Renal histological changes in relation to renal function and urinary protein excretion in IgA nephropathy

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Abstract

Renal function and urinary protein excretion (UPE) were investigated at the time of kidney biopsy in 24 children with IgA nephropathy. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by clearances of inulin and para-aminohippuric acid. For UPE albumin, IgG, β_2 microglobulin, and creatinine were analysed. Glomerular global/segmental sclerosis and crescents in the biopsy specimens were assessed, and glomerular and tubulointerstitial changes classified on a five degree scale.

The patients with tubulointerstitial or mesangial biopsy changes or glomerular sclerosis had significantly lower GFR than those without corresponding lesions. Patients with segmental sclerosis also had higher excretion rates of IgG, which increased with increasing segmental sclerosis. Six patients had GFRs below 2SD of the controls. Within the group of patients with reduced GFR overt albuminuria, a raised excretion rate of IgG, interstitial fibrosis, and advanced mesangial lesions were more frequent. A rising excretion rate of IgG seems to indicate both reduced GFR and increasing segmental glomerulosclerosis and may be a marker of progressive disease.

Since the first report in 1968 by Berger and Hinglais[1] IgA nephropathy has become recognised as a common form of chronic glomerulonephritis.[2 3] The growing awareness that IgA nephropathy is a progressive kidney disease in a certain percentage of cases leading to end stage renal failure has inspired a search for prognostic markers.[4–7] Glomerular sclerosis and interstitial fibrosis are histological features associated with an increased risk of developing renal failure.[2 6 8 9] An older age at the onset of the disease, heavy proteinuria, hypertension, and male gender are clinical predictors of a poorer prognosis.[2 4 8–11] IgA nephropathy may also lead to uraemia in childhood.[12–14] This study was designed to characterise the morphological changes in kidney biopsy specimens from children and adolescents with IgA nephropathy, and to correlate the findings with the pattern of urinary protein excretion (UPE) as well as with renal haemodynamics at the time of the kidney biopsy, and to evaluate the diagnostic and prognostic significance of UPE and renal haemodynamics.

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Patients and methods

UPE and renal haemodynamics were studied in 24 children and adolescents (16 boys, eight girls) with IgA nephropathy within 34 days of a kidney biopsy, mean (SEM) 2·8 (1·4) days. The mean (SEM) age of the patients was 13·3 (0·7) years and the investigations were performed 0·13–12·3 (mean 3·9 (0·7)) years after the first signs or symptoms of kidney disease. The patients were all in a stable condition without signs of current infection.

Renal biopsies were performed percutaneously using a Tru-Cut biopsy needle having an outer diameter of 2·0 mm (Travenol). Tissue for light microscopy was fixed in 3% buffered formaldehyde, and embedded in paraffin. Two to 3 micron thick sections were stained with haematoxylin and eosin, Ladewigs trichrome stain, and silver methenamine. Tissue for immunofluorescence microscopy was frozen immediately in liquid nitrogen. Unfixed frozen sections were incubated with fluorescein isothiocyanate (FITC)-conjugated antisera (Dako) against human IgG, IgM, IgA, C3, and C1q.

All biopsy specimens were examined without knowledge of other laboratory data or patient histories. The percentage of glomeruli showing global sclerosis, segmental sclerosis, and cellular crescents was calculated. A number of glomerular and tubulointerstitial parameters were then assessed semiquantitatively on a five degree scale (0, ±, +, ++, +++). These included glomerular mesangial expansion, mesangial cell proliferation, interstitial fibrosis and tubular atrophy, interstitial inflammation, and vascular lesions.

Urine analyses for UPE were based on timed short term collections, and blood was drawn in connection with the sampling period. Albumin, IgG, β_2 microglobulin, and creatinine were measured in serum and urine, and the respective excretion rates, clearances, and fractional clearances were calculated.

Renal function was determined as the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) and measured as clearances of inulin and para-aminohippuric acid during water diuresis using a standard clearance technique.[15] This includes a priming dose and continuous infusion of inulin and para-aminohippuric acid. The filtration fraction and fractional sodium excretion were also determined.

Serum albumin was analysed by an automated dye binding method with bromcresol purple[16]; urinary albumin and IgG, and serum IgG were analysed by an automated immunonephelometric method (Behring Nephelometer Analyser). β_2 Microglobulin was assayed by a

radioimmunological method (Phadebas, β2-micro Test, Pharmacia Diagnostics AB). Creatinine was determined by a kinetic method based on a modified Jaffé reaction,[17] and serum sodium with ion selective electrodes and urinary sodium by flame photometry. Inulin was analysed by the anthrone method[18] and para-aminohippuric acid by a modified Smith technique.[19]

To evaluate the distribution of proteinuria, the patients' urines were screened by the routine laboratory detection limits for the different proteins (albumin ≥30 mg/l, IgG ≥10 mg/l, β_2 microglobulin ≥100 μg/l). For statistical analyses the patients were grouped according to the type and extent of morphological changes in the biopsy specimen and according to the urinary excretion rates of the respective proteins: (1) urinary excretion rate of albumin <20 μg/min/1·73 m^2 (or urinary albumin <30 mg/l) (non-albuminuria), 20–200 μg/min/1·73 m^2 (microalbuminuria) and >200 μg/min/1·73 m^2 (albuminuria); (2) urinary excretion rate of IgG <10 and ≥10 μg/min/1·73 m^2, and (3) urinary excretion rate of β_2 microglobulin <200 and ≥200 ng/min/1·73 m^2.

The results in the different groups were expressed as the mean (SEM) and were compared by means of the Mann-Whitney non-parametric test. The χ^2 test was peformed for associations of categorical variables, and the correlation between variables was assessed by linear regression analysis. Thirty six healthy children and adolescents without any signs of kidney disease (mean age 12·8 (0·9) years, range 3·5–21·5 years) served as controls regarding renal haemodynamics.

