Diabetologia (1996) 39: 1584–1589

Diabetologia

Glomerular hyperfiltration in microalbuminuric NIDDM patients

P. Vedel[1], **J. Obel**[1], **F.S. Nielsen**[1], **L.E. Bang**[2], **T.L. Svendsen**[2], **O.B. Pedersen**[1], **H.-H. Parving**[1]

[1] Steno Diabetes Center, Gentofte, Denmark
[2] Department of Internal Medicine, County Central Hospital, Næstved, Denmark

Summary Glomerular hyperfiltration and microalbuminuria are both regarded as risk factors for the development of diabetic nephropathy in insulin-dependent diabetic patients. Information on glomerular hyperfiltration is scarse in microalbuminuric non-insulin-dependent diabetic (NIDDM) patients. Therefore, we performed a cross-sectional study of glomerular filtration rate (single i.v. bolus injection of ^{51}Cr-EDTA, plasma clearance for 4 h) in 158 microalbuminuric NIDDM patients compared to 39 normoalbuminuric NIDDM patients and 20 non-diabetic control subjects. The groups were well-matched with regard to sex, age and body mass index. The uncorrected (ml/min) and the adjusted ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) glomerular filtration rate were both clearly elevated in the microalbuminuric patients: 139 ± 29 and 117 ± 24 as compared to 115 ± 19 and 99 ± 15; 111 ± 23 and 98 ± 21 in normoalbuminuric NIDDM patients and control subjects, respectively ($p < 0.001$). The glomerular filtration rate ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) in NIDDM patients who had never received antihypertensive treatment was also clearly elevated in the microalbuminuric patients ($n = 96$): 119 ± 22 as compared to 100 ± 14 and 98 ± 21 in normoalbuminuric NIDDM patients ($n = 27$) and control subjects ($n = 20$), respectively ($p < 0.001$). Glomerular hyperfiltration (elevation above mean glomerular filtration rate plus 2 SD in normoalbuminuric NIDDM patients) was demonstrated in 37 (95 % confidence interval 30–45)% of the microalbuminuric patients. Multiple regression analysis revealed that HbA_{1c}, 24-h urinary sodium excretion, age and known duration of diabetes were correlated with glomerular filtration rate in microalbuminuric NIDDM patients ($r^2 = 0.21$, $p < 0.01$). Our cross-sectional study indicates that NIDDM patients at high risk of developing diabetic nephropathy are also characterized by an additional putative risk factor for progression, glomerular hyperfiltration. [Diabetologia (1996) 39: 1584–1589]

Keywords Glomerular hyperfiltration rate, microalbuminuria, hyperfiltration, NIDDM.

Thirty to forty percent of all insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetic patients develop incipient and/or overt diabetic nephropathy [1–3]. A number of factors have been considered to contribute to the initiation and progression of diabetic nephropathy, including genetic and racial predisposition, glycaemic and other metabolic abnormalities, alterations in systemic and renal haemodynamics and various cytokines and growth factors, as reviewed by Parving et al. [3]. Originally, Brenner's group advocated the concept that glomerular hyperfiltration/hypertension plays an important role in the initiation and development of diabetic glomerulopathy [4, 5]. Glomerular hyperfiltration is frequently present in IDDM patients [6–9] while some but not all studies in normoalbuminuric NIDDM patients have demonstrated elevated glomerular filtration rate (GFR) [10–17]. Originally, Schmitz et al. [18] reported a normal GFR in a small group of microalbuminuric NIDDM patients ($n = 19$).

Corresponding author: Dr. H.-H. Parving, Niels Steensensvej 2, DK-2820 Genofte, Denmark
Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; GFR, glomerular filtration rate; UAE, urinary albumin excretion.

The aim of our cross-sectional study was to measure GFR in NIDDM patients at high risk of developing diabetic nephropathy as compared to normoalbuminuric NIDDM patients and non-diabetic control subjects.

