CHAPTER 2

Extracellular matrix in human diabetic nephropathy: reduced expression of heparan sulphate in skin basement membrane*

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Abstract

In diabetic nephropathy, expression of glycosaminoglycan side chains of heparan sulfate proteoglycan in the glomerular basement membrane is reduced proportionally to the degree of proteinuria. We performed a cross-sectional study to evaluate whether non-vascular basement membranes also show a decrease in heparan sulfate side chain staining in patients with diabetic nephropathy.

We evaluated the skin basement membrane for extracellular matrix components in the following groups: control subjects (*n* = 16); patients with type 1 diabetes and normoalbuminuria (*n* = 17), microalbuminuria (*n* = 7), and macroalbuminuria (*n* = 16); patients with type 1 diabetes and diabetic nephropathy undergoing renal replacement therapy (*n* = 13); and non-diabetic patients undergoing renal replacement therapy (*n* = 21). The following antibodies were used for this immunohistochemical study : monoclonal antibodies against the heparan sulfate side chain (JM403) and core protein (JM72) of the glomerular heparan sulfate proteoglycan; polyclonal antibodies against the core protein (B31); polyclonal antibodies against collagen types I, III, and IV, fibronectin, and laminin; and monoclonal antibodies against the non-collagenous domain of α1(collagen IV) and α3(collagen IV), against transforming growth factor β (2G7), and against advanced glycosylation end products (4G9).

Expression of heparan sulfate side chains was reduced in the skin basement membrane of patients with overt diabetic nephropathy, of those with type 1 diabetes undergoing renal replacement therapy, and those with non-diabetic renal failure. Increased intensity of staining was found for collagen type I and advanced glycosylation end products in patients with diabetic nephropathy.

Changes in the extracellular matrix of the skin basement membrane seem to be similar to those in the glomerular basement membrane. These findings support the suggestion that patients with diabetic nephropathy also have altered heparan sulfate and collagen staining in extrarenal basement membranes. However, patients with non-diabetic renal failure also had reduced expression of heparan sulfate in the skin basement membrane, suggesting that this finding is not specific for diabetic nephropathy.

Introduction

In type 1 diabetes mellitus all tissues are exposed to hyperglycemia, but only a subset (30 - 40%) of diabetic patients develop diabetic kidney disease (1,2). According to the Steno hypothesis, albuminuria and associated complications result from a genetic polymorphism of enzymes involved in the metabolism of heparan sulfate proteoglycans (HSPG) (2).

HSPG consists of a core protein with heparan sulfate (HS) glycosaminoglycan side chains. Sulfate groups covalently to the repeating disaccharides of HS give the glycosaminoglycan chains their high negative charge. HS is thought to be important to the structure and function of glomerular basement membrane; it prevents clogging and determines the sieving function (3). HS is mainly responsible for the negative charge barrier in the glomerular basement membrane, which prevents albumin passing through the capillary wall (3). The key role that HS has in the normal function of the glomerular basement membrane is illustrated by the in vivo finding that removal of HS by heparinitase (3) or shielding of HS by a monoclonal antibody (4) generates albuminuria.

In patients with diabetic nephropathy, staining for HS in the glomerular basement membrane is reduced proportionally to the amount of proteinuria, but staining for the core protein is not altered (5,6). Similar findings have been observed in membranes of small vessels, such as muscle capillary basement membrane (7), and of large vessels, such as the aorta (8).

Reduced synthesis and sulfation of HSPG proteoglycans by podocytes and mesangial cells, when cultured under high glucose conditions, has been reported (9). Further clinical evidence supporting the Steno hypothesis comes from observations of the efficacy of treatment with heparin and HS in animal models and humans with diabetic nephropathy (10-14).

Since the diabetic milieu affects all body tissues, we wondered whether non-vascular lining and more easily accessible basement membrane tissue, obtained with simple skin punch biopsies techniques, show the same pattern of changes of extracellular matrix. Such a generalised reduction in heparan sulfate expression supports the study of changes in the extracellular matrix of the glomerulus without evaluation of the glomerulus itself.

Accordingly, we embarked on this cross-sectional study to evaluate the expression of extracellular matrix components of the epidermo-dermal junction basement membrane zone, a non-endothelial membrane, in patients with different stages of diabetic nephropathy, and patients with non-diabetic renal failure.

