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Higher urinary IgM excretion in type 2 diabetic nephropathy compared to type 1 diabetic nephropathy

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Higher urinary IgM excretion in type 2 diabetic nephropathy compared to type 1 diabetic nephropathy.

Background. Proteinuria, due to impairment of the charge- and/or size selectivity of the glomerular capillary wall (GCW) is the earliest clinical evidence of diabetic nephropathy (DN). To study the pathophysiological differences between patients with DN in type 1 diabetes mellitus (type 1 DN) and type 2 diabetes mellitus (type 2 DN), we compared the patterns of urinary proteins of different size and charge in the two entities of diabetic kidney disease.

Methods. Urine concentrations of albumin, IgG₂, IgG₄ and IgM were assessed in 22 (15 males and 7 females) patients with type 1 DN, and in 20 (18 males and 2 females) patients with type 2 DN. Comparisons with one control group of 13 (12 males and one female) patients with nephrosclerosis due to systemic hypertension and a second control group of 16 (14 males and 2 females) healthy controls were made.

Results. The urine excretion of IgG₂ and IgM and the ratio of IgG₂ to IgG₄ (IgG₂/IgG₄), were significantly higher in type 2 DN compared to type 1 DN ($P < 0.01$). Patients with type 2 DN and patients with nephrosclerosis had significantly higher urine excretion of IgG and IgM compared to the age-matched healthy subjects ($P < 0.001$). The IgG₂/IgG₄ ratio was higher in type 2 DN compared to nephrosclerosis and healthy controls ($P < 0.01$).

Conclusion. The increased urine excretion of IgG and IgM that accompanies albuminuria in type 2 DN suggests that the dominant pathophysiological mechanism of proteinuria in type 2 DN might be an alteration of the size selective properties of the glomerular capillary wall, including the occurrence of non-discriminatory “shunt pathways.” The charge selective properties of the glomerular capillary wall seem to be intact in type 2 DN, as indicated by the high IgG₂/IgG₄ ratio. The mechanisms of proteinuria in type 1 DN seem to be merely a consequence of an impaired charge selectivity of the glomerular capillary wall.

Diabetic nephropathy (DN) is a severe complication of both type 1 and type 2 diabetes mellitus. The disease has become the most common single cause of end-stage

renal failure in Europe and U.S. [1]. However, several clinical findings indicate that the pathophysiological mechanisms of proteinuria might differ between DN in type 1 diabetes mellitus (type 1 DN) and type 2 diabetes mellitus (type 2 DN) [2–4]. For example, angiotensin-converting enzyme (ACE) inhibitors decrease proteinuria in patients with type 1 DN but have no influence on proteinuria in type 2 DN [5].

Transport of proteins across the glomerular capillary wall is determined by the size- and charge-selectivity of the filtration barrier and by the size, shape and charge of the transported macromolecules [6, 7]. The widely used description of this transport is based on hypothetical, water-filled, cylindrical pores perforating the glomerular filter [8]. In a “two-pore with a shunt” model the pores consist of two populations [9]. The vast majority of the pores are “small pores” of radius 2.9–3.1 nm *vis-à-vis* negatively charged rigid spherical proteins. The remaining pore population consists of a small number of “large pores” of radius 8 to 9 nm [10]. The small pores are essentially impermeable to proteins the size of albumin (mol radius 3.6 nm) or larger. Such macromolecules are normally transported by convection across the large pores [11]. In addition to the two populations of pores, the intact glomerular capillary wall exhibits very sporadic physiological “membrane defects” or “shunts” large enough to allow transport of very large proteins and red blood cells [10, 11].

The loss of negative charges of the glomerular capillary wall causes the “effective” small pore radius *vis-à-vis* negatively charged macromolecules to increase to ~4.5 nm, which allows passage of albumin. Larger proteins, such as IgG (mol radius 5.5 nm) or IgM (mol radius 12 nm) are still unable to pass across this pathway. IgG passes the glomerular capillary walls through the large pores while IgM can permeate the glomerular capillary wall solely through the shunts [10, 12]. Thus, increased transport of IgG indicates increased density of large pores and increased concentration of urine IgM indicates increased density of shunts in the glomerular capillary wall [12].

Our previous findings show that the large pore radius

Key words: albuminuria, IgG, IgM, macromolecular transport, proteinuria, charge selectivity, glomerular capillary wall, diabetes mellitus.

