

Pattern of Proteinuria in Tubular Injury and Glomerular Hyperfiltration

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Abstract

Proteinuria is one of the bad prognostic indices in renal disease. This study compares the pattern of protein excretion in 10 patients with IgA nephropathy (IgAN), 10 patients with chronic glomerulonephritis approaching end-stage renal failure (ESRF) who still had proteinuria and 10 other patients with diabetic nephropathy (DN) with proteinuria but normal renal function. The pattern of proteinuria was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF) and assayed for orosomucoid, alpha-1-microglobulin, retinol-binding protein, lysozyme, beta-2-microglobulin and N-acetyl-beta-D-glucosaminidase activity. Our data showed much similarity in the pattern of proteinuria between the DN and ESRF groups but significant differences with the IgAN group. The pattern of proteinuria in the IgAN group reflects glomerulonephritis whereas the similar pattern between the ESRF and DN groups may reflect hyperfiltration as well as tubular injury.

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Introduction

Proteinuria is one of the bad prognostic indices in patients with renal disease.¹⁻³ It has been previously shown that patients with IgA nephropathy with selective proteinuria tend to respond to steroids in contrast to those with non-selective proteinuria.⁴⁻⁷ Recent reports^{8,9} also documented the pattern of proteinuria in these patients using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) and demonstrated various distinct patterns of proteinuria which affect response to therapy and prognosis.

This study compares the pattern of protein excretion in patients with IgA nephropathy (IgAN) using SDS-PAGE and IEF with the pattern of proteinuria in patients with chronic glomerulonephritis approaching end-stage renal failure (ESRF) who still had moderate amounts of proteinuria due to tubular injury and glomerular hyperfiltration and patients with diabetic nephropathy (DN) where the mechanism of proteinuria is attributed to glomerular hyperfiltration.

Patients and Methods

Patients

Ten patients with biopsy proven IgA nephropathy had their proteinuria evaluated by SDS-PAGE, IEF and

estimation of 5 low molecular weight (LMW) proteins and a urinary enzyme. The diagnosis of IgA nephropathy was based on the demonstration of IgA as the predominant immunoglobulin in the mesangium on immunofluorescence and exclusion of systemic diseases such as systemic lupus erythematosus, Henoch-Schönlein purpura, cirrhosis of the liver or systemic vasculitis. All 10 patients had normal renal function at the time of the study. Table I shows the profile of these patients.

Another 10 patients with chronic glomerulonephritis who were approaching ESRF (Table II), serum creatinine 12.4 ± 3.8 mg/dl (range 6.8 to 18.2 mg/dl) who still had significant proteinuria 2.5 ± 1.2 gAlb/gCr and 10 other patients with diabetic nephropathy with proteinuria 1.8 ± 1.3 gAlb/gCr with normal renal function and no nephrotic syndrome (Table III) were similarly studied using selectivity index (SI), SDS-PAGE, IEF and LMW protein to assess their pattern of proteinuria. The ESRF patients allowed us to study the effects of renal failure and tubular dysfunction on the pattern of proteinuria and the diabetics with normal renal function enabled us to document the pattern of proteinuria in hyperfiltration. None of the patients received treatment with prednisolone, cyclophosphamide or any other medication apart from hypotensive agents but not angiotensin converting enzyme (ACE) inhibitors for those with systemic hypertension.

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TABLE I: PROFILE OF PATIENTS WITH IgA NEPHROPATHY (IgAN)

No.	Sex	Age (year)	Hypertension (mg/dl)	Serum Creatinine	SI	SDS-PAGE (pattern)	IEF (pattern) (g/gCr)	Urine Albumin (mg/gCr)	Urine Orosomucoid (au/gCr)	Urine A1M (au/gCr)	Urine RBP (au/gCr)	Urine Lysozyme (au/gCr)	Urine B2M (u/gCr)	Urine NAG
1	M	35	N	1.2	0.03	1	SC:UA	0.8	29	28	0	0	0	0.7
2	M	28	Y	1.2	0.16	1	SC:UA	0.8	33	18	0	0	0	0.2
3	M	31	N	0.9	0.12	3	SC:UA	2.3	68	24	0	0	0	0.3
4	F	46	N	1.1	0.27	3	SC:UA	1.1	70	34	9	0	0	1.1
5	F	32	N	1.1	0.28	3	SC:UC	2.5	102	41	0	0	0	1.9
6	M	27	N	1.1	0.26	3	SC:UA	3.3	205	27	0	0	0	0.2
7	F	43	Y	1.4	0.27	3	SC:UA	1.1	90	26	0	0	0	0.6
8	M	19	N	1.1	0.15	3	SC:UA	0.7	51	20	0	0	0	0.8
9	F	18	N	0.9	0.29	1	SC:UA	1.0	36	0	0	0	0	0.3
10	M	33	N	1.2	0.20	3	SC:UA	1.0	42	11	0	0	0	0.8
Mean		31		1.1	0.20			1.5	73	24	2	0	0	0.7
±SD		9		0.2	0.09			0.9	53	10	3			0.5