Subjects and methods

Subjects

Control specimens comprised incisional abdominal skin biopsies from a skin bank derived from nine patients who had undergone autopsy and seven otherwise healthy patients who had had abdominal plastic surgery. Control subjects did not have diabetes, renal disease or skin diseases, but other demographic characteristics were not available because of the anonymous character of the bank.

We included skin biopsies from the following patients with type 1 diabetes mellitus: 17 patients with normoalbuminuria (albumin excretion rate (AER) <30 mg/24 h), 7 patients with microalbuminuria (AER 30-300 mg/24 h), 16 patients with macroalbuminuria (AER >300 mg/24 h), and 13 patients with diabetic nephropathy who were undergoing renal replacement therapy. A second control group comprised skin biopsies from 21 patients, who were undergoing renal replacement therapy and had no diabetes mellitus or skin disease. In this group of patients, the underlying renal disease were as follows: IgA nephropathy (n = 3), mesangiocapillary glomerulonephritis (n = 1), adult polycystic kidney disease (n = 3), focal segmental glomerulosclerosis (n = 4), interstitial nephritis and analgesic nephropathy (n = 4), hemolytic uremic syndrome (n = 1), and unknown (n = 5). Before renal replacement therapy, 14 of these patients had had proteinuria (>1 g/l), three had no proteinuria, and in four patients the urinary protein status was unknown. Abdominal skin biopsy was performed with a disposable 4 mm punch device (Stiefel, Wächtersbach, Germany), after adequate local anesthesia and outside areas of insulin injection. The demographic characteristics of the subjects are shown in Table 1.

Table1. *Clinical characteristics of study subjects*

Group	***Number of cases*** ***(women)***	***Age*** ***(years)***	***Diabetes duration*** ***(years)***	***Mean arterial Blood pressure (mmHg)***	***HbA_{1c}*** ***(%)***	***Creatinine clearance*** ***(ml/min)***	***Albuminuria*** ***(mg/24 h)***
Type 1 diabetes: Normoalbuminuria	17 (5)	38 (25-65)	21 (8-46)	100 (74-113)	7.7 (5.6-9.4)	103 (64-167)	8 (2-22)
Type 1 diabetes: Microalbuminuria	7 (4)	40 (32-56)	24 (15-40)	103 (92-124)	9.0 (7.8-10.3)	88 (39-121)	130 (30-265)
Type 1 diabetes: Macroalbuminuria	16 (6)	42 (29-49)	25 (19-36)	107 (98-140)	9.0 (5.3-12.7)	22 (11-114)	1522 (544-5181)
Type 1 diabetes: Renal replacement therapy	13 (2)[a]	42 (26-57)	26 (14-43)	113 (100-133)	9.5 (6.9-13.2)		
No diabetes: RRT	21 (9)[b]	44 (19-69)	0	110 (71-127)	5.6 (4.8-6.7)		

Data are median (range). [a]*peritoneal dialysis (PD) 6, hemodialysis (HD) 7,* [b]*PD 10, HD 11.*

All patients had longstanding type 1 diabetes mellitus, documented by a glucagon test or a typical clinical history relating to the onset of the disease. Dermatological diseases were excluded by medical history and inspection of the skin. Diabetic nephropathy was defined by the presence of microalbuminuria or macroalbuminuria in sterile urine and of retinopathy. Patients with hematuria, urinary tract infections, cardiac failure or other renal disease were excluded from the study. A general medical history (including the duration of diabetes and dermatological history) was taken, and blood pressure was measured while the patient was in the sitting position. Serum creatinine, glucose, HbA_{1c} (the mean of four previous measurements for patients with type 1 diabetes), and creatinine and albumin concentrations in two, 24 h urinary collections were measured. The mean results of these two urine collections had to agree with previous results determined at the outpatient clinic. Urinary albumin was assessed using immunonephelometry on an autoanalyser (Array Protein system, Beckman Instruments Inc., Brea, Calif., USA) with specific antibodies. Urinary and serum creatinine concentrations were assessed with a Hitachi 747 or 911 autoanalyser (Boehringer Mannheim, Germany). HbA_{1c} was assessed by high performance liquid chromatography after hemolysis.
The type of dialysis treatment was not different for diabetic and non-diabetic patients. The groups of patients were adequately matched for age, duration of type 1 diabetes mellitus (where appropriate) and mean arterial blood pressure (Table 1).