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is in the range of 9 nm [10, 13]. Thus, the protein traffic through the large pores is, in parallel with the small pore pathway, influenced by the charge properties of the transported macromolecules and the glomerular capillary wall [14]. Since IgG₂ is a neutral and IgG₄ a negative charged molecule, high value of urine IgG₂ to IgG₄ ratio (IgG₂/IgG₄ ratio) reflects preserved charge selectivity of the glomerular capillary wall. In parallel, a low IgG₂/IgG₄ ratio suggests a loss of the charge selectivity of the glomerular capillary wall.

With these considerations in mind we investigated the transport of macromolecules across the glomerular filter in type 1 DN and type 2 DN. We used urine IgG as marker of the large pore pathway and urine IgM as marker of the shunt transport. Urine albumin and the IgG₂/IgG₄ ratio were used to study the charge influence on the transport through the small pores and the large pores, respectively.

METHODS

Twenty-five patients (22 males and 3 females) with type 2 DN, who participated in a renal biopsy study conducted at Lund University Hospital between February 1997 and December 1999, were invited to take part in the current investigation. Twenty patients (18 males and 2 females) with persistent macroalbuminuria (albumin creatinine index >20 mg/mmol) entered the study. The patients had typical DN findings on the light microscopy examination of the renal biopsy, such as nodular and diffuse glomerulosclerosis and diffuse arteriolar hyalinosis. Global glomerulosclerosis was found in 14 patients, affecting on average 24% (8 to 55%) of the glomeruli. Moderate-to-severe interstitial fibrosis was seen in five patients, and four patients had a normal interstitium. The known duration of diabetes mellitus was 17 (6 to 35%) years and the median HbA_{1c} level was 8.6% (5.5 to 10.7%). The diagnosis of type 2 diabetes mellitus was established if the patient did not need insulin for at least two years after the diagnosis of diabetes. All patients except one were treated with insulin during the time of the study. The daily average insulin dose was 62 (range 16 to 138) IU. Fifty-six percent of the patients had a history of medically treated hypertension before or at the diagnosis of the diabetic disease and 83% of the patients were receiving ACE inhibitors during the study.

Twenty-two patients with type 1 DN (15 males and 7 females) were recruited to the study among the patients at the outpatients' diabetic department at Lund University Hospital. The inclusion criteria were macroalbuminuria (albumin creatinine index >20 mg/mmol in at least two out of three consecutive urine samples) and serum creatinine concentration below 250 µmol/L. The mean duration of the diabetic disease was in this group 25 (15 to 41) years. The daily insulin dose was 41 (24 to 88) IU,

and the median HbA_{1c} level was 8.6% (6.8 to 13). None of the patients in the group had a history of hypertension before the onset of diabetes. Fifty-eight percent of the patients were on ACE inhibitors during the time of the investigation.

Because of the usually high incidence of essential hypertension in patients with type 2 diabetes mellitus, an age matched control group consisting of 13 patients (12 males and one female) with biopsy verified nephrosclerosis secondary to hypertension, was selected. The patients participated in a large investigative program of all forms of glomerular diseases conducted at the Department of Nephrology at Lund University Hospital between 1993 and 1997. The renal biopsy findings in this group showed, on average, 45% of glomeruli affected with global glomerulosclerosis and moderate-to-severe interstitial fibrosis in 69% of the biopsies. The estimated duration of the medical treatment for high blood pressure was 15 (2 to 37) years.

An additional control group consisting of 16 (14 males and 2 females) apparently healthy individuals was used in the study. The inclusion criteria for the healthy controls, in addition to laboratory test results within the reference limits used at our laboratory [14], were no history of ongoing diseases and no current medication. The blood pressure had to be less than 165 mm Hg systolic and 90 mm Hg diastolic pressures. All the subjects gave informed consent. Table 1 shows the patient demographics.

Type 2 DN patients were older than type 1 DN patients ($P < 0.01$; Table 1). There was no difference in regard to the sex distribution, age, serum creatinine, and creatinine clearance (C_{Cr}) between type 2 DN, nephrosclerosis and the healthy control subjects. Mean arterial blood pressure was significantly higher in type 2 DN and nephrosclerosis compared to healthy subjects (Table 1).

