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Progression of diabetic nephropathy in normotensive type 1 diabetic patients

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Progression of diabetic nephropathy in normotensive type 1 diabetic patients.

Background. The first aim of our long-term study was to describe the natural history of diabetic nephropathy in 59 normotensive type 1 diabetic patients. Secondly, we evaluated genetic and nongenetic progression promoters.

Methods. The following progression promoters were determined: the insertion/deletion polymorphism in the angiotensin converting enzyme (ACE) gene, blood pressure, albuminuria, hemoglobin A_{1c}, cholesterol, smoking, height, and gender. We studied the natural history by measuring ^{51}Cr-EDTA plasma clearance at yearly intervals at least three times during [median (range)] 5.5 (2.2 to 18.3) years.

Results. At baseline the three groups (II, $N = 11$; ID, $N = 25$, and DD, $N = 23$) had comparable GFR (103 ± 16; 99 ± 19; 113 ± 22 ml/min/1.73 m^2, respectively; mean ± SD), arterial blood pressure, albuminuria, and hemoglobin A_{1c}. During the follow-up there was a median rate of decline in GFR in all 59 patients of 1.2 (range 12.9 to –4.4) ml/min/year. During the study period no significant differences were observed in: the rate of decline in glomerular filtration rate [median (range) 0.9 (10.6 to –1.9); 2.5 (12.9 to –4.4); 1.4 (10.8 to –1.9 ml/min/year)], arterial blood pressure, albuminuria, hemoglobin A_{1c} or cholesterol between the three groups (II, ID and DD), respectively. At baseline, multiple linear regression analysis including the above-mentioned putative risk factors revealed that albuminuria, short stature, and male gender independently predict an enhanced decline in GFR [R^2 (adjusted) = 0.33; $P < 0.002$]. During the follow-up period, only albuminuria acted as an independent progression promoter [R^2 (adjusted) = 0.37; $P < 0.0001$].

Conclusions. Our study revealed a rather slow progression of kidney disease in normotensive type 1 diabetic patients with diabetic nephropathy. Albuminuria, short stature, and male gender act as progression promoters in such patients.

Studies of the natural history of diabetic nephropathy have been carried out in small numbers of mainly hypertensive (diastolic BP > 90 mm Hg) patients, revealing a large variation in the decrease in glomerular filtration rate (GFR): 0 to 24 ml/min/year, mean 12 to 15 ml/min/year [1–3]. Several risk factors for loss of filtration power, so-called progression promoters, have been described in the above-mentioned studies and in investigations dealing with type 1 diabetic patients receiving antihypertensive treatment, as reviewed by Rossing [4]. Numerous trials have demonstrated a beneficial effect of effective lowering of blood pressure (BP) on albuminuria, rate of decline in GFR and the prognosis of diabetic nephropathy [5–9]. The progression of renal disease in spontaneously normotensive type 1 diabetic patients suffering from diabetic nephropathy is not known. This question deserves attention, since as many as 25% of type 1 diabetic patients with this complication are normotensive [6, 10].

The aim of our long-term observational follow-up study was to describe the natural history of diabetic nephropathy in normotensive type 1 diabetic patients. Furthermore, we evaluated genetic and nongenetic progression promoters.

METHODS

Patients

We examined the records of all adult Caucasian type 1 diabetic patients with persistent albuminuria (>200 μg/min in 2 out of 3 consecutive determinations carried out within a 6-month period) attending the outpatient clinic at Steno Diabetes Center between 1993 and 1996 who had diabetic nephropathy and who had their GFR measured during the same period. Diabetic nephropathy was diagnosed clinically if there were persistent albuminuria, diabetic retinopathy, diabetes duration of more than 10 years and absence of any clinical or laboratory evidence of other kidney or renal tract disease [11]. Patients fulfillling the following criteria could be enrolled: diabetic nephropathy, normotension, no renoprotective therapies including angiotensin converting enzyme (ACE) inhibitors, at least three GFR measurements during at least two years of observation without antihypertensive treatment. Fifty-nine consecutive patients ful-

Key words: angiotensin converting enzyme gene, glomerular filtration rate, albuminuria, blood pressure, type 1 diabetes.