Ethics
The medical ethical committee of the Leiden University Medical Center approved the protocol of the study, and all patients gave informed consent.

Antibodies
We used the following antibodies (see Table 2) to evaluate semiquantitatively the HSPG expression of the epidermal-dermal junction basement membrane zone as follows: JM403 (a monoclonal antibody of the IgM class against rat glomerular HS that cross-reacts with human HS) (4,15); JM72 (a monoclonal antibody of the IgG1 class directed against the core protein of HSPG) (15); and a goat polyclonal antibody B31 directed against the HSPG core protein (16).
To elucidate other putative changes that occur in the extracellular matrix, we used goat anti-collagen types I, III, and IV (Sera-lab, Sussex, UK) and monoclonal antibodies (α1(IV)NC and α3(IV)NC) against the non-collagenous domain of α1(IV) and α3(IV) (a kind gift by Dr. J. Wieslander, Lund, Sweden) (17). Affinity purified goat anti-human fibronectin antibodies were purchased from Sigma (St. Louis, Mo., USA).

Table 2. *Antibodies and their characterisation*

Antigen	*Antibody*	*Ig(sub)clas*	*Mono/Polyclonal antibody (animal source)*	*Source*	*Ref. no*
Heparan sulfate	JM403	IgM	Mono (mouse)	University of Nijmegen	(4,15)
HSPG-core	JM72	IgG1	Mono (mouse)	University of Nijmegen	(15)
HSPG-core	B31	IgG	Poly (goat)	University of Nijmegen	(16)
Laminin	Laminin	IgG-fraction	Poly (rabbit)	Leiden University Medical Center	
Fibronectin	Fibronectin	Ig-fraction	Poly (goat)	Sigma	
TGF-ß 1,2,3	2G7	IgG1	Mono (mouse)	Leiden University Medical Center	(18)
Collagen I	Collagen I	Ig-fraction	Poly (goat)	Sera-lab	
Collagen III	Collagen III	Ig-fraction	Poly (goat)	Sera-lab	
Collagen IV	Collagen IV	Ig-fraction	Poly (goat)	Sera-lab	
Collagen IV∀1NC	a1(IV)NC	IgG1	Mono (mouse)	Biocarb	(17)
Collagen IV∀3NC	a3(IV)NC	IgG1	Mono (mouse)	Biocarb	(17)
AGE	4G9	IgG1	Mono (mouse)	Picower Institute	(19)

Polyclonal rabbit anti-laminin antibodies were raised in our own laboratory using human laminin (Sigma, St. Louis, Mo., USA) as immunogen. Monoclonal anti-transforming growth factor β (TGF-β) antibodies (2G7) the specifically recognise human TGF-β 1,2,3 were used (18). The monoclonal antibodies against advanced glycosylation end products antibody 4G9 (mouse IgG1 subclass) came from the Picower Institute for Medical Research (Manhasset, NY, USA) (19).

Immunohistological methods.

The skin biopsies specimens were embedded in cellophane capsules filled with Tissue-Tek (Miles, Elkhart, Ind., USA) and stored at -80°C until processed. Specimens were cut into 4 µm sections in a Reichert Jung 2800 cryostat at -20°C. After defrosting and fixation with acetone, the slides were rinsed with phosphate buffered saline (PBS), 30% H_2O_2 and 10% sodium-azide. Thereafter, the slides were incubated with the primary antibody in PBS 1% bovine serum saline. After rinsing with PBS, incubation with the second antibody conjugated with horse radish peroxidase (DAKO, Glastrup, Denmark) was performed in a solution of 20% Δ normal human serum in PBS 1% bovine seum saline for

30 minutes. After rinsing again with PBS, the slides were incubated with the third antibody conjugated with horse radish peroxidase in a solution of PBS 1% bovine serum saline for 30 minutes. The slides then were rinsed with PBS and acetate buffer. Subsequently, 3 amino-9 ethyl carbozol was applied for 7 min, followed by hematoxylin. Finally, the sections were counterstained with Mayer's hematoxylin and mounted with Kaiser's glycerin gelatin (both from Merck, Darmstadt, Germany). Slides of normal kidney tissue from human kidneys condidered unsuitable for transplantation for anatomical reasons were used as reference. All specimens were processed by the same laboratory technician and each series of staining with a specific antibody was performed without interruption under the same conditions.