Serum and urine creatinine was determined enzymatically using a Kodak Ektachem 700 XR-C system (Eastman Kodak, Rochester, NY, USA). Morning urine samples from patients with no signs of urinary tract infection and menstruation, and with negative urine strip for hemoglobin were used. Urine samples were stored in 3.5-mL polypropylene plastic tubes and kept at -20°C . Urine albumin, urine $\alpha 1$ -microglobulin and serum albumin, serum IgG and serum IgM were determined by immunoturbidimetry, using a Cobas Mira S system (Roche Inc.) and monospecific rabbit antisera obtained from Dako (Copenhagen, Denmark) [14–16]. Agarose gel electrophoresis of plasma was done on all blood samples to exclude the presence of the M component. Urine IgG₂, IgG₄, and IgM were measured by the ELISA technique presented earlier [13, 17], and the lower detection limits for IgG₂ and IgM was 0.05 mg/L, and for IgG₄ was 0.1 mg/L.

Calculations

Creatinine clearance (C_{Cr}) was calculated using the Cockcroft and Gault formula [18], where:

Table 1. Characteristics of patients with diabetic nephropathy of type 1 (1 DN) and type 2 (2 DN) diabetes, healthy control subjects and patients with nephrosclerosis

	Healthy	Nephrosclerosis	2 DN	1 DN	P value
Number	16	13	20	23	NS
Sex male/female	14/2	12/1	18/2	15/8	NS
Age years	60 (± 3.3)	57 (± 3.5)	59 (± 2.2)	38 (± 1.6)	<0.001
MAP mm Hg	93.6 (± 2.2)	117.8 (± 4.6) ^a	105.6 (± 2) ^a	102 (± 2)	NS
Creatinine $\mu\text{mol/L}$	85 (± 2.8)	143 (± 14.4) ^a	113 (± 11.3)	130 (± 11.4)	NS
Creatinine clearance mL/min	89.7 (± 8.7)	57.8 (± 6.6)	75.8 (± 8)	76.5 (± 6.7)	NS

P value represents the difference between type 1 and type 2 diabetic patients. Significance level was at $P \leq 0.01$ (two-tailed).

^aSignificant difference in comparison to healthy control subjects

Table 2. Mean value of fractional clearance and urinary excretion of albumin, IgG₂, IgG₄, IgM and α_1 -microglobulin and the IgG₂/IgG₄ ratio, in type 1 (1 DN) and type 2 (2 DN) diabetes with nephropathy, nephrosclerosis patients and healthy subjects

	Healthy	Nephrosclerosis	2 DN	1 DN	P value
Fractional protein clearance ($\times 10^{-6}$) of					
Albumin	1.8 (± 0.36)	499 (± 172) ^a	379 (± 92) ^a	327 (± 79)	NS
IgG ₂	2.5 (± 1.2)	100 (± 32) ^a	370 (± 77) ^a	185 (± 93)	<0.001
IgG ₄	3.1 (± 2.1)	104 (± 41) ^a	159 (± 52) ^a	211 (± 122)	NS
IgM	0.15 (± 0.07)	4.5 (± 2.9) ^a	2.6 (± 0.65) ^a	0.4 (± 0.09)	0.004
Urine protein creatinine index (mg/mmol) of					
Albumin	0.81 (± 0.15)	132 (± 27) ^a	106 (± 20) ^a	82 (± 12.7)	NS
IgG ₂	0.19 (± 0.14)	6.9 (± 2.6) ^a	36.3 (± 7.5) ^a	10.2 (± 4.45)	<0.001
IgG ₄	0.35 (± 0.25)	9.3 (± 4.5) ^a	13.6 (± 4.1) ^a	13.5 (± 6.3)	NS
IgM	1.8 (± 0.74) $\times 10^{-3}$	24.1 (± 7.6) $\times 10^{-3}$	21.4 (± 5) $\times 10^{-3}$	4 (± 0.95) $\times 10^{-3}$	0.011
α_1 -microglobulin	0.5 (± 0.07)	3.2 (± 0.86) ^a	2.1 (± 0.43) ^a	2.8 (± 0.87)	NS
IgG ₂ /IgG ₄ ratio	2.3 (± 0.71)	1.12 (± 0.26)	15.95 (± 7.1) ^a	3.74 (± 2.6)	<0.001

P value is difference between type 1 DN and types 2 DN. Significance level was at $P \leq 0.01$ (two tailed).