Results

By definition, all biopsy specimens included in this study showed a positive immunofluorescence reaction in the glomeruli with IgA as the predominant immunoglobulin, which mainly occurred in the mesangial areas. In the vast majority of cases (92%) C3 was present in a similar pattern while C1q was usually negative. No patient showed any evidence of systemic disease associated with glomerular IgA deposits.

The distribution of the patients according to tubulointerstitial and glomerular changes is given in table 1. No biopsy specimen showed significant arteriolosclerosis. Thirteen of the 24 patients (54%) had glomerular sclerosis or crescent formations: nine exhibited glomeruli with global sclerosis (up to 10% glomeruli affected), eight segmental sclerosis (up to 19% glomeruli affected), and two had focal crescent formations.

There were no significant differences in UPE when the patients were grouped according to mesangial changes (volume expansion and cell proliferation). The patients with modest/moderate (+/++) mesangial changes (volume expansion as well as cell proliferation) had a lower GFR than those with no mesangial alterations (table 2). Mesangial volume expansion associated significantly with mesangial cell proliferation (fig 1). The duration of the IgA nephropathy was not longer in the four patients with mesangial volume expansion predominating over mesangial cell proliferation.

The patients with glomerular sclerosis (global as well as segmental) had significantly lower GFR than those without corresponding glomerular changes (fig 2). The patients with segmental sclerosis also had higher urinary excretion rates of IgG (55·1 (13·4) μg/min/1·73 m^2, $p<0·05$), higher IgG clearance, higher fractional IgG clearance, and higher serum β_2 microglobulin concentrations (2·5 (0·4) mmol/l, $p<0·01$) compared with the patients with no segmental sclerosis (IgG excretion rate 14·8 (5·0) μg/min/1·73 m^2 and serum β_2 microglobulin 1·3 (0·1) mmol/l). There were highly significant correlations between the proportions

Table 1 Distribution of the patients according to the extent (semiquantitative score) of tubulointerstitial and glomerular mesangial damage

Score	Interstitial fibrosis	Interstitial inflammation	Mesangial expansion	Mesangial cell proliferation
0	13	20	6	8
±	8	1	8	7
+	3	3	7	7
++	0	0	3	2
+++	0	0	0	0

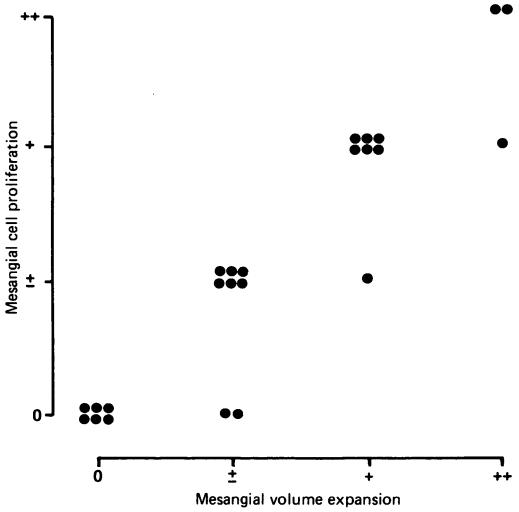

Figure 1 Correlation between the extent of mesangial cell proliferation and mesangial volume expansion (semiquantitative score).

Table 2 Mean (SEM) GFR ml/min/1·73 m^2 in the patients grouped according to extent (semiquantitative score) of glomerular mesangial and tubulointerstitial biopsy findings

Group	Extent of mesangial and interstitial changes		
	0 [n]	± [n]	+/++ [n]
Measangial volume expansion	122 (6) [6]	113 (5) [8]	100 (7) [10]*
Measangial cell proliferation	118 (5) [8]	119 (6) [7]	96 (7) [9]*†
Interstitial fibrosis	119 (4) [13]	105 (5) [8]*	76 (12) [3]**
Interstitial inflammation	115 (3) [20]‡	64 [1]	90 (17) [3]

*p<0·05 compared with 0, **p<0·01 compared with 0, †p<0·05 compared with ±, ‡p<0·05 compared with ±/+/++.

Table 3 Coefficients of correlation (r) between the extent of glomerular sclerosis and excretion rates, clearances, and fractional clearances of albumin and IgG respectively

	Segmental sclerosis (%)	*Segmental and global sclerosis (%)*	*Global sclerosis (%)*
Albumin excretion rate	0·80***	0·86***	0·59
IgG excretion rate	0·87***	0·84***	0·44
Albumin clearance	0·78***	0·88***	0·67**
IgG clearance	0·88***	0·90***	0·58*
Albumin clearance/inulin clearance	0·72**	0·85***	0·71**
IgG clearance/inulin clearance	0·83***	0·90***	0·68*

*p<0·05, **p<0·01, ***p<0·001.

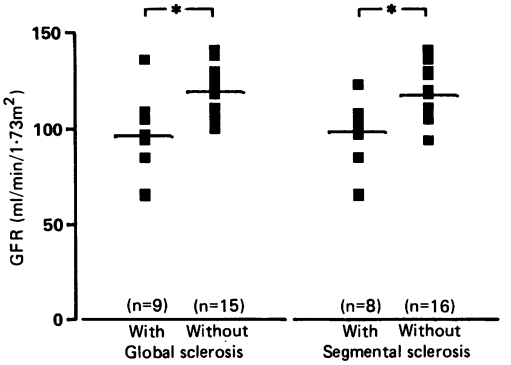

Figure 2 GFR in patients grouped according to the presence of global and segmental glomerular sclerosis respectively; p<0·05.