SI: selectivity index; SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis; IEF: iso-electric focusing; SC:UA: serum cationic albumin, urine anionic albumin; SC:UC: serum cationic albumin, urine cationic albumin; A1M: alpha-1-microglobulin; RBP: retinol-binding protein; B2M: beta-2-microglobulin; NAG: N-acetyl-beta-D-glucosaminidase; SD: standard deviation

TABLE II: PROFILE OF PATIENTS WITH END-STAGE RENAL FAILURE (ESRF) DUE TO CHRONIC GLOMERULONEPHRITIS

No.	Sex	Age (year)	Hypertension (mg/dl)	Serum Creatinine	SI	SDS-PAGE (pattern)	IEF (pattern) (g/gCr)	Urine Albumin (mg/gCr)	Urine Orosomucoid (au/gCr)	Urine A1M (au/gCr)	Urine RBP (au/gCr)	Urine Lysozyme (au/gCr)	Urine B2M (u/gCr)	Urine NAG
1	M	57	Y	14.0	0.39	4	SC:UC	2.4	90	522	117	58	0	3.5
2	M	21	N	9.7	0.93	4	SC:UC	2.6	73	701	218	77	114	2.3
3	M	39	Y	9.4	0.22	4	SC:UA	1.6	62	208	133	28	256	2.1
4	M	42	Y	13.2	0.77	4	SC:UC	1.5	168	691	213	89	0	4.3
5	M	32	Y	6.8	0.42	4	SC:UC	3.3	162	207	45	0	100	3.6
6	M	43	Y	18.2	0.71	4	SC:UC	4.5	201	631	127	43	111	12.1
7	M	43	Y	15.6	0.44	4	SC:UC	1.2	122	306	231	32	134	3.7
8	F	32	Y	8.0	0.57	4	SC:UC	2.9	159	272	158	8	102	5.2
9	F	34	Y	12.8	0.37	4	SC:UC	4.1	152	1079	579	97	417	4.8
10	M	46	Y	16.0	0.28	4	SC:UA	1.2	51	330	110	0	200	1.2
Mean		39		12.4	0.51			2.5	124	495	193	43	143	4.3
±SD		10		3.8	0.23			1.2	52	283	147	36	123	3.0

SI: selectivity index; SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis; IEF: iso-electric focusing; SC:UA: serum cationic albumin, urine anionic albumin; SC:UC: serum cationic albumin, urine cationic albumin; A1M: alpha-1-microglobulin; RBP: retinol-binding protein; B2M: beta-2-microglobulin; NAG: N-acetyl-beta-D-glucosaminidase; SD: standard deviation

Selectivity Index (SI)

Selectivity Index (SI) was calculated from the ratio of IgG clearance over transferrin clearance.^{4,10} Serum and urine IgG and transferrin concentrations were measured by radial immunodiffusion technique. An SI of <0.2 indicates selective proteinuria while one of >0.2 indicates nonselectivity.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

This was performed on the PhastSystem (Pharmacia, Sweden) using the precast PhastGel, gradient 8-25 together with PhastGel SDS Buffer strips and stained with Coomassie Brilliant Blue. The pattern of proteinuria was categorized as previously described.⁸ Briefly, Pattern 1

shows middle molecular weight (MMW) proteins only (inclusive of albumin and transferrin); Pattern 2 shows MMW + low molecular weight (LMW) proteins (smaller than albumin); Pattern 3 shows MMW + high molecular weight (HMW) proteins (larger than transferrin); Pattern 4 shows MMW + LMW + HMW proteins.