^aSignificant difference in comparison to healthy control subjects

$C_{Cr} = [88 \times (145 - \text{age}) / \text{serum creatinine } (\mu\text{mol/L})] - 3$
and where it is 15% lower for women. (Eq. 1)

Fractional clearance of proteins was calculated according to the formula:

$$\frac{\text{Urine protein (g/L)} \times \text{serum creatinine } (\mu\text{mol/L})}{\text{Serum protein (g/L)} \times \text{urinary creatinine } (\mu\text{mol/L})} \quad (\text{Eq. 2})$$

The IgG₂/IgG₄ ratio was calculated as:

$$\frac{\text{Urine IgG}_2 \text{ concentration (mg/L)}}{\text{Urine IgG}_4 \text{ concentration (mg/L)}} \quad (\text{Eq. 3})$$

The albumin creatinine index (mg/mmol) was defined as ratio of urine albumin (mg/L) to urine creatinine (mmol/L). The IgG creatinine index (mg/mmol) was defined as ratio of urine IgG (mg/L) to urine creatinine (mmol/L), while the IgM creatinine index (mg/mmol) was defined as ratio of urine IgM (mg/L) to urine creatinine (mmol/L). The α_1 -microglobulin creatinine index (mg/mmol) was defined as ratio of urinary α_1 -microglobulin (mg/L) to urinary creatinine (mmol/L).

Statistical methods

The data in the Tables are expressed as medians followed by ranges. Statistical comparisons between the patient groups were performed with the non-parametric

Mann-Whitney test. Correlations were tested using the Spearman correlation coefficient. For urinary concentrations of IgM below the detection limits, 0.01 mg/L was used. The statistical package for social science (version 10; SPSS, Chicago, IL, USA) was used. $P \leq 0.05$ was selected as the level of significance.

RESULTS

Patients with type 1 DN and type 2 DN did not differ in regard to the degree of albuminuria, serum creatinine, C_{Cr} , urine protein α_1 microglobulin concentrations, levels of HbA1c, or the mean arterial blood pressure (Tables 1 and 2).

The urine IgG₂/IgG₄ ratio was higher ($P < 0.01$) in type 2 DN compared to type 1 DN patients (Fig. 1). Type 2 DN patients had increased urine excretion of IgM and IgG₂ compared to type 1 DN patients ($P < 0.01$; Table 2). Urine concentration of IgM was below the detection limit in 19 out of 22 type 1 DN patients (86%) and only in 7 out of 20 (35%) type 2 DN patients (Fig. 2). Urinary excretion of IgG₂ correlated to urinary IgG₄ in type 1 DN ($r = 0.82$, $P < 0.01$) but not in type 2 DN ($r = 0.38$, NS). Increased urine excretion of albumin in type 2 and type 1 DN correlated to decreased C_{Cr} ($r = 0.55$, and 0.56 , respectively, $P < 0.05$) but not to urinary excretion of IgG or IgM.

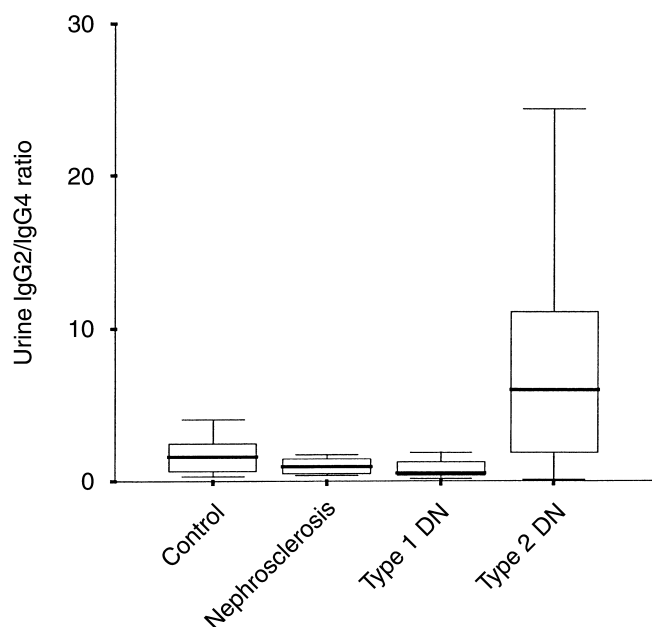


Fig. 1. Urine IgG₂ to IgG₄ ratio in diabetic nephropathy due to type 1 and type 2 diabetes and in control groups.