Table 1. Clinical and laboratory data at baseline in 59 normotensive type 1 patients with diabetic nephropathy according to insertion(I)/ deletion (D) polymorphism in the ACE-gene (ACE/ID genotypes)

	II genotype	ID genotype	DD genotype	*P* value
Sex *men/women*	7/4	13/12	14/9	NS
Age *years*	34 (11)	35 (10)	29 (10)	NS
Duration of diabetes *years*	19 (7)	20 (9)	20 (9)	NS
N of patients with retinopathy (simplex/proliferative)	8/3	15/10	15/8	NS
Insulin dose *IU/kg/24 hr*	0.6 (0.1)	0.6 (0.2)	0.7 (0.2)	NS
Height *cm*	173.6 (2.6)	169.6 (1.3)	171.7 (1.6)	NS
Body mass index kg/m^2	22.8 (1.4)	23.0 (3.3)	23.5 (2.3)	NS
Smoking *yes/no*	4/7	16/9	17/6	NS
GFR $ml/min/1.73\ m^2$	103 (16)	99 (19)	113 (22)	0.07
Serum creatinine *μmol/liter*[a]	72 (66–78)	79 (73–85)	73 (67–79)	NS
Blood pressure *mm Hg*	134/83 (9/5)	135/83 (13/5)	128/79 (11/6)	0.08
Albuminuria *μg/min*[a]	550 (332–939)	550 (386–783)	397 (315–501)	NS
Hemoglobin A_{1C}%	8.7 (1.5)	8.9 (1.2)	9.0 (1.4)	NS
Serum cholesterol *mmol/liter*	4.9 (0.9)	5.1 (1.2)	5.5 (1.2)	NS

Values are means (SD).
[a] Geometric mean with 95% CI

filled these criteria and all gave fully informed consent. In our clinic, GFR is measured routinely approximately once a year from onset of diabetic nephropathy in all type 1 diabetic patients. Baseline data are based on values from the year of the first GFR measurement (Table 1). Median time from onset of diabetic nephropathy to first GFR determination was one year (range 0.1 to 9 years). Arterial hypertension was diagnosed according to WHO criteria, and antihypertensive medication prescribed if at least three consecutive recordings revealed a systolic BP >160 mm Hg and/or a diastolic BP >95 mm Hg. The experimental design was approved by the local ethical committee.

Methods

Glomerular filtration rate and other physiological investigations were carried out 3 to 24 (median 7) times during 2.2 to 18.3 (5.5) years (NS between ACE/ID groups). The GFR was measured applying a single intravenous injection of ^{51}Cr-EDTA (3.7 MBq) and thereafter following the plasma clearance of the tracer for four hours [12]. The mean day to day coefficient of variation in GFR in individuals is 4% in our laboratory. The urinary albumin concentration was determined by an enzyme immunoassay during the four-hour clearance period and from all 24-hour urine collections made at home (about four times a year) [13]. Arterial blood pressure was measured with a standard clinical mercury sphygmomanometer (cuff 25 cm × 12 cm) on the right arm while the patient was sitting after 10 minutes of rest. Diastolic blood pressure was recorded at the disappearance of the Korotkoff sounds (phase V). From venous blood samples HbA_{1C} concentration (normal range 4.3 to 6.2%) was determined by high performance liquid chromatography (DIAMAT Analyzer; Bio-Rad, Richmond, CA, USA). Serum creatinine concentration was assessed by the kinetic Jaffé method. Serum cholesterol concentrations were measured enzymatically using CHOD-PAP reagens from Boehringer-Mannheim GmbH (Mannheim, Germany). Genotyping was performed as described in detail previously [14] and subjects were classified according to the presence (I) or absence (D) of a 287 base pair insertion in intron 16 of the ACE-gene into II, ID or DD genotypes. Diabetic retinopathy was assessed by fundus photography after pupillary dilation. Patients were on a diabetic diet (45 to 55% carbohydrates, 30 to 35% fat, and 15 to 20% protein) without restriction in sodium or protein intake.

Statistical analysis

Values are given as means (SD), except for urinary albumin concentration and serum creatinine, which are expressed as geometric means with 95% confidence interval (CI) owing to the skewed distribution. Other non-normally distributed variables are given as medians (range). Linear regression analysis was used to assess the rate of decline in GFR from all values obtained without antihypertensive treatment. Calculations of baseline blood pressure, urinary albumin excretion rate and HbA_{1C} are based on all values from the year of the first measurement of GFR. Analysis of the three above-mentioned variables during follow-up are based on all values until the date of the last GFR measurement included. For normally distributed variables, including logarithmically transformed values of albuminuria and serum creatinine, the three groups were compared by a one-way analysis of variance (ANOVA). For non-normally distributed continuous variables the three groups were compared by Kruskal–Wallis test. When the patients were divided in two groups comparisons with an unpaired Students *t*-test and Mann–Whitney test were used depending on sample size and distribution. Two separate multivariate stepwise linear regression analyzes of putative risk factors (at baseline and during the follow-up period) influ-