Patients and methods

The cohort for this study was based on all (n = 192) microalbuminuric NIDDM patients under 66 years of age attending the Steno Diabetes Center during 1993. The patients were asked to collect six 24-h urine samples within 4 months and persistent microalbuminuria was confirmed if urinary albumin excretion rate (UAE) was between 30 and 300 mg/24 h in four out of the six samples. Urine collection was carried out during unrestricted daily life. For collection of the urine sample, a 2-liter plastic container together with clear instructions on how to collect a 24-h urine sample were given to each patient at a prior visit to the outpatient clinic. Patients were asked to collect the 24-h urine sample on the last 2 weeks before the clinical investigation. They were instructed to pour a sample from the total urine portion into a 10-ml plastic tube and bring it to the clinic. Statement of timing and the total amount (to the nearest 10 ml) of urine passed was indicated on the tube. If bacterial growth was found, urine collection was repeated after treatment. Thirty-four of these 192 NIDDM patients were excluded from the study for the following reasons: non-attenders (n = 12), glucagon (1 mg i.v.) stimulated C-peptide < 0.6 pmol/l /n = 9), persistent albuminuria (UAE > 300 mg/24 h, n = 2), normoalbuminuria (UAE < 30 mg/24 h, n = 7), chronic cystitis (n = 2), allergy towards chrome (n = 1) and biopsy-proven mesangioproliferative glomerulonephritis (n = 1). Thus, the final number of microalbuminuric NIDDM patients was 158. From a previously described cohort of 55 persistently normoalbuminuric (UAE < 30 mg in three consecutive 24-h urine collections) NIDDM patients [19] all patients (n = 39) less than 66 years of age were selected as a control group. Furthermore, 20 non-diabetic control subjects matched to the NIDDM patients for sex, age and body mass index served as a non-diabetic control group [20]. These patients were recruited from a population study where the patients were randomly selected from the local community register in an age and gender stratified fashion. Individuals with a history of hypertension, diabetes, kidney disease, current illness in the previous 2 weeks, pregnancy or performing night-duty were excluded, as well as persons taking medication influencing blood pressure.

The patients were considered to have NIDDM if they were treated with diet alone, or in combination with oral hypoglycaemic agents, or if they were treated with insulin and had an onset of diabetes after the age of 40 years and a body mass index above normal ($\geq$ 25 kg/m^2 in females, $\geq$ 27 kg/m^2 in males) at the time of diagnosis [21]. All insulin-treated patients had a glucagon test performed, and NIDDM was diagnosed if a stimulated plasma C-peptide value was 0.60 pmol/ml or more [22]. The glucagon/C-peptide test was carried out at 08.00 hours after an overnight fast. Blood samples for plasma C-peptide determination were obtained before and 6 min after an i.v. bolus injection of 1 mg glucagon (Novo Nordisk, Bagsværd, Denmark) as described previously [22]. All microalbuminuric NIDDM patients had a glucagon/C-peptide test performed. All subjects included in the study were Caucasians, and all gave informed consent to participate in the study. The study was approved by the regional ethics committee.

Methods

As part of our previous 24-h blood pressure study [19] the 39 normoalbuminuric NIDDM patients were investigated after having discontinued antihypertensive treatment for 2 weeks (if on treatment, n = 12). All patients were normoalbuminuric before and after stopping this therapy. The microalbuminuric NIDDM patients continued their usual antihypertensive medication (62 out of 158 patients).

The GFR was measured over a 4-h period after a single i.v. injection of 3.7 MBq ^{51}Cr-labelled-EDTA at 08.30 to 09.00 hours by determining the radioactivity in venous blood samples taken from the other arm 180, 200, 220, and 240 min after the injection [23, 24]. The mean day-to-day coefficient of variation (CV) in GFR of each patient was 4 %. The results are given as ml · min^{-1} · m^{-1} height or standardized for 1.73 m^2 body surface area. Patients rested supine during the entire investigation, except when voiding. Patients drank 150–200 ml tap water per hour during the clearance.

Urinary albumin concentration was measured using an enzyme immunoassay and expressed as median of 24-h collections [25]. The 24-h urinary urea excretion was used to calculate protein intake from the nitrogen content of the urea and an estimated value of non-urea nitrogen of 31 mg · kg^{-1} · day^{-1} [26]. Assuming a constant nitrogen balance, nitrogen intake equals the nitrogen content of urinary urea plus non-urea nitrogen; protein intake (in grams per day) equals nitrogen intake times 6.25 [27]. Urine concentrations of sodium were measured using the flame photometric method. HbA_{1c} was measured by high performance liquid chromatography (HPLC) (Bio Rad DIAMAT, Richmond, Calif., USA) (normal range 4.3–6.2 %). Serum creatinine was measured by a reaction rate kinetic technique eliminating pseudo-creatinines [28].