Isoelectric Focusing (IEF)

This was also done on the PhastSystem using small precast PhastGel IEF 4-6.5 (Pharmacia, Sweden) as previously reported.⁹ Briefly, paired serum and urine samples were examined at the same time with 1 ul aliquots applied, focused at 2000 Volts, 8°C for a total of 1000 volt-hours and stained with Coomassie Brilliant Blue. The three IEF patterns were then determined as: SC:UA

TABLE III: PROFILE OF PATIENTS WITH DIABETIC NEPHROPATHY (DN)

No.	Sex	Age (year)	Hypertension (mg/dl)	Serum Creatinine	SI	SDS-PAGE (pattern)	IEF (pattern) (g/gCr)	Urine Albumin (mg/gCr)	Urine Orosomucoid (au/gCr)	Urine A1M (au/gCr)	Urine RBP (au/gCr)	Urine Lysozyme (au/gCr)	Urine B2M (u/gCr)	Urine NAG
1	F	60	N	1.4	0.32	4	SC:UA	1.2	115	61	0	0	0	2.4
2	M	46	Y	1.4	0.19	3	SC:UC	1.8	65	48	6	0	0	1.3
3	F	51	Y	1.4	0.39	4	SC:UC	4.4	310	155	0	0	0	8.6
4	M	61	Y	1.4	0.32	4	SC:UA	0.4	42	145		0	13	2.2
5	M	31	Y	1.1	0.30	3	SC:UA	1.3	46	42	0	0	0	2.9
6	F	55	Y	1.2	0.46	4	SC:UC	3.4	275	259	22	0	17	5.3
7	M	49	Y	1.3	0.10	4	SC:UC	1.4	82	76	0	0	0	1.8
8	M	37	Y	1.4	0.33	4	SC:UC	1.6	67	121	10	0	0	3.5
9	M	59	Y	1.4	0.10	1	SC:UA	0.4	31	43	0	0	0	2.9
10	M	57	Y	1.4	0.23	4	SC:UC	2.4	115	90	0	0	0	0.0
Mean		50		1.3	0.27			1.8	115	104	5	0	3	3.1
± SD		10		0.1	0.12			1.3	98	68	7		6	2.4

SI: selectivity index; SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis; IEF: iso-electric focusing; SC:UA: serum cationic albumin, urine anionic albumin; SC:UC: serum cationic albumin, urine cationic albumin; A1M: alpha-1-microglobulin; RBP: retinol-binding protein; B2M: beta-2-microglobulin; NAG: N-acetyl-beta-D-glucosaminidase; SD: standard deviation

(serum cationic albumin and urine anionic albumin); SA:UC (serum anionic albumin and urine cationic albumin); SC:UC (serum cationic albumin and urine cationic albumin).

Low Molecular Weight Proteins and Urine Enzyme Quantitation

The 5 LMW proteins were quantitated by radial immunodiffusion in gel containing 1% Agarose type L (Behring, Germany) plus 3% polyethylene glycol 6000 (BDH, England) in phosphate buffered saline. Orosomucoid (ORO, 44 kD) was quantitated against Human Serum Protein Calibrator (Dako, Denmark) and concentration expressed in mg/l. In the absence of commercial standards, alpha-1-microglobulin (A1M, 27 kD), retinol-binding protein (RBP, 21 kD), lysozyme (LYS, 15 kD) and beta-2-microglobulin (B2M, 12 kD) were quantitated against individual urine specimens with high specific protein concentration (arbitrary units assigned, 100 au/l) and concentrations were therefore expressed in au/l. N-acetyl-beta-D-glucosaminidase (NAG, 180 kD) was assayed using a test kit, standard and control from Boehringer Mannheim (Germany). This assay was done at room temperature (25°C) and activity expressed as u/l. All urinary protein measurements were expressed as units/gCr to standardise based on creatinine excretion so as to eliminate the need to collect timed urine."

Statistics

Data were expressed as means ± standard deviation (SD). Mann-Whitney test was used for comparing differences between the IgAN, ESRF and diabetic groups. Analysis of data in SDS-PAGE and IEF patterns was done with Pearson chi-square. SPSS for windows was used to calculate all statistics.

Results

Previous studies have established the pattern of proteinuria using SDS-PAGE and IEF in patients with IgA nephropathy.^{8,9} In the present study, the 10 patients with IgAN had either pattern 1 (MMW) or 3 (HMW and MMW) and none had pattern 2 (MMW and LMW) or pattern 4 (HMW, MMW and LMW). This is expected as all the 10 patients had normal renal function and would not be expected to have LMW proteinuria as in patterns 2 or 4 typically found in patients later on in the disease when they already have renal impairment.⁸ The pattern of proteinuria based on IEF showed 9 patients with SC:UA and only 1 with SC:UC pattern. Among 5 types of LMW proteins measured in the patients with IgAN none showed significant excretion of LMW proteinuria.

The SDS-PAGE pattern in the IgAN group differed significantly ($X^2 = 20$, $P < 0.001$) from the ESRF group (Table IV). The IEF pattern also differed between the IgAN and ESRF groups ($X^2 = 9.9$, $P < 0.005$). All 5 LMW proteins and 1 enzyme studied were present in significantly larger amounts in the ESRF group compared to the IgAN group (Table IV). Selectivity index was higher in the ESRF group compared to the IgAN group.

