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Risk factors for development of proteinuria by type II (non-insulin dependent) diabetic patients

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1. Renal involvement in non-insulin dependent diabetes mellitus patients is the single most important cause of renal failure. The aim of this study was to evaluate the clinical features and to assess the risk factors for the development of proteinuria by non-insulin dependent diabetic patients.

2. Risk factors (expressed as an odds ratio) were calculated by multiple logistic regression analysis taking into account age, sex, body mass index, known duration of diabetes, presence of arterial hypertension, fasting plasma glucose, cholesterol and triglycerides as independent variables and proteinuria as the dependent variable. Sixty-four normoalbuminuric (24-h albumin excretion rate <30 µg/min, 27 females, mean age 53.7 years) and 53 proteinuric (24-h proteinuria >0.5 g, 31 females, mean age 59.3 years) were studied.

3. Proteinuric patients were older, with a longer mean known duration of diabetes (12.4 *vs* 5.6 years), higher mean fasting plasma glucose (214 *vs* 168 mg/dl) and plasma creatinine (1.5 *vs* 1.1 mg/dl) and more frequently presented diabetic retinopathy (94% *vs* 23%), peripheral neuropathy (94% *vs* 23%) and arterial hypertension (73% *vs* 16%) than normoalbuminuric patients. Age >50 years, body mass index >28.6 kg/m², known duration of diabetes >10 years, presence of arterial hypertension, and fasting plasma glucose >160 mg/dl were significantly and independently associated with development of proteinuria.

Key words: diabetes mellitus, proteinuria, renal failure.

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Introduction

Diabetic nephropathy is the single most important cause of renal failure in the western world, accounting for about 28% of new cases of end-stage renal failure in the United States (Eggers, 1988). The contribution of non-insulin dependent diabetic patients (NIDDM) to end-stage renal programs is substantial. Approximately 50 to 90% of diabetic patients enrolled in these programs are NIDDM patients (Rettig and Teutsch, 1984; Grenfell et al., 1988; Humphrey et al., 1989).

The prevalence of proteinuria in NIDDM is extremely variable. A review of several epidemiological studies disclosed figures ranging from 25 to 63% (Pugh, 1989). Considerable evidence implicates poor glycemic control in the pathogenesis of diabetic nephropathy (Larkins and Dunlop, 1992). However, it is not clear why some patients are spared, but probably genetic and environmental factors are involved. The vast majority of the literature regarding clinical diabetic nephropathy refers to insulin-dependent diabetic patients (IDDM). There are few data about NIDDM patients; some of the studies deal with the issue in selected minorities such as the Pima Indians, and others do not analyze some factors such as weight or serum lipids, frequently found to be altered in non-insulin dependent diabetic patients. Although there is no evidence to suggest that the nature of glomerular disease is different in the two types of diabetes, proteinuric NIDDM patients seem to present some peculiarities. We recently reported that the decline in glomerular filtration rate of a cohort of proteinuric NIDDM patients was slower than observed in IDDM patients (Friedman and Gross, 1991). A better knowledge of the clinical features of proteinuric NIDDM patients and of risk factors for the development of clinical nephropathy will permit proper therapeutic management of these patients and the use of intervention measures to prevent this complication.

The aim of the present study was to determine the clinical and laboratory features of proteinuric NIDDM patients and to evaluate the risk factors for the development of clinical nephropathy.

Patients and Methods

Patients

This cross-sectional study was performed on NIDDM patients consecutively attending the outpatient clinic of the Endocrine Unit of Hospital de Clínicas de Porto Alegre. Patients were considered to have NIDDM when 1) diabetes was diagnosed after 30 years of age, 2) there was no record of ketoacidosis, 3) ketonuria was persistently absent, 4) insulin was used because of secondary failure of oral

agents or to improve glycemic control, 5) insulin was introduced after 5 years of disease.

The presence of proteinuria was established when 24-h urinary protein excretion was >0.5 g on at least three separate occasions, 2 to 4 weeks apart, in the absence of urinary tract infection, heart failure or other glomerulopathies. Absence of urinary tract infection was characterized by a negative urine culture. Heart failure was ruled out by clinical examination and chest films. Hemoglobinuria, erythrocyte casts, previous history of nephropathies, and serious systemic hypertension (beginning before the 4th decade and before the diagnosis of diabetes) were considered to be evidence of other glomerulopathies. Normoalbuminuria was defined as urinary albumin excretion rate <30 µg/min. Only normoalbuminuric and proteinuric NIDDM patients were included in this study. The normoalbuminuric patients were treated by diet only (N = 25), sulphonylurea (N = 31) and insulin (N = 8). It is the policy of our service to treat proteinuric patients with insulin only.