Arterial blood pressure was measured (two or three measurements) with a random zero sphygmomanometer (Hawksley, Lancing West Sussex, UK) and expressed as the mean value. A cuff size 25 × 12 cm in lean and 30 × 15 cm in obese patients was used. Diastolic blood pressure was recorded at the disappearance of the Korotkoff sounds (phase V).

The degree of diabetic retinopathy was scored from fundus photos (Canon CFD-602) through dilated pupils as none, background or proliferative changes. Smokers were defined as subjects smoking more than 1 cigarette per day, all others were classified as non-smokers. Body mass index (BMI) was calculated as weight (kg) divided kg weight (m)^2.

Statistical analysis

All normally distributed values are given as mean ± SD all other values are given as median (range).

In comparison of the non-normally distributed variables the Kruskal-Wallis test of variance was used to test for differences between the three groups. If differences were found, the Mann-Whitney test was used for comparisons between two groups. For all other normally distributed variables analysis of variance (ANOVA) was performed in order to test for differences between the three groups. If differences were found, the Student's t-test was used for comparison between two groups. The chi-square test was used for evaluating frequencies.

When considering possible association to GFR, regression analysis was used. Univariate results were calculated for continuous and class variables. All variables significant at a 10 % level were included in the multiple regression analysis, and stepwise forward selection was used. UAE was logarithmically

Table 1. Clinical data in NIDDM patients with or without microalbuminuria and in control subjects

	NIDDM with microalbuminuria	NIDDM with normoalbuminuria	Control subjects	*p*-value
Sex (male/female)	117/41	31/8	14/6	NS
Age (years)	55 ± 7	58 ± 6	57 ± 8	NS
Known duration of diabetes (years)	6 (0–30)	9 (2–38)		a
Body mass index (kg/m^2)	29.8 ± 4.4	29.1 ± 5.7	27.3 ± 3.8	NS
HbA_{1c} (%)	8.6 ± 1.6	8.1 ± 1.4	5.7 ± 0.4	b, c
Fasting blood glucose (mmol/l)	10.4 ± 2.9	10.3 ± 3.1	4.7 ± 0.5	b, c
Systolic blood pressure (mm Hg)	148 ± 20	140 ± 19	137 ± 25	d, e
Diastolic blood pressure (mm Hg)	85 ± 11	83 ± 9	82 ± 11	NS
Antihypertensive treatment (1/2/3/4 drugs) *(n)*	27/29/5/1	8/3/1/0		b, c
ACE inhibitor/beta blocker/Ca antagonist/diuretic/ other agents *(n)*	29/12/20/39/4	3/4/1/6/3		
Antidiabetic treatment (diet/oral hypoglycaemic agent/insulin) *(n)*	56/13/69	13/19/7		c, d
Retinopathy (%) (nil/simplex/proliferative)	66/29/5	40/53/7		e
Smokers (%)	34	45	49	NS

Mean ± SD or median (range) indicated, [a] $p < 0.001$ NIDDM patients with vs without microalbuminuria, [b] $p < 0.001$ control subjects vs NIDDM patients with microalbuminuria, [c] $p < 0.001$ control subjects vs NIDDM patients with normoalbuminuria, [d] $p < 0.05$ control subjects vs NIDDM patients with microalbuminuria, [e] $p < 0.05$ NIDDM patients with vs without microalbuminuria

transformed before inclusion in the analysis. A *p*-value (two-tailed) less than 0.05 was considered statistically significant. All calculations were made with a commercially available program (Statgraphic; STSC, Rockville, Md., USA).