The SDS-PAGE pattern in the IgAN group was different from that of the DN group ($X^2 = 10.7$, $P < 0.005$) (Table IV). The IEF pattern was also significantly different between the IgAN and DN groups ($X^2 = 5.5$, $P < 0.02$). Of the 5 LMW proteins and 1 enzyme estimated in the urine, alpha-1-microglobulin and N-acetyl-beta-D-glucosaminidase (NAG) activity were present in significantly larger amounts in the DN group compared to the IgAN group ($P < 0.001$ and $P < 0.005$, respectively).

Comparing the DN group with the ESRF group (Table IV) there was no difference in their NAG activity and the SDS-PAGE and IEF patterns but the excretion of 4 LMW

TABLE IV: COMPARING DATA BETWEEN THE DIFFERENT PATIENT GROUPS

Group	Sex (M/F)	Age (year)	Hypertension	Serum Creatinine (mg/dl)	SI	SDS-PAGE (Nos.) (patterns 1, 3/2, 4)	IEF (Nos.) (pattern SC: UA /SC:UC)
Means $\pm$ SD							
IgAN (n = 10)	6/4	31 $\pm$ 9	2/8	1.1 $\pm$ 0.2	0.20 $\pm$ 0.09	10/0	9/1
ESRF (n = 10)	8/2	39 $\pm$ 10	9/1	12.4 $\pm$ 3.8	0.51 $\pm$ 0.23	0/10	2/8
DN (n = 10)	7/3	50 $\pm$ 10	9/1	1.3 $\pm$ 0.1	0.27 $\pm$ 0.12	3/7	4/6
<i>P</i> values							
IgAN versus ESRF	ns	ns	<0.005 X ² = 9.9	<0.001	<0.001	<0.001 X ² = 20	<0.005 X ² = 9.9
versus DN	ns	<0.001	<0.005 X ² = 9.9	<0.01	ns	<0.005 X ² = 10.7	<0.02 X ² = 5.5
DN versus ESRF	ns	<0.05	ns	<0.001	<0.02	ns	ns

TABLE IV: *contd.*

Group	Urine Albumin (g/gCr)	Urine Orosomucoid (mg/gCr)	Urine A1M (au/gCr)	Urine RBP (au/gCr)	Urine Lysozyme (au/gCr)	Urine B2M (au/gCr)	Urine NAG (u/gCr)
Means $\pm$ SD							
IgAN (n = 10)	1.5 $\pm$ 0.9	73 $\pm$ 53	24 $\pm$ 10	2 $\pm$ 3	0	0	0.7 $\pm$ 0.5
ESRF (n = 10)	2.5 $\pm$ 1.2	124 $\pm$ 52	495 $\pm$ 283	193 $\pm$ 147	43 $\pm$ 36	143 $\pm$ 123	4.3 $\pm$ 3.0
DN (n = 10)	1.8 $\pm$ 1.3	115 $\pm$ 98	104 $\pm$ 68	5 $\pm$ 7	0	3 $\pm$ 6	3.1 $\pm$ 2.4
<i>P</i> values							
IgAN versus ESRF	<0.05	<0.05	<0.001	<0.001	<0.005	<0.005	<0.001
versus DN	ns	ns	<0.001	ns	ns	ns	<0.005
DN versus ESRF	ns	ns	<0.001	<0.001	<0.005	<0.005	ns

SI: selectivity index; SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis; IEF: iso-electric focusing; SC:UA: serum cationic albumin, urine anionic albumin; SC:UC: serum cationic albumin, urine cationic albumin; A1M: alpha-1-microglobulin; RBP: retinol-binding protein; B2M: beta-2-microglobulin; NAG: N-acetyl-beta-D-glucosaminidase; SD: standard deviation; ns: not significant

proteins (AIM, RBP, Lysozyme and B2M) were significantly higher in the ESRF group ($P < 0.005$)

Discussion

We have previously shown¹² that in patients with IgA nephropathy, as the disease progresses and proteinuria increases with declining renal function, the pattern of proteinuria as assessed by SDS-PAGE and IEF undergoes changes. For SDS-PAGE, the change was attributed to an increase in LMW proteinuria. In a previous publication it has been shown that patients with LMW proteinuria on SDS-PAGE had an increased incidence of developing chronic renal failure. In the present study none of the 10 patients with IgAN had LMW proteinuria as they all had normal renal function.