Patients were evaluated for the presence of other long-term complications of diabetes. Arterial pressure was measured in the sitting position in the left arm after a 5-min rest with a standard 12.5-cm cuff sphygmomanometer (phases 1-5 of the Korotkoff sounds). Hypertension was defined as an arterial blood pressure >160/95 mmHg in untreated patients, or any value in patients under antihypertensive treatment. Fundus examination was performed after mydriasis and the patients were classified as having no signs of retinopathy or presenting retinopathy. Peripheral neuropathy was considered to be present whenever the vibratory threshold was impaired (tuning fork test), especially if it was associated with a diminished Achilles tendon reflex test and a compatible history. Body mass index (BMI) was calculated as weight (kg)/height^2 (m). On the day of the clinical evaluation, the patients brought in a carefully timed 24-h urine sample for measurement of protein. A fasting blood sample was drawn for glucose, cholesterol, triglycerides, and creatinine measurements. A fresh urine sample was collected for urinalysis and culture.

The study protocol was approved by the Ethics Committee of the Hospital, and all patients gave informed consent to participate.

Methods

Urinary protein was measured by the turbidimetric method of Denis and Ayer (Schriever and Gambino, 1965) and albumin excretion rate in timed 24-h urine by radioimmunoassay (Diagnostic Product Corporation, Los Angeles, CA; interassay coefficient of variation = 2.3%, intra-assay coefficient of variation = 2.8%).

Serum and urinary creatinine was quantified by the method of Jaffé using an autoanalyzer (Chason et al., 1961). Glucose was measured by a glucose-oxidase

method (Trinder, 1969) and cholesterol and triglycerides by enzymatic methods (Burstein et al., 1970; McGowan et al., 1983).

Statistical analysis

The results are reported as means ± SD. Comparison of means between groups was performed by the unpaired *t*-test and the clinical characteristics were compared by the chi-square test. A P value of less than 0.05 was considered to be significant.

The risk factors (expressed as an odds ratio) were calculated by univariate and multiple logistic regression analyses. These analyses were performed using the EGRET software (Epidemiological Graphics Estimation Testing, Statistical and Epidemiological Research Corporation, Seattle, Washington) to assess the association of age, sex, BMI, known duration of diabetes, presence of arterial hypertension, fasting plasma glucose, cholesterol and triglycerides as independent variables and proteinuria as the dependent variable.

Results

Sixty-four normoalbuminuric and 53 proteinuric NIDDM patients fulfilled the inclusion criteria. The clinical and laboratory features of these patients are shown in Tables 1 and 2, respectively. Proteinuric patients were older (unpaired *t*-test, t = -3.94, P<0.0001) and presented a known longer duration of diabetes (unpaired *t*-test, t = -5.56, P<0.0001) than normoalbuminuric patients. There were no differences in BMI (unpaired *t*-test, t = -1.22. P = 0.23) or sex distribution (chi-square test = 0.15) between the two groups. The values of fasting plasma glucose (unpaired *t*-test, t = -3.65, P<0.0001) and plasma creatinine were higher (Mann-Whitney test, 25.5, P<0.0001) in proteinuric than in normoalbuminuric patients. Cholesterol (unpaired *t*-test, t = -1.64, P = 0.1) and triglyceride levels (unpaired *t*-test, t = -1.52, P = 0.2) were not significantly different in the two groups.

Table 1 - *Clinical features of normoalbuminuric and proteinuric NIDDM patients.*

Data are reported as means ± SD. *P<0.0001 compared to normoalbuminuric group (Student *t*-test).

	Normoalbuminuric patients (N = 64)	Proteinuric patients (N = 53)
Age (years)	53.7 ± 7.2	59.3 ± 8.5*
Sex (female/male)	27/37	31/22
Duration of diabetes (years)	5.6 ± 4.7	12.4 ± 5.1*
BMI (kg/m²)	24.7 ± 2.9	25.9 ± 4.8

Diabetic retinopathy and peripheral neuropathy occurred in 94% of proteinuric patients but were diagnosed in only 23% of normoalbuminuric patients (Table 3; chi-square test = 58.1 and 47.14, respectively, P<0.0001). Arterial hypertension was more prevalent in proteinuric than in normoalbuminuric patients (Table 3; chi-square test = 37.67, P<0.0001).