Results

Table 1 shows pertinent clinical data for the three groups. The groups were well matched with regard to sex, age, and body mass index. The normoalbuminuric NIDDM patients had a longer known duration of diabetes as compared with the microalbuminuric NIDDM patients ($p < 0.001$). Antihypertensive treatment was more prevalent in the two diabetic groups as compared with the non-diabetic control subjects ($p < 0.001$). Diabetic retinopathy was more prevalent comparing NIDDM patients with normoalbuminuria to patients with microalbuminuria, probably due to longer diabetes duration in the former group ($p < 0.05$). Kidney function is shown in Table 2. Regardless of correction for height and body surface area GFR was elevated in the microalbuminuric NIDDM patients as compared to normoalbuminuric NIDDM patients and non-diabetic control subjects ($p < 0.001$). No differences were found between the latter two groups. The GFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) in NIDDM patients who had never received antihypertensive treatment was also clearly elevated in the microalbuminuric patients ($n = 96$): 119 ± 22 as compared to 100 ± 14 and 98 ± 21 in normoalbuminuric NIDDM patients ($n = 27$) and control subjects ($n = 20$), respectively, $p < 0.001$. When considering hyperfiltration as present if GFR exceeded mean plus 2 SD of the value found in the normoalbuminuric NIDDM group 37 (95 % confidence interval 30–45)% of the microalbuminuric patients had hyperfiltration ($p < 0.0005$). If considering hyperfiltration as present if GFR exceeded mean plus 2 SD of the value found in the control subjects 29 (95 % confidence interval 22–37)% of the microalbuminuric patients had hyperfiltration ($p < 0.0005$). Twenty-four hour protein intake was comparable in NIDDM patients with and without microalbuminuria, and in control subjects; 1.1 ± 0.5, 1.0 ± 0.3 and 0.9 ± 0.2 $g \cdot kg^{-1} \cdot 24\ h^{-1}$, respectively (NS). Urinary sodium excretion was higher in the microalbuminuric and normoalbuminuric NIDDM patients as compared with the non-diabetic control subjects; 214 ± 92, 200 ± 79 and 157 ± 69 mmol/24 h, respectively ($p < 0.005$ comparing microalbuminuric NIDDM patients with non-diabetic control subjects, $p < 0.05$ comparing NIDDM patients with normoalbuminuria with non-diabetic control subjects). Correlations between GFR and age, known duration of diabetes, 24-h urinary sodium excretion and HbA_{1c} were demonstrated with univariate regression analysis in the microalbuminuric NIDDM patients. We found no correlation between the following variables and GFR in the microalbuminuric NIDDM patients; systolic and diastolic blood pressure, 24-h protein intake, antihypertensive treatment, diabetic retinopathy or UAE. Multiple regression analysis revealed that age, known duration of diabetes, 24-h urinary sodium excretion and HbA_{1c} correlated to GFR in microalbuminuric NIDDM patients ($r^2 = 0.21$, $p < 0.01$) (Table 3). Covariance

Table 2. Kidney function in NIDDM patients with or without microalbuminuria and in control subjects

	NIDDM with microalbuminuria	NIDDM with normoalbuminuria	Control subjects	*p*-value
GFR (ml/min)	139 ± 29	115 ± 19	111 ± 23	a, b
GFR (ml · min^{-1} · m)	80 ± 17	66 ± 10	64 ± 12	a, b
GFR (ml · min^{-1} · 1.73 m^{-2})	117 ± 24	99 ± 15	98 ± 21	a, b
Albuminuria (mg/24 h)	76 (31–292)	9 (2–29)	6 (2–25)	a, b

Mean ± SD or median (range) indicated, [a] $p < 0.001$ NIDDM patients with vs without microalbuminuria, [b] $p < 0.001$ control subjects vs NIDDM patients with microalbuminuria

Table 3. Multiple regression analysis between GFR and variables significant at a 10 % level in a univariant regression analysis of 158 NIDDM patients with microalbuminuria

Variable	Slope	*p*-value
Age (years)	– 1.09	< 0.001
Known duration of diabetes (years)	– 0.65	< 0.05
Urinary sodium excretion (mmol/24 h)	0.04	< 0.05
HbA_{1c} (%)	2.98	< 0.001

Slope given as change in GFR (ml · min^{-1} · 1.73 m^{-2}) per unit of variable

analysis with the above-mentioned four variables as covariates confirmed that GFR was significantly higher in microalbuminuric NIDDM patients as compared with normoalbuminuric NIDDM patients ($p < 0.001$). The slightly shorter known diabetes duration in the microalbuminuric NIDDM patients will tend to increase GFR with 2 ml (0.65 ml × 3 years, Table 3).