The intensity of staining of the epidermal-dermal junction basement membrane zone was scored as previously described for other tissues on a semiquantative scale where: 0 = negative, ½ = slightly positive, 1 = positive, 2 = positive to strongly positive and 3 = strongly positive (6,20-22). All slides were numbered randomly so that the two independent observers could examine them without knowledge of the sample origin and clinical data.

Statistical analysis.

Using the model in which a deviation of one class in scoring intensity was accepted, weighted Cohen's kappa statistics were calculated. The final scoring results were obtained after conference without knowledge of the sample origin. Antibody staining intensity scores from the control group with no diabetes mellitus or renal disease were compared with scores from the diabetic patients. These five patient groups were compared with regard to the antibody staining intensity scores using non-parametric statistical tests and the ordinal semiquantative scale of both the patient groups and the scoring system (the Mantel-Haenszel χ^2 test for linear association). In a separate analysis we used the same procedure to compare the antibody staining intensity scores from patients with diabetic nephropathy undergoing renal replacement therapy with scores from non-diabetic patients on renal replacement therapy. Correlation coefficients were calculated with the Spearman's rank correlation test. Results are given as medians with ranges, unless otherwise indicated. Statistics were performed with the SPSS for Windows, version 6.0. Significance was accepted at the 0.05 level.

Results

Scoring of staining intensity between observers was reproducible (weighted Cohen's kappa median 0.70, range 0.49 -1.00; HS (JM403): 0.81 and collagen type I 0.77).

Table 3. *Staining intensities (number of biopsies in relation to score) for different parameters in skin biopsy specimens from patients and controls*

Parameters	***Controls*** ***(n = 16)***	***Type 1 diabetes: normo-albuminuria*** ***(n = 17)***	***Type 1 diabetes: micro-albuminuria*** ***(n = 7)***	***Type 1 diabetes: macro-albuminuria*** ***(n = 16)***	***Type 1 diabetes: Renal replacement therapy*** ***(n = 13)***	***No diabetes: Renal replacement therapy*** ***(n = 21)***
	0 ½ 1 2 3	**0 ½ 1 2 3**	**0 ½ 1 2 3**	**0 ½ 1 2 3**	**0 ½ 1 2 3**	**0 ½ 1 2 3**
HS (JM403)	0 0 2 9 5[1] (87)[a]	0 0 0 7 9[1] (100)	0 0 0 3 4[1] (100)	0 1 4 4 6[1] (67)	0 1 5 6 1[1] (54)	0 1 8 8 4 (57)
HSPG (JM72)	0 0 0 8 8 (100)	0 0 1 7 9 (94)	0 0 0 5 2 (100)	0 0 2 7 6 (87)	0 0 0 5 8 (100)	0 0 0 13 7 (100)
HSPG (B31)	0 0 4 12 0 (80)	0 0 4 6 7 (76)	0 0 0 4 3 (100)	0 1 2 5 8 (81)	0 2 4 5 2 (54)	0 7 7 7 0 (33)
Laminin	0 2 9 5 0 (31)	0 1 12 3 1 (24)	0 0 5 2 0 (29)	0 2 10 3 0 (20)	0 1 7 5 0 (38)	0 7 8 6 0 (29)
Fibronectin	0 4 9 0 2 (13)	0 0 8 7 2 (53)	0 1 4 2 0 (29)	0 1 9 3 2 (33)	0 3 6 4 0 (33)	0 5 9 7 0 (33)
TGF-ß	2 4 2 7 0 (47)	5 4 6 2 0 (13)	1 4 2 0 0 (0)	2 8 3 1 1 (13)	0 2 5 4 1 (42)	2 4 6 7 1 (40)
Collagen I	0 3 4 9 0[2] (56)	0 0 9 8 0[2] (47)	0 0 2 5 0[2] (71)	0 1 2 12 0[2] (80)	0 0 2 9 2[2, 3] (85)	0 1 9 11 0[3] (52)
Collagen III	0 0 8 7 0 (53)	0 0 6 10 1 (65)	0 0 2 4 0 (67)	0 0 7 8 0 (53)	0 2 6 5 0 (38)	0 0 14 7 0 (33)
Collagen IV	0 0 1 8 7 (94)	0 0 0 4 12 (100)	0 0 0 1 6 (100)	0 0 1 3 11 (93)	0 0 0 8 5 (100)	0 0 1 14 6 (95)
α1(IV)NC	0 0 1 4 10 (93)	0 0 0 6 11 (100)	0 0 0 1 6 (100)	0 0 1 4 11 (94)	0 0 0 4 9 (100)	0 0 1 11 9 (100)
α3(IV)NC	4 4 2 2 4 (38)	4 5 4 2 2 (24)	2 1 2 2 0 (29)	2 3 2 6 2 (53)	1 3 3 4 2 (46)	1 3 5 4 7 (55)
AGE (4G9)	2 2 5 0 7 (44)	1 7 5 1 2[4] (19)	0 1 3 2 0[4] (33)	0 1 6 4 3[4] (50)	1 1 1 6 4[4] (77)	2 5 3 3 7 (50)