In a previous study⁹ it was established that SC:UC pattern reflected patients with nephrotic syndrome who were unlikely to respond to treatment with steroid or cyclophosphamide. We have also shown in a more recent study¹² that the change in the pattern of proteinuria based on IEF was due to transformation from SC:UA pattern to SC:UC pattern (cationic albumin) and that patients with SC:UC pattern have a more severe disease. All 6 patients who had SC:UC pattern developed renal impairment. This change from anionic albumin to cationic albumin therefore reflects an adverse prognosis.

In the present study, the data showed that diabetics have the SC:UC pattern with loss of cationic albumin and LMW protein in the urine. This is consistent with the findings of other workers.^{13,14} Ghiggeri et al¹³ postulated

that glycation of serum albumin and the subsequent cationization may induce microalbuminuria in diabetics. A relationship between the amount of glycated albumin and its cationized form and the development and progression of diabetic nephropathy has been reported by Bundschuh et al.¹⁴

The excess loss of alpha-1-microglobulin¹⁵ and NAG activity¹⁶ in the urine of our patients with DN represents tubular damage. The loss of LMW proteins revealed by SDS-PAGE was recently reported by Pun et al¹⁷ as indicative of tubular damage. Alternatively, these changes could reflect proteinuria resulting from glomerular hyperfiltration^{18,19} as this pattern was similar to that of patients with IgAN studied 5 years later when they developed renal failure and increasing proteinuria presumably due to hyperfiltration.¹² In rats with 5/6 nephrectomies after 30 or 60 days of chronic renal failure, elevation of single nephron glomerular filtration rate due to increase in glomerular plasma flow and hydraulic pressure was observed. These hemodynamic alterations were followed by a rise in proteinuria and glomerulosclerosis. The conclusion was that preglomerular vasodilatation with glomerular hypertension was responsible at least in part for the presence of proteinuria and glomerular sclerosis.¹⁸

The comparative data of the group of patients with IgAN with the group of patients with ESRF who still had albuminuria 2.5 ± 1.2 g/gCr showed that the pattern between IgAN and ESRF was different. These data together with previous reports^{18,19} suggest that the presence of moderate to severe amounts of proteinuria in these patients with ESRF could be the result of protein excretion from the underlying glomerular disease as well as residual nephrons undergoing glomerular hyperfiltration and tubular dysfunction. This would explain why their pattern of proteinuria is different from those with IgAN but similar to those with DN though the ESRF group had more proteinuria with LMW proteinuria as the disease was more severe. Proteinuria observed when functioning nephrons are reduced has been attributed to elevation of glomerular plasma flow and pressure and the intensive transit of macromolecules through the glomerulus.¹⁸ It has been postulated that this intensive transit of macromolecules through the glomerular mesangium is an important factor inducing glomerular sclerosis. This mechanism of proteinuria is different from immune mediated models of chronic renal failure like IgA nephropathy and lupus nephritis where the immune complex mediated proteinuria consists of higher molecular weight and more anionic albumin in contrast to hyperfiltration where proteinuria is mainly LMW and more cationic albumin. This phenomenon would explain why the pattern of proteinuria in patients approaching ESRF is different from those of IgAN in our study.

Further data that may suggest hyperfiltration were obtained from a group of 10 patients with established DN with proteinuria but normal renal function to exclude proteinuria related to tubular injury secondary to renal failure. It has been suggested that proteinuria in DN (with normal renal function) could be the result of glomerular hyperfiltration.^{20,21} Our data showed that there was no basic difference in the pattern of proteinuria between the patients with DN and ESRF as assessed by SDS-PAGE and IEF though the ESRF group excreted significantly more LMW proteins in the urine as there is presumably more tubular injury in these patients compared to the DN group who still had normal renal function. There was a significant difference between the DN and IgAN groups; proteinuria in the DN group being attributed to glomerular hyperfiltration and tubular injury while that in the IgAN group was due to immune complex mediated proteinuria. We concluded that the data in this study showed that the pattern of proteinuria in ESRF and DN was the same (SDS-PAGE, IEF pattern) suggesting hyperfiltration injury as the main mechanism of proteinuria in these 2 groups. The characteristic features of proteinuria in these patients are the presence of LMW proteinuria on SDS-PAGE (patterns 2 & 4), SC:UC pattern (cationic albumin) on IEF and excess amounts of LMW proteins in the urine such as orosomucoid, alpha-1-microglobulin, retinol-binding protein and beta-2-microglobulin. These features typify the pattern of proteinuria in glomerular hyperfiltration with tubular injury.

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