Discussion

Our cross-sectional study revealed elevated GFR (approximately 20 %) in microalbuminuric NIDDM patients as compared with normoalbuminuric NIDDM patients and control subjects. Frank glomerular hyperfiltration was present in one third of the microalbuminuric patients, despite many of the patients having received antihypertensive treatment, a modality known to reduce GFR in normo- and hypertensive NIDDM patients with microalbuminuria [29, 30]. Furthermore, hyperfiltration was also demonstrated in the microalbuminuric NIDDM patients who did not receive antihypertensive treatment. It should be stressed that GFR was elevated both when expressed as crude values and when index to body height or surface area. Poor glycaemic control and increased sodium intake were both associated with higher GFR values.

Originally, Schmitz et al. [31] reported a lack of glomerular hyperfiltration and renal hypertrophy in normoalbuminuric Caucasian NIDDM patients. This finding was challenged by studies demonstrating elevated GFR in native Americans, black Americans and Polynesians suffering from uncomplicated NIDDM [14–16]. Vora et al. [17] clearly demonstrated elevated GFR and effective renal plasma flow (expressed to 1.73 m^2) in a large group ($n = 76$) of newly presenting non-proteinuric normotensive Caucasian NIDDM patients. Furthermore, they demonstrated that improved glycaemic control produces a reduction in GFR, thus confirming and extending the results of Schmitz et al. [11]. A significant reduction in kidney volume as measured by ultrasound scanning has also been reported after 3 months of strict glycaemic control [11]. Recently, Gragnoli et al. [13] reported a normal GFR and kidney volume using ^{99m}Tc-DTPA scintigraphy and ultrasonography in normo- and microalbuminuric Caucasian NIDDM patients with a mean HbA_{1c} below 7 %. Differences in glycaemic control may partly explain the above-mentioned controversy in relation to glomerular hyperfiltration in NIDDM patients. Comparing groups with different body mass index/body surface area may also contribute to the diversities in relation to GFR [32]. Thus, the study from Myers et al. [16] only indicated elevated GFR if the crude values were used, while indexed GFR values did not differ due to a much higher body mass index in NIDDM patients as compared to control subjects. Gragnoli et al. [13] reported unchanged GFR (ml · min^{-1} · 1.73 m^{-2}) values in obese normo- and microalbuminuric NIDDM patients as compared to lean non-diabetic control subjects. Schmieder et al. [32] showed that obesity was not a determinant of renal plasma flow and when related to body surface area, inappropriately low values of renal plasma flow were calculated for obese patients. Therefore, they recommend using renal plasma flow/height criteria to correct renal plasma flow by a measure of body size and suggested that these considerations might be extended to adjusting GFR for body size.

Our findings provide no insight into the mechanism by which GFR became elevated in our NIDDM patients. Previous studies of glomerular haemodynamics in experimental diabetes and in IDDM patients have suggested elevated renal plasma flow, increased glomerular ultrafiltration coefficient and increased glomerular hydraulic pressure as reviewed [4, 5, 9, 33]. Many mediators of hyperfiltration in diabetes have been identified, including glycaemic control, as reviewed by Christiansen [9] and Parving et al. [3].

Glomerular hyperfiltration and enhanced transglomerular passage of plasma proteins in the course of diabetes have both been suggested as factors which might predispose to the accumulation within glomeruli of extracellular matrix and sequential development of glomerulosclerosis [4, 5, 34]. Our finding of increased glomerular permeability to protein along with an elevated GFR is predicted to enhance the rate of protein flux across the glomerular capillary wall. Increased passage of proteins could stimulate glomerular cells to overproduce extracellular matrix leading to expansion of the mesangium with subsequent reduction in the glomerular capillary surface area for filtration [34, 35]. NIDDM patients with microalbuminuria suffer a great risk of later development of diabetic nephropathy [36, 37]. It remains to be established if glomerular hyperfiltration accelerates the rate of progression from micro- to macroalbuminuria in NIDDM patients. Our group have initiated a longitudinal study of glomerular function in all the present microalbuminuric patients. While longitudinal studies in NIDDM are lacking, several but not all longitudinal studies in IDDM patients suggest glomerular hyperfiltration as a risk factor for development of micro- and macroalbuminuria as reviewed by Mogensen [38].

In conclusion, our cross-sectional study indicates that NIDDM patients at high risk of developing diabetic nephropathy are also characterized by an additional putative risk factor for progression, glomerular hyperfiltration.

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