[a] *Percentage in each category with a staining score ≥ 2.* [1] *$p < 0.009$ for heparan sulfate between controls and all diabetic groups;* [2] *$p < 0.005$ for collagen type 1 between controls and all diabetic groups;* [3] *$p < 0.02$ for collagen type I between patients with type 1 diabetes on renal replacement therapy and non-diabetic patients on renal replacement therapy;* [4] *$p < 0.004$ for AGE (advanced glycosylation end products) between the various diabetic groups.*

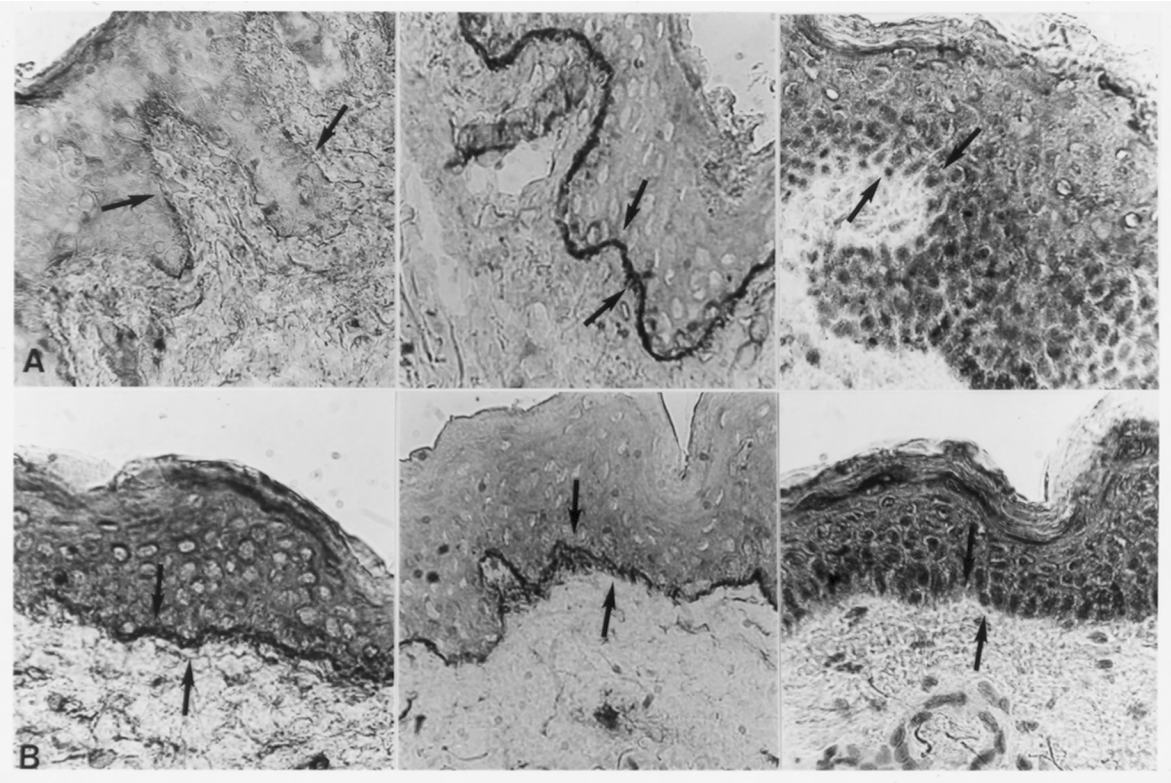

Figure 1 A,B. *Immunoperoxidase staining of the epidermal-dermal junction of skin biopsy specimens (original magnification x 400). (**A**) JM403 score 1 (left), JM72 score 3 (middle) and negative control (right). (**B**) JM403 score 3 (left), JM72 score 3 (middle) and negative control (right)*

HS (JM403), HSPG (JM72, B31), and anti-fibronectin staining in the sections was predominantly at the level of the epidermal-dermal junction basement membrane zone, although some activity also was observed in the epidermis (especially HS).