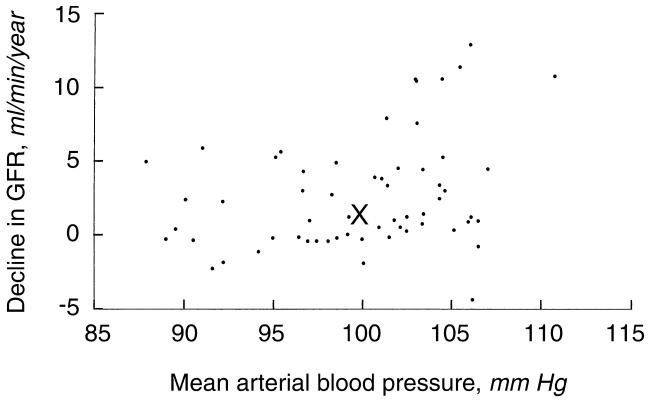

Fig. 1. Rate of decline in glomerular filtration rate (GFR) versus mean arterial blood pressure in 59 normotensive type 1 diabetic patients with diabetic nephropathy. None of the patients received blood pressure lowering treatment. Median is indicated by the (X).

encing the rate of decline in GFR were performed with backward selection. The following putative progression promoters were included in the analyses: ACE/ID genotype, BP, albuminuria, HbA_{1c}, cholesterol, smoking, height and gender. The two latter variables were excluded in the analysis during the follow-up period. ACE/ID genotype was entered as an ordered categorical variable (II = 1, ID = 2, DD = 3) due to the relationship between genotype and serum ACE levels. The R^2 value is adjusted for the number of levels introduced into the model. A *P*-value (two sided) of < 0.05 was considered to be significant. All calculations were performed using SPSS (SPSS, Chicago, IL, USA).

RESULTS

At baseline the patient groups with different genotypes were similar with regard to all recorded variables (Table 1).

During follow-up we demonstrated a rate of decline in GFR in all 59 patients of [median (range)] 1.2 (12.9 to −4.4) ml/min/year (Fig. 1). No significant difference in the rate of decline in the GFR between the groups with different ACE/ID genotypes (II, ID, DD) was observed (ml/min/year): 0.9 (10.6 to –1.9); 2.5 (12.9 to −4.4) and 1.4 (10.8 to −1.9), respectively. The nongenetic progression promoters BP, albuminuria, HbA_{1C} and serum cholesterol concentration were similar in the three groups during the whole follow-up of the study (Table 2).

Thirty-one of the patients (53%) developed hypertension and therefore had their observation period terminated. The time from onset of diabetic nephropathy to antihypertensive medications were prescribed was [median (range)] 7 (3 to 14) years.

The 31 patients who later developed hypertension had a rate of decline in GFR of [median (range)] 2.5 (12.9 to −4.4) ml/min/year while still normotensive, as compared to 1.2 (10.8 to −2.3) in the 28 patients who did not become hypertensive (NS). Baseline and follow-up BP, HbA_{1C} and albuminuria did not differ between the two groups. The five patients with II genotype who remained normotensive had a rate of decline in GFR of 0.2 (2.7 to −1.9) ml/min/year compared to 3.0 (12.9 to −4.4) ml/min/year in the 25 patients with ID and DD who later developed hypertension ($P = 0.16$).

At baseline a multiple linear regression analysis of putative risk factors revealed that albuminuria, short stature and male gender independently predict an accelerated rate of decline in GFR ($R^2 = 0.33$; $P < 0.002$), whereas ACE/ID genotype, BP, HbA_{1C}, serum cholesterol concentration and smoking did not contribute significantly. The only independent progression promoter during the follow-up period was albuminuria [R^2 (adjusted) = 0.37; $P < 0.0001$]. Patients with albuminuria in the lowest tertiles had rate of decline in GFR of only [median (range)] 0.2 (10.6 to –4.4) ml/min/year, whereas the rate of decline was 3.0 (12.9 to –0.8) ml/min/year in those patients with albuminuria in the highest tertiles ($P < 0.01$; Table 3). It should be mentioned that HbA_{1c} was nearly identical in the three groups.

DISCUSSION

Our six-year observational follow-up study of normotensive type 1 diabetic patients with diabetic nephropathy revealed a median loss in GFR of ≈1 ml/min/year, a GFR reduction equal to that observed with the natural aging process. Increased baseline albuminuria, short stature and male gender predicted a steeper rate of decline in GFR, while albuminuria was the only independent progression promoter during the follow-up period.

To obtain a valid determination of the rate of decline in GFR in individual patients with chronic progressive kidney disease, the following requirements should be fulfilled: the applied GFR method should have a good accuracy and precision, repeated GFR determination should be performed, and the observation period should be extended to at least two years [15]. These requirements were fulfilled in our study.

