria and progressive renal failure, there are changes in the plasma lipoprotein concentration (12–14) which are thought to contribute to the increased cardiovascular risk. Recently, in a case-control study (15), the concentrations of lipoproteins, apolipoproteins, fibrinogen and factor VII were compared in insulin-dependent diabetic patients with (n = 20) and without (n = 20) microalbuminuria under similar glyco-metabolic control (HbA_1).

In this study we have investigated the correlation between the microalbuminuria and the following parameters: several lipid fractions (cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol); parameters for glyco-metabolic control (HbA_{1c} and fructosamine) (16, 17); the routine clotting test fibrinogen; sensitive indicators for clotting activation like fibrin monomers and the thrombin-antithrombin-III complex (18); and the fibrinolysis factors, plasminogen activator inhibitor (19, 20) and D-Dimer, the latter being a sensitive indicator of the onset of reactive fibrinolysis (21). The aim of the study was to investigate the possibility that biochemical parameters other than urinary microalbumin excretion rates possess predictive value for the occurrence of diabetic angiopathy.

Materials and Methods

Methods

The serum samples were analysed with a Cobas Bio centrifugal fast analyser and commercial test kits according to the manufacturer's instructions were used.

— Serum triacylglycerol:

Hoffmann La Roche, Basel, Switzerland, product No. 0710865

— total serum cholesterol:

Boehringer Mannheim, Germany, product no. 237574

— Serum HDL cholesterol:

Boehringer Mannheim, Germany, product No. 543004

— Serum LDL cholesterol:

calculated by applying the *Friedewald* equation

— HbA_{1c} in haemolysate:

Bio Rad ion exchange column chromatography, product No. 192-8001

— Serum fructosamine:

Hoffmann La Roche, Basel, Switzerland, product No. 0711217.
The test was performed according to the method of *Johnson* et al. (17) on a Cobas Bio centrifugal analyser

— Plasma D-Dimer:

For the Elisa D-dimer determinations we used the test kit of Boehringer Mannheim, product No. 998117. The measurements were carried out on the Bio Rad micro-titre reader system. The reactions were performed in polystyrene micro-titre plates of the Nunc Corporation

— Plasma plasminogen activator inhibitor:

Test kit of Kabi Vitrum COA-SET PAI.

— Plasma thrombin-antithrombin III:

Elisa test kit Enzygnost-TAT, Behring, Hoechst, Marburg (Germany), product No. OURC 10/11

— Fibrinogen:

Turbidimetric method of Behring, using the CTS-fibrinogen reagent on the Chromotime System (Behring).

— Fibrin monomers:

chromogenic COA-set FM test Kabi Vitrum Diagnostica.

— Urinary microalbumin:

immunoturbidimetric test of Orion Diagnostica Corporation, product No. 67352

Patients

One hundred and sixteen insulin-treated diabetic patients, consisting of 47 males (mean age 55.3 years, range 20–80) and 69 females (mean age 60.1 years, range 30–82) were included in the study. The percentage of patients with microalbuminuria (i.e. 20–200 μg urinary albumin excreted per min) was 26.7%. Patients with a urinary albumin exceeding 200 μg per min were excluded from the study. The co-medication of the patients included in the study consisted mainly of oral anticoagulants or acetyl-salicylic acid. Patients treated with antihypertensive drugs or corticosteroids were excluded. The number of true type 1 subjects was 62, equally divided between the two albumin excretion groups. The general characteristics of the patients are given in table 1. Retinopathy was diagnosed by fundoscopy and fluorescence angiography. Neuropathy was diagnosed by measurement of the vibration sense and the presence of sensory disturbance in the extremities. Blood samples were collected when the patients attended the hospital (St. Gregorius hospital, Brunssum, The Netherlands) for their routine diabetic control in the department for internal medicine. The reference values were obtained from 50 subjectively healthy individuals, ranging in age from 24 to 50 years. Reference ranges were taken to be the mean values ± 2 SD.

Samples

We collected venous blood samples between 8.30 and 9.00 a. m. and prepared citrated plasma by centrifugation of nine volumes of freshly drawn blood with one volume of trisodium citrate (0.11 mol/l) for 30 min at 25 °C (1600 g). The plasma was used immediately or stored at –70 °C in plastic tubes and thawed with tap water for 5 min before use. For the glyco-metabolic determinations washed red cells were haemolysed and the haemolysate as such was used for the chromatographic separation of HbA_{1c}. Urine samples for urinary albumin determination were obtained from a morning portion after collecting overnight, and the time of collection was recorded.