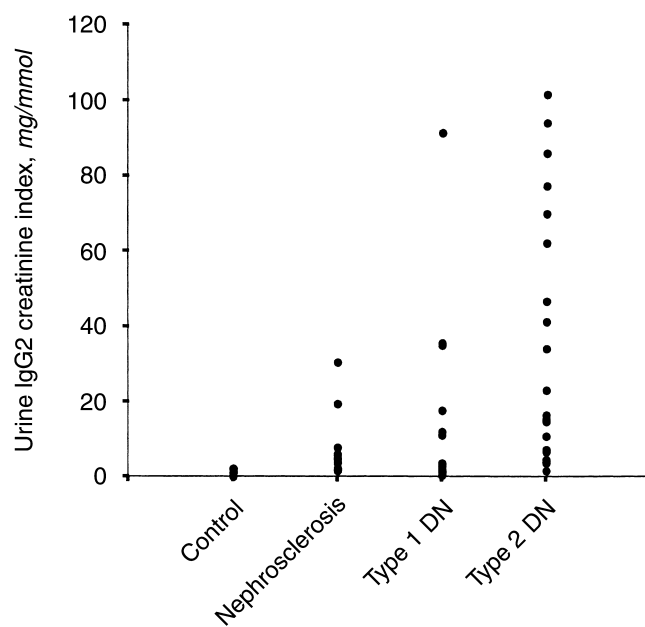


Fig. 3. Urinary IgG₂ excretion in diabetic nephropathy due to type 1 and type 2 diabetes and in control groups.

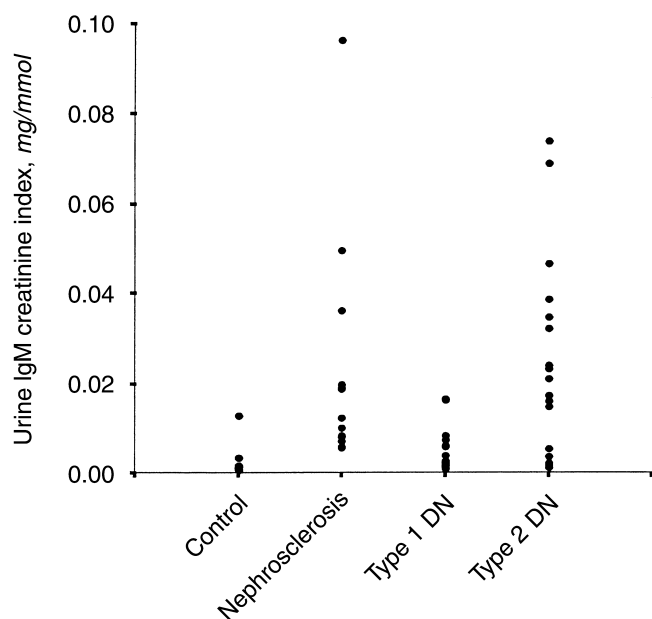


Fig. 2. Urinary IgM excretion in diabetic nephropathy due to type 1 and type 2 diabetes and in control groups.

Type 2 DN patients had a significantly higher degree of albuminuria and many-fold higher urinary IgG and urinary IgM concentrations than the healthy control subjects ($P < 0.001$; Table 2). There were no differences in the urinary excretion of albumin, IgM or α_1 -microglobulin between patients with type 2 DN and nephrosclerosis. However, type 2 DN patients had an increased urinary excretion of IgG₂ compared to the nephrosclerosis pa-

tients ($P < 0.01$; Table 2 and Fig. 3). The increased urine α_1 -microglobulin excretion in type 2 DN and nephrosclerosis did not correlate with increased urine IgG and IgM excretion ($r = 0.12$ and 0.25 , respectively, NS). The IgG₂/IgG₄ ratio was higher in type 2 DN compared to the nephrosclerosis ($P < 0.001$) as well as healthy subjects ($P < 0.01$; Fig.1).

DISCUSSION

Proteinuria is widely regarded as a hallmark of nephropathy and is the most common clinical manifestation of DN in both type 1 and type 2 diabetes. Many experimental studies in diabetic disease have demonstrated that decreased heparan sulfate content causes a reduction of the fixed negative charges of the glomerular barrier resulting in increased urine albumin excretion. Since IgG is transported mainly through the large pores of the glomerular capillary wall, the finding of increased urine concentration of IgG in type 2 DN is suggestive of increased number of large pores. This increase might be the major pathophysiological mechanism of albuminuria in type 2 DN. This assumption is further supported by the preserved charge-selectivity of the glomerular barrier as indicated by the high IgG₂/IgG₄ ratio in type 2 DN. With the intact charge-selectivity of the glomerular capillary wall, the small pore pathway is virtually impermeable for albumin and the main transport of the protein occurs through the large pore pathway.