Anti-TGF-β, anti-AGE, and anti-laminin stained positively in a granular pattern along the basal lining of the keratinocytes. Anti-collagen type I, III and IV, anti-∀1(IV)NC and anti-∀3(IV)NC stained only along the junction basement membrane zone (the latter was granular, the other antibodies directed against collagens were more continuous).

Representative examples of staining for HS (JM403), HSPG (JM72) and negative controls are shown in Figure 1. Table 3 shows the results of the antibody staining intensities for HS side chain, HSPG (JM72), HSPG (B31), laminin, fibronectin, TGF-β 1,

2 and 3, collagen types I, III, and IV; ∀1(IV)NC, ∀3(IV)NC and AGE. Since each series of staining with a specific antibody was performed without interruption and under the same conditions, on rare occasions (maximum of 1 per patient group) a slide with a skin biopsy sample lacked enough basement membrane for scoring and was therefore judged as a missing value. Only HS and collagen type I showed a significant difference between the groups of patients (Fig. 2). An increase in staining intensity for collagen type I was observed in relation to the stage of diabetic nephropathy. Results for AGE did not reach significance, but when only the diabetes patients were compared, increased intensity of staining was found in relation to the severity of the nephropathy (p = 0.004). The intensity of staining for the antibody directed against HS side chains (JM403) was significantly lower in diabetic patients with overt proteinuria and in diabetic patients undergoing renal replacement treatment. All seven patients with microalbuminuria had a staining score of 2 or more.

A correlation was found (r = 0.56, p = 0.007) between CrCl and the staining intensity for HS. The ratio of albuminuria to creatinine clearance, reflecting the albumin loss in still-functioning glomeruli, correlated with the intensity of staining for HS (r = -0.47, p < 0.03). A significant result was also found for AGE: AGE ratio of albuminuria over creatinine clearance r = 0.42, p = 0.01. We found no statistically significant correlations between the intensity of staining for HS, HSPG, and AGE and age, duration of type 1 diabetes mellitus and HbA_{1c} in the diabetic patients.

We found no differences in the staining intensity of the extracellular matrix components in the two groups undergoing renal replacement therapy, except for collagen type I (p < 0.02). The non-diabetic patients with renal failure had a normal collagen type I expression when compared with normal control subjects (p = 0.86).

There were no differences in respect of the nature of renal disease and the intensity of staining of HSPG (JM72) (p = 0.75), and HS (p = 0.47) in the non-diabetic patients with renal failure. Seven of 14 non-diabetic patients who had proteinuria before renal replacement therapy had a score of 1 (IgA nephropathy (n = 1), mesangiocapillary glomerulonephritis (n = 1), adult polycystic disease (n = 1), focal segmental glomerulosclerosis (n = 3) and interstitial nephritis and analgesic nephropathy (n =1). The remainder had a score of 2 or more. The three patients without proteinuria before renal replacement therapy all had scores for HS 2 or more.

Discussion

This study aimed to determine whether the non-vascular basement membrane of the skin in patients with diabetic nephropathy exhibits changes in extracellular matrix components

that are similar to those observed in the glomerular basement membrane (6).

We chose immunohistochemical staining with semiquantiative scoring to estimate the distribution of the extracellular matrix components. Several studies have previously shown that this is a useful technique for observing alterations in intensity, and therefore changes, in the quantities of a specific antigen (6,15,20). We found a significant reduction in HS expression in the skin basement membrane in relation to the severity of diabetic nephropathy. However, staining of B31 and JM72 - antibodies directed against the core protein of HSPG - remained unaltered.