In agreement with previous studies of the natural history of diabetic nephropathy in mainly hypertensive type 1 diabetic patients [1–3, 16], we found a considerable variation in the rate of decline in GFR in our normotensive patient group. In an attempt to explain this variation we investigated some genetic and several nongenetic risk factors, so-called progression promoters.

Recently, we have shown that hypertensive type 1 diabetic patients treated with an ACE inhibitor have a rate of decline in GFR of 5.7 ml/min/year if they are homozygous for the D allele as compared to 2.6 ml/min/year in patients having the ID or II genotype [17]. Similar findings have been reported in type 2 diabetic patients

Table 2. Progression of diabetic nephropathy in 59 normotensive type 1 patients according to ACE/ID genotypes

	II genotype	ID genotype	DD genotype	*P* value
Follow-up *years*[a]	4.2 (2.9 to 12.4)	4.8 (2.2 to 14.1)	7.7 (2.5 to 18.3)	NS
Decrease in GFR *ml/min/year*[a]	0.9 (10.6 to −1.9)	2.5 (12.9 to −4.4)	1.4 (10.8 to −1.9)	NS
Blood pressure *mm Hg*	138/82 (10/4)	136/83 (10/6)	134/81 (10/6)	NS
Albuminuria *μg/min*[b]	786 (438–1410)	574 (423–778)	548 (429–699)	NS
Hemoglobin A_{1C} %	8.9 (1.0)	8.7 (1.1)	9.0 (0.7)	NS
Serum cholesterol *mmol/liter*	5.2 (0.9)	5.5 (1.3)	5.2 (1.0)	NS

Values are means (SD).
[a] Median (range)
[b] Geometric mean with 95% CI

Table 3. Impact of albuminuria on progression of diabetic nephropathy in 59 normotensive type 1 diabetic patients

	Tertiles			
	Lowest	Middle	Highest	*P*
Albuminuria *μg/min*	<420	420–809	>809	
Decrease in GFR *ml/min/year*[a]	0.2 (10.6 to −4.4)	2.8 (7.9 to −1.9)	3.0 (12.9 to −0.8)	<0.01
Hemoglobin A_{1C} %	8.7 (0.9)	9.0 (0.9)	8.7 (1.1)	NS

Values are means (SD).
[a] Median (range)

with overt nephropathy [18], and in various types of nondiabetic kidney disease [19]. However, in the present study dealing with normotensive subjects not receiving BP lowering medication, the ACE/ID polymorphism had no significant impact on the deterioration of kidney function.

The present study is the first to demonstrate that short stature and male gender act as independent progression promoter in normotensive type 1 diabetic patients with diabetic nephropathy. The concept that low birth weight, known to correlate directly with adult height [20], is associated with a reduced number of nephrons and therefore may act as a risk factor for renal disease was originally suggested by Brenner and Chertow [21]. In support of this concept two studies have demonstrated an increased risk for development of diabetic nephropathy in women born with a low birth weight [20] and in men with short stature [22]. In contrast, it should be mentioned that we were not able to show that low birth weight acted as a progression promoter in diabetic nephropathy in a group of hypertensive type 1 diabetic patients receiving antihypertensive treatment (abstract; Jacobsen et al, *J Am Soc Nephrol* 7:1359, 1996). Several studies have demonstrated that male gender plays a significant role in the progression of several nondiabetic renal diseases [23]. The exact mechanism of this relationship is not known, but differences in renal hemodynamic, and dietary factors have been proposed [23].

Albuminuria at baseline and during follow-up was the strongest independent risk factor for loss of filtration power, a finding in agreement with the original demonstration from Watkins et al [24]. Several studies have confirmed that albuminuria plays an important role as progression promoter both in type 1 and in type 2 diabetic patients. Remuzzi and Bertani have suggested that various types of glomerulosclerosis including diabetic are the consequence of altered glomerular permeability to proteins [25].

Numerous studies have demonstrated that elevated BP is an important progression promoter in diabetic nephropathy [2, 9, 16]. However, this impact is reduced and sometimes even completely lacking in patients receiving aggressive antihypertensive treatment leading to normal blood pressure levels [26, 27]. Our study confirms and extends this observation to include normotensive type 1 diabetic patients not receiving blood pressure lowering agents.

In conclusion, our long-term observational study revealed a rather slow progression of kidney disease in normotensive type 1 diabetic patients with diabetic nephropathy. Albuminuria, short stature, and male gender act as progression promoters in such patients.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin converting enzyme; ACE/ID, insertion/deletion polymorphism in the angiotensin converting enzyme-gene; BP, blood pressure; BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; HbA_{1C}, hemoglobin A_{1C}; PCR, polymerase chain reaction; type 1 diabetes, insulin-dependent diabetes mellitus.

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