The appearance of IgM in the final urine found in type 2 DN reflects a markedly increased population of the highly

nonselective pathways, that is, "shunts." This is an additional support for the hypothesis of the size selective dysfunction of the glomerular barrier in type 2 DN. We suggest that an increased size in pore area and an increased number of shunts occur in parallel in type 2 DN.

The significantly smaller amount of the urinary excretion of IgG₂ and IgM in patients with type 1 DN compared to that in type 2 DN, despite a similar degree of albuminuria and kidney function impairment, suggests that the size selective properties of the glomerular capillary wall are relatively intact in type 1 DN. However, the impairment of the charge-selectivity of the glomerular capillary wall in type 1 DN, indicated by the low IgG₂/IgG₄ ratio, causes an increase in the effective small pores radius. The increased small pore radius facilitates the escape of albumin, but not IgG or IgM, into the urinary space. Thus, the impairment of charge selectivity of the glomerular capillary wall is probably the major cause of albuminuria in type 1 DN.

Proximal tubular cell injury leads to an increase of urinary content of the neutral IgG₂ compared to the negatively charged IgG₄ resulting in an increased IgG₂/IgG₄ ratio [19]. Thus, an impairment of the tubular reabsorption may have contributed to the increased IgG₂/IgG₄ ratio in type 2 DN compared to healthy controls. However, the finding of an increased IgG₂/IgG₄ ratio in type 2 DN patients compared to the patients with nephrosclerosis cannot be attributed to a tubular disease, since the degree of interstitial fibrosis as well as the renal function impairment was similar in both groups. Urine excretion of α 1-microglobulin correlated to the degree of interstitial fibrosis, which predicts the degree of kidney function impairment [20, 21]. In our study, patients with type 1 DN and type 2 DN had a similar degree of tubulointerstitial damage; they showed the same degree of renal function impairment and protein α 1-microglobulin urine excretion. Again, the lack of association between increased urinary protein α 1-microglobulin excretion and both IgG and IgM excretion further indicates that the increased urinary excretion of IgG and IgM in type 2 DN is primarily due to increased transglomerular transport.

Thus, we suggest that it is the increased large pore number together with the maintained charge selectivity of the glomerular capillary wall that explains the increased IgG₂/IgG₄ ratio in type 2 DN. At the same time, it is the decreased charge selectivity of the glomerular capillary wall that allows passage of IgG₄ in nephrosclerosis and type 1 DN.

According to the Debye-Hückel theory of charged solute interactions, a negatively charged molecule of 5.5 nm radius will behave as a 6.3 nm ($= 5.5 + 0.8$) radius uncharged molecule at physiological ionic strength (≈ 290 mmol/L). A negatively charged pore of 9 nm radius will behave as an 8.2 nm ($= 9 - 0.8$) radius uncharged pore [22]. Assuming the radius of large pores

of the GCW to be 9 nm, the expected clearance of anionic IgG should increase fourfold in comparison to the clearance of the neutral IgG when the charge selectivity of the capillary wall is completely lost. The decrease in the IgG₂/IgG₄ ratio obtained in this study in patients with type 1 DN compared to type 2 DN in fact corresponds to what could be expected from the above calculations. The mean IgG₂/IgG₄ ratio was 3.7 ± 2.5 in type 1 DN and 16 ± 7.1 in type 2 DN patients. In at least 80% of patients with type 2 diabetes mellitus, hypertension or abnormal circadian blood pressure profile is found at the diagnosis of the diabetic disease [23]. In hypertensive patients, blood pressure correlates to the decrease in renal function [24]. The level of blood pressure before the onset of diabetes is an important predictor of albuminuria in diabetic patients [25]. Proteinuria and glomerulosclerosis were reported to occur in patients with renovascular disease [26]. The increase in renal vascular resistance of patients with the metabolic syndrome of insulin resistance, hypertension and high blood glucose may induce ischemia and structural changes in the glomeruli [27].

Until now, few studies have compared type 1 and type 2 DN [28]. Indeed, the urinary IgM excretion, as a marker of a size selective injury in type 2 DN, to our knowledge has not been studied before at all.

In conclusion, proteinuria in patients with type 1 DN is mainly due to impaired charge-selective properties of the glomerular capillary wall, while proteinuria in type 2 DN is predominantly caused by a decreased size selectivity of the glomerular capillary wall. This difference in the patterns of proteinuria in type 1 and type 2 DN obtained in our study, in addition to the clinical and functional differences, suggests different pathophysiological mechanisms of nephropathy in the two entities of diabetic renal disease.

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