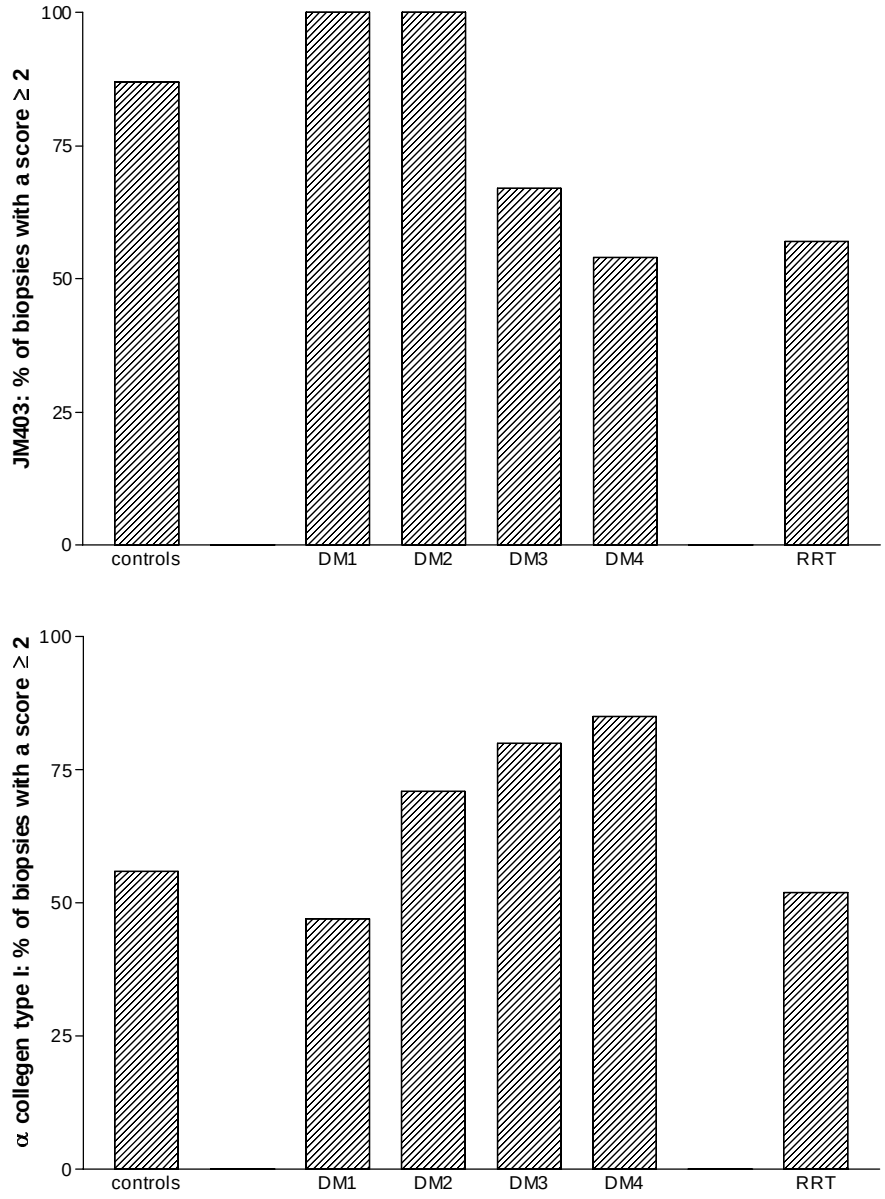

Figure 2. *DM1 denotes IDDM and AER < 30 mg/24 hrs, DM2 denotes IDDM and AER 30-300 mg/24 hrs, DM3 denotes IDDM and AER > 300 mg/24 hrs, DM4 denotes IDDM and RRT. RRT denotes the patients without DM on RRT.*

We do not, in our institution, perform renal biopsies in patients with a clinical diagnosis of diabetic nephropathy (defined by the presence of retinopathy and microalbuminuria or macroalbuminuria without hematuria or urinary tract infection). As a result, we could not correlate our findings in the skin basement membrane with those in the glomerular basement membrane.

We have shown recently that the JM403 binding to HS depends on the presence of an N-unsubstituted glucosamine unit in HS, and that the expression of the JM403 epitope in HS preparations from various sources is inversely correlated with HS sulfation (24). Reduced staining for JM403 might therefore be explained by a decreased synthesis and/or increased degradation of HS, or an altered structure of HS resulting in loss of the JM403 epitope. The HS chains are modified post-transcriptionally by N-substitution of the glucosamine units, facilitated by the key enzyme glucosaminyl N-deacetylase/N-sulfotransferase. A reduction in this enzyme has been found in diabetes (25). Thus, loss of HS staining might be explained by a reduction in N-unsubstituted glucosamine units of HS or an absolute reduction in HS.