Table 2 - *Fasting plasma glucose, cholesterol, triglycerides and creatinine in normoalbuminuric and proteinuric non-insulin dependent diabetic patients.*

Data are reported as means ± SD. *P<0.0001 (Student *t*-test); +P<0.0001 (Mann-Whitney test).

	Normoalbuminuric patients (N = 64)	Proteinuric patients (N = 53)
Glucose (mg/dl)	168 ± 63	214 ± 74*
Cholesterol (mg/dl)	224 ± 60	246 ± 71
Triglycerides (mg/dl)	175 ± 123	207 ± 185
Creatinine (mg/dl)	1.1 ± 0.2	1.5 ± 0.8+

The results of the univariate analyses are reported in Table 4. Age above 50 years, BMI greater than 28.6 kg/m², known duration of diabetes longer than 10 years, cholesterol levels above 250 mg/dl, fasting plasma glucose levels above 160 mg/dl and the presence of arterial hypertension were significantly associated with proteinuria. Sex and triglyceride levels were not associated with proteinuria. However, when the interdependence of the variables was taken into account in the multiple logistic regression analysis (Table 5), plasma cholesterol levels >250 mg/dl and age ceased to be significantly associated with proteinuria. BMI, known duration of diabetes, fasting plasma glucose and presence of arterial hypertension still remained significantly and independently associated with proteinuria.

Discussion

Since all but 3 of the proteinuric NIDDM patients presented diabetic retinopathy, it is unlikely that patients with non-diabetic glomerulopathy would have been included in this group, particularly after the thorough initial clinical and laboratory investigation.

Table 3 - *Prevalence of diabetic retinopathy, peripheral neuropathy and arterial hypertension in normoalbuminuric and proteinuric non-insulin dependent diabetic patients.*

*P<0.0001 compared to normoalbuminuric patients (chi-square test).

	Normoalbuminuric patients (N = 64)	Proteinuric patients (N = 53)
Diabetic retinopathy	15 (23%)	49 (94%)*
Peripheral neuropathy	15 (23%)	49 (94%)*
Arterial hypertension	10 (16%)	38 (73%)*

Although renal diseases other than diabetic glomerulosclerosis account for proteinuria in 14 to 26% of NIDDM patients (Fabre et al., 1982; Grenfell and Watkins, 1986) recent data indicate that all albuminuric Type 2 diabetic patients with diabetic retinopathy present histopathological evidence of diabetic glomerulosclerosis (Parving et al., 1992). The presence of peripheral neuropathy is another evidence of diabetic nephropathy since we have reported previously that retinopathy, neuropathy and nephropathy usually occur more or less simultaneously (Kruter et al., 1982).

The other characteristics of the proteinuric NIDDM patients were older age, longer known duration of diabetes, poorer metabolic control, and increased prevalence of arterial hypertension.

Table 4 - *Univariate analysis of risk factors for proteinuria in non-insulin dependent diabetes.*

The Wald test was used for statistical analysis.

Variable	Odds ratio	95% CI	P-value
Age (years)			
38-49	1.0		
50-59	2.1	0.7-6.1	
60-76	6.6	2.1-20.8	0.002
BMI (kg/m²)			
16.9-28.5	1.0		
28.6-41.1	3.9	1.4-11.3	<0.001
Duration of diabetes (years)			
0-9	1.0		
10-33	7.3	3.1-17.2	<0.001
Sex			
Male	1.0		
Female	1.8	0.9-3.7	0.12
Arterial hypertension			
No	1.0		
Yes	9.1	3.8-21.8	<0.01
Plasma glucose (mg/dl)			
47-159	1.0		
160-415	4.5	20-10.1	<0.001
Cholesterol (mg/dl)			
105-249	1.0		
250-482	2.8	1.2-6.1	0.01
Triglycerides (mg/dl)			
33-249	1.0		
250-840	1.9	0.8-4.5	0.14

A strong positive association was found between the diagnosis of arterial hypertension and the presence of proteinuria. This has been consistently demonstrated by other investigators (West et al., 1980; Klein et al., 1988; Marshall and Alberti, 1989; Gall et al., 1991). Assessment of a possible causal relationship between arterial hypertension and proteinuria in NIDDM patients is complex.