Statistics

Correlations between the different parameters and urinary microalbumin were investigated by the *Spearman* rank test. The significance of the differences was assessed by the *Mann-Whitney-Wilcoxon* test. The sensitivity and specificity of HbA_{1c} and D-dimer were calculated according to the presence or absence of microalbuminuria as the decision standard (cut-off level 20 μg/min). Receiver Operating Characteristic (ROC) curves for assessing the power of the tests were constructed as described earlier (22).

Tab. 1. General characteristics of the patients according to the albumin excretion rate. Values are means (SD). The number of the patients with retinopathy, and neuropathy are given as percentages.

		Diabetic patients with albumin excretion rate ≤ 20 µg/min; mean 9.7, range 0—20 (n = 85)		Diabetic patients with albumin excretion rate 20—200 µg/min; mean 47.0, range 21—142 (n = 31)	
Age (years)		55.3	(16.1)	60.1	(14.8)
Body mass index	(kg/m²)	24.3	(3.5)	24.1	(4.6)
Diastolic pressure	(mmHg)	83	(6.9)	85	(8.1)
Systolic pressure	(mmHg)	128	(10.4)	128	(15.8)
Duration of diabetes	(years)	14.0	(8.7)	14.5	(9.7)
Serum creatinine	(µmol/l)	82.1	(12.0)	82.1	(15.8)
Retinopathy		15.3%		29.0%	
Neuropathy		7.1%		12.9%	

Results

The *Spearman* rank collection between urinary microalbumin concentrations and several biochemical parameters is given in table 2. Significant correlations with microalbuminuria were found only for HbA₁c and D-dimer, with r values of 0.232 (p = 0.008 and 0.278 (p = 0.002) respectively. In table 3 the diabetic patient groups without ($\leq$ 20 µg/min) and with (> 20 µg/min) albuminuria are compared with a healthy volunteers group without albuminuria. No significant differences for either diabetic group were found for cholesterol, cholesterol/HDL-cholesterol ratio, LDL-cholesterol, thrombin-antithrombin III complex and plasminogen activator inhibitor. Additionally, fibrinogen levels were not significantly altered in the group with a high albumin excretion rate. The other parameters showed highly significant differences between the two patient groups and the volunteer group. Mutual comparison of the two patient groups as given in table 4 showed a significant decrease for HDL-

cholesterol (p < 0.05), and significant increases for HbA₁c (p < 0.01) and D-dimer (p < 0.01).

To assess the predictive power of the HbA₁c in relation to the microalbuminuria, a ROC-curve (fig. 1) was calculated at different cut-off levels of the test against microalbuminuria as the reference parameter at the cut-off decision level 20 µg/min. For the HbA₁c range from 6% to 12% the sensitivity ranged from 38% to 100%, the specificity from 85% to 65%.

Tab. 2. *Spearman* rank correlation between urinary microalbumin and several biochemical parameters in diabetic patients.

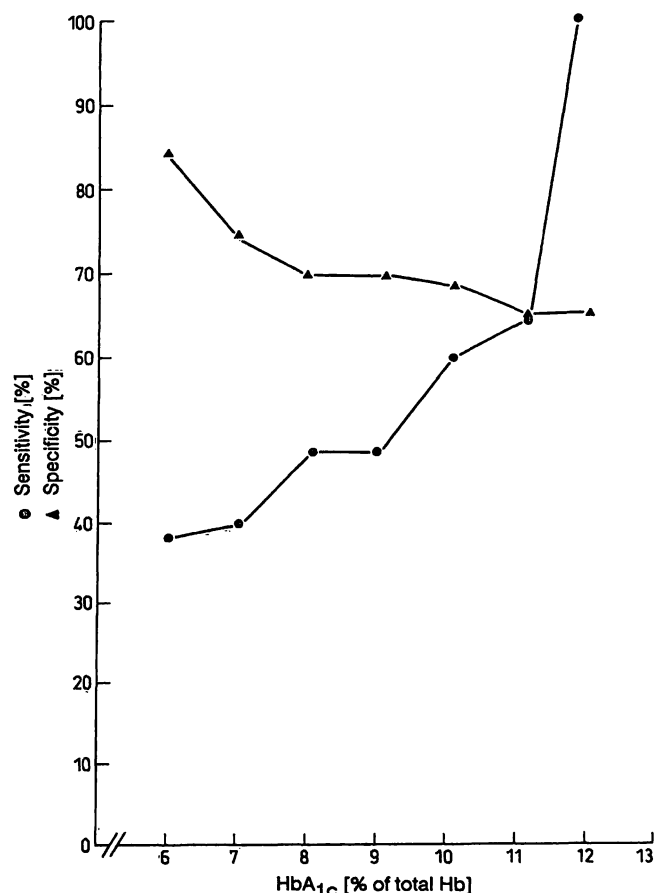| Microalbumin versus parameter	r	p
Cholesterol	0.04	0.65
Cholesterol/HDL-cholesterol ratio	0.01	0.26
HDL-cholesterol	−0.14	0.11
LDL-cholesterol	0.03	0.79
Triacylglycerols	0.13	0.14
HbA₁c	0.23	0.008
Fructosamine	0.07	0.42
Fibrinogen	0.18	0.06
Fibrin monomers	0.13	0.17
Thrombin-antithrombin-III	0.11	0.23
Plasminogen activator inhibitor	0.05	0.60
D-Dimer	0.28	0.002

Fig. 1. Sensitivity (●) and specificity (▲) at different HbA₁c decision levels (reference cut-off parameter : urinary microalbumin 20 µg/min).

Tab. 3. Comparison of the healthy volunteers (group 1) with the two diabetic groups with (group 3) and without (group 2) microalbuminuria (Mann-Whithey-Wilcoxon test).

		Albumin excretion rate							
		≤ 20 µg/min		≤ 20 µg/min		20−200 µg/min		Significance level: p-value	
		Group of healthy volunteers Group 1 (n = 50)		Diabetics Group 2 (n = 85)		Diabetics Group 3 (n = 31)			
Variables		Mean	(SD)	Mean	(SD)	Mean	(SD)	G2/G1	G3/G1
Cholesterol	(mmol/l)	5.8	(0.9)	6.2	(1.5)	6.0	(1.4)	n. s.	n. s.
Cholesterol/HDL-cholesterol	ratio	5.3	(0.9)	5.2	(2.0)	6.0	(2.7)	n. s.	n. s.
HDL-cholesterol	(mmol/l)	1.5	(0.13)	1.27	(0.44)	1.1	(0.3)	<0.001	<0.0001
LDL-cholesterol	(mmol/l)	4.0	(0.5)	3.9	(1.2)	3.9	(1.1)	n. s.	n. s.
Triacylglycerols	(mmol/l)	1.4	(0.3)	2.49	(2.80)	2.24	(1.31)	<0.01	<0.0001
HbA$_{1c}$	(%)	5.5	(0.75)	7.9	(1.6)	8.8	(1.9)	<0.0001	<0.0001
Fructosamine	(mmol/l)	2.9	(0.3)	2.2	(0.6)	3.5	(0.8)	<0.0001	<0.0001
Fibrinogen	(g/l)	3.8	(0.3)	3.4	(0.8)	3.7	(1.2)	< 0.01	n. s.
Fibrin monomer	(nmol/l)	10.8	(1.2)	13.9	(0.4)	13.7	(5.2)	<0.0001	<0.0001
Thrombin-antithrombin-III	(µg/l)	2.6	(0.8)	4.4	(11.8)	5.6	(10.8)	n. s.	n. s.
Plasminogen activator inhibitor	(10³ AU/l)	12.7	(6.3)	14.8	(11.1)	18.3	(11.4)	n. s.	n. s.
D-Dimer	(µg/l)	238	(100)	412	(356)	567	(468)	<0.0001	<0.0001

Tab. 4. Mutual comparison of the diabetic groups without (group 1) and with (group 2) microalbuminuria (Mann-Whitney-Wilcoxon test).

Diabetics		Albumin excretion rate				
		≤ 20 µg/min		20−200 µg/min		
Variable		Group 2		Group 3		Significance level: p-value
		x̄ (SD)		x̄ (SD)		
Cholesterol	(mmol/l)	6.2	(1.5)	6.0	(1.4)	n. s.
Cholesterol/HDL-cholesterol ratio		5.2	(2.0)	6.0	(2.7)	n. s.
HDL-cholesterol	(mmol/l)	1.27	(0.44)	1.1	(0.3)	<0.05
LDL-cholesterol	(mmol/l)	3.9	(1.2)	3.9	(1.1)	n. s.
Triacylglycerols	(mmol/l)	2.49	(2.80)	2.24	(1.31)	n. s.
HbA$_{1c}$	(%)	7.9	(1.6)	8.8	(1.9)	<0.01
Fructosamine	(mmol/l)	3.3	(0.6)	3.5	(0.8)	n. s.
Fibrinogen	(g/l)	3.4	(0.8)	3.7	(1.2)	n. s.
Fibrin monomer	(nmol/l)	13.9	(4.9)	13.7	(5.2)	n. s.
Thrombin-antithrombin III	(µg/l)	4.4	(11.8)	5.6	(10.8)	n. s.
Plasminogen activator inhibitor	(10³ AU/l)	14.8	(11.1)	18.3	(11.4)	n. s.
D-Dimer	(µg/l)	412	(356)	567	(468)	<0.01

Discussion

The general characteristics of the studied diabetic population, as shown in table 1, contain data that need some clarification. The reported mean values for blood pressure and urinary albumin excretion in patients with a mean duration of diabetes mellitus of 14 years indicate, as mentioned in the patients section, that patients with hypertension and "macroalbuminuria" were excluded from the study. Possibly therefore we found no differences in mean systolic and diastolic blood pressure between the diabetic patients with and without microalbuminuria. The correlation between microalbuminuria and several biochemical parameters was shown to be significant only for HbA$_{1c}$ and D-dimer (tab. 2). These results suggest an increased prevalence of microalbuminuria associated with poorer glycaemic control and augmented reactive fibrinolysis (probably after preceding coagulation activation). Recently, we described an increase of coagulation activation with a decreased efficiency of glyco-metabolic control (23), as well as a relative decrease of the elevated reactive fibrinolysis with increasing HbA$_{1c}$ (24) in a large population of insulin-dependent diabetic patients. Thus, the D-dimer level might be an indirect indicator of long term sequelae of diabetes mellitus such as microalbuminuria.

Comparison of the insulin-dependent diabetics with or without microalbuminuria with a group of healthy individuals showed significant alterations in some lipid parameters (HDL-cholesterol, triacylglycerol) and in some coagulation and fibrinolysis parameters (fibrin monomer, D-dimer). Several factors might be responsible for the differences between the three groups, but most probable is the influence of the glycaemic control. In young insulin-dependent diabetics, the glyco-metabolic control state has been shown to influence the lipoprotein serum concentrations (25–27) as well as the prevalence of microalbuminuria, whereas changes in the lipid fractions have been described in diabetics with microalbuminuria and with poor glycaemic control (13).

Recently triacylglycerol has been reported to enhance the activation of coagulation factor VII (28), leading to thrombin formation and hence to fibrin monomer formation and reactive fibrinolysis (D-dimers). Because serum triacylglycerol correlates with plasminogen activator inhibitor, fibrinolysis might be impaired when triacylglycerol is elevated (29). Moreover increased serum triacylglycerol as well as elevated plasminogen activator inhibitor levels have been observed to be strongly related to an enhanced risk of ischaemic heart disease (30–34). The two diabetic groups of the present study differ from the reference group with respect to those parameters known to be correlated

with an enhanced risk of cardiovascular disease. In the mutual comparison of the two diabetic patient groups with and without microalbuminuria (tab. 4) the HDL-cholesterol concentration, a known risk factor for cardiovascular disease, was significantly decreased in the microalbuminuria group. Moreover, there appeared to be an enhanced clotting activation (fibrin monomers) with reactive fibrinolysis (D-dimer) in both diabetic groups, whereas HbA$_{1c}$ was higher in the microalbuminuria group, indicating poorer glycaemic control. In view of the relative decrease of the fibrinolysis level with increasing HbA$_{1c}$ which we reported earlier (24), there might, however, be insufficient reactive fibrinolysis for the extent of the signalized coagulation activation. For the testing of the power of HbA$_{1c}$ as a predictive parameter for microalbuminuria we constructed a ROC curve for HbA$_{1c}$ at different cut-off levels. The ROC curve showed 100% sensitivity for the prediction of microalbuminuria (> 20 µg/min) at a cut-off level of 12% HbA$_{1c}$. Moreover, it should be noted that between 11% and 12% HbA$_{1c}$ there is a jump in the prediction sensitivity from 66.7 to 100%; which might be another indication for setting the goal of glycaemic control at least below the value of 11% HbA$_{1c}$. Nevertheless it seems worthwile from the course of the ROC curve to pursue a normoglycaemia, because in that range a decrease of the sensitivity is achievable from 66.7% (at a HbA$_{1c}$ level of 11%) to 37.8% (at a HbA$_{1c}$ level of 6%). This finding agrees with the results of Klein et al. (35), who demonstrated a strong and consistent relation between hyperglycaemia and the incidence and progression of retinopathy, and who emphasized the importance of normoglycaemia in diabetics.

Conclusion

The results of this study show that in insulin-treated diabetics with microalbuminuria glycaemic control is significantly poorer than in the patients without microalbuminuria. Several abnormalities are significantly more pronounced in the microalbuminuria than in the non-microalbuminuria group; the lipid fractions contain lower HDL-cholesterol and higher triacylglycerol, which are prerequisites for the development of atherosclerosis. Of the coagulation and fibrinolysis factors, the elevation of the fibrin monomer and the D-dimer values should be noted, because they indicate an activation of coagulation, possibly with relatively insufficient reactive fibrinolysis in poor glycaemic control, thereby possibly also constituting risk factors for coronary heart disease. We did not, however, observe a direct correlation between coagulation and fibrinolysis and the existence of microalbuminuria in the diabetic patient groups.

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