The skin biopsy specimens from microalbuminuric patients showed a normal intensity of anti-HS staining. Using cuprolinic blue to demonstrate HS-associated anionic sites, Vernier et al. (5) also found a normal pattern of expression in the glomerular basement membrane of patients with microalbuminuria. Unfortunately, both our study and that of Vernier et al. included only a few patients with microalbuminuria (seven and three respectively). However, supplementation of heparin and a low-molecular weight heparin showed a reduction in albuminuria in type 1 diabetic patients with microalbuminuria, suggesting that these patients might already have an altered HS content in the glomerular basement membrane (13). In a study of HS expression in muscle capillaries, microalbuminuria was associated with an undersulfation of HS, while an absolute reduction in HS staining was observed, (as in our study) in type 1 diabetic patients with macroalbuminuria (7). These findings are underlined by similar observations in experimental diabetic nephropathy (25). Thus, our observations and those of others may argue against the hypothesis that reduced HS expression in the glomerular basement membrane precedes microalbuminuria.

Non-diabetic patients with renal failure also had low HS expression. All these patients were euglycemic, as demonstrated by normal serum glucose concentrations and a normal HbA_{1c} value. Van den Born et al. (15) also reported reduced expression of HS in several non-diabetic renal diseases such as lupus nephritis, crescentic nephritis and minimal change disease. Changed HS expression may occur via several possible pathways, and it is tempting to speculate that it is a relevant factor in proteinuria and/or the progression of renal insufficiency, in general, rather than a specific marker for the development of diabetic nephropathy.

With regard to diabetic nephropathy, reduced HS expression might be explained by long-standing high glucose concentrations, non-enzymatic glycosylation and the functional changes of the renin-angiotensin system. A high glucose concentration induces a change in HS production (both qualitatively and quantitatively) in cultured renal cells (9). We found a significant increase in AGE staining in relation to the severity of the nephropathy in patients with type 1 diabetes. Our results agree with those from other studies: AGE accumulation, determined by immunohistochemical methods in glomeruli and arterioles (26) and by immunochemical procedures in the skin collagen of diabetic patients, was found to increase as normal renal status advanced to microalbuminuria and macroalbuminuria (27).

With regard to the renin-angiotensin system, it has been reported that in patients with diabetic nephropathy, angiotensin converting enzyme inhibition has a better anti-proteinuric effect than other antihypertensive treatments that provide equal blood pressure effects (28). This suggests that inhibition of the renin-angiotensin system may correct structural glomerular changes independently of hemodynamic changes.

Staining for collagen type I was increased in relation to the severity of the diabetic nephropathy. Non-diabetic patients undergoing renal replacement therapy did not show this increase. Hyalinised and disorganised collagen can be found in thick diabetic skin (defined as an increase in whole skin thickness). Diabetic patients with and without thick skin showed a predominance of large collagen fibres in biopsies evaluated with electron microscopy (29). Furthermore, enhanced collagen synthesis by cultured skin fibroblasts from type 1 diabetic patients with diabetic nephropathy has been reported (30), which seems to agree with our results on tissue sections.

Our findings suggest that HS expression in the skin epidermal-dermal junction basement membrane zone may reflect not only what occurs in the glomerular basement membrane but the fact that in vitro studies of skin cells such as keratinocytes and fibroblasts may offer a useful model for what happens with autologous renal cells.

In conclusion, we found a decreased expression of the heparan sulfate side chains using a semi-quantative immunohistochemical evaluation in the basement membrane zone of the skin of patients with overt diabetic nephropathy and of patients with non-diabetic renal failure showed a reduced expression of HS side chains. Collagen type I and AGE were increased in the basement membrane zone of patients with diabetic nephropathy. Our findings support the Steno hypothesis with regard to the diabetic patients - suggesting that changes in skin composition of the epidermal-dermal junction basement membrane zone correspond closely to changes in the extracellular matrix in the glomerular basement membrane in diabetic nephropathy. However, patients with non-diabetic renal failure also had reduced expression of HS in the skin basement membrane, wich suggests that this finding is not specific for diabetic nephropathy.

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