Increased blood pressure is a consequence of renal disease, but hypertension can precede and cause proteinuria in diabetic patients. This and previous studies are cross-sectional, and therefore they cannot determine which comes first: renal disease or hypertension. However, it was demonstrated that treatment of hypertension reduces protein excretion and the rate of deterioration of glomerular filtration rate in proteinuric IDDM patients (Parving, 1991). Unfortunately, there are no long-term studies on NIDDM patients.

Increased BMI was also strongly associated with proteinuria. This was also described before by Gall et al. (1991). Isolated proteinuria has been described to be more prevalent in obese people (Cohen, 1975).

Another independent and significant risk factor for proteinuria in NIDDM patients was known duration of more than 10 years. This is a well known factor and probably one of the most important determinants of the development of chronic complications in diabetic patients (Pirart, 1978). However, some recent studies have failed to

Table 5 - *Multiple logistic regression analysis of risk factors for proteinuric non-insulin dependent diabetes.*

The odds ratio was adjusted to age, BMI, duration of diabetes, sex, cholesterol, triglycerides and presence of arterial hypertension. Wald was the statistical test used.

Variable	Odds ratio	95% CI	P-value
Age (years)			
38-49	1.0		
50-59	1.8	0.7-8.6	
60-76	4.7	1.5-22.6	0.09
BMI (kg/m²)			
16.9-28.5	1.0		
28.6-41.1	3.0	0.7-12.5	<0.001
Duration of diabetes (years)			
0-9	1.0		
10-33	4.0	1.3-12.5	<0.01
Sex			
Male	1.0		
Female	1.3	0.4-4.0	0.6
Arterial hypertension			
No	1.0		
Yes	8.2	2.7-24.5	<0.001
Cholesterol (mg/dl)			
105-249	1.0		
250-482	1.5	0.5-4.7	0.5
Triglycerides (mg/dl)			
33-249	1.0		
250-840	0.8	0.2-3.1	0.8
Plasma glucose (mg/dl)			
47-159	1.0		
160-415	3.4	1.1-10.3	0.02

identify duration of diabetes as a significant risk factor for proteinuria in NIDDM patients, probably because the actual beginning of the disease is so difficult to establish.

Fasting hyperglycemia was also identified as a risk factor for proteinuria. This association has been reported in other studies (Pirart, 1978; Fabre et al., 1982). One interesting aspect was that only fasting plasma glucose above 160 mg/dl was significantly associated with proteinuria in these patients. There appeared to be a "threshold effect" (fasting plasma glucose >160 mg/dl) above which the development of proteinuria was more likely. It has not yet been determined what degree of glycemic control is necessary to prevent or delay diabetic complications in NIDDM patients. In a retrospective study of IDDM adolescents (Kalk et al., 1990) a threshold effect of glycemic control (mean HbA1c >12.0%) for the development of microalbuminuria was also suggested.

Male gender was not found to be associated with proteinuria. This was also reported by Marshall and Alberti (1989) in a study of 524 NIDDM patients. However, other authors (Klein et al., 1988; Ballard et al., 1988; Gall et al., 1991) found a higher rate of proteinuria in NIDDM men.

Age has been considered to be a major risk factor for diabetic nephropathy by Ballard et al. (1988), but not by other authors (West et al., 1980, 1982). In the present study NIDDM patients aged >60 years were 4.7 times more likely to have proteinuria. Although the confidence interval was greater than 1.0 (1.5-22.6) the statistical significance was marginal (P = 0.09). In fact, aging is associated with an increase in the number of sclerotic glomeruli (Kaplan et al., 1975) and a decrease in glomerular filtration rate (Gross et al., 1992), but little information is available about permeability occurring in the elderly. A reduction in the number of functional glomeruli brought about by aging leads to elevation of pressure in the remaining glomeruli (Zatz and Brenner, 1986). The addition of diabetes and the associated glomerular hyperfiltration (Silveiro et al., 1993) can impair the permselective properties of the membrane and injure the component cells of the glomerulus (Andersen and Brenner, 1986).

In conclusion, NIDDM patients at risk for the development of clinical nephropathy can be identified. The intensification of therapeutic intervention with the objective of reducing BMI, blood pressure, and improving glycemic control could prevent or delay the development of proteinuria in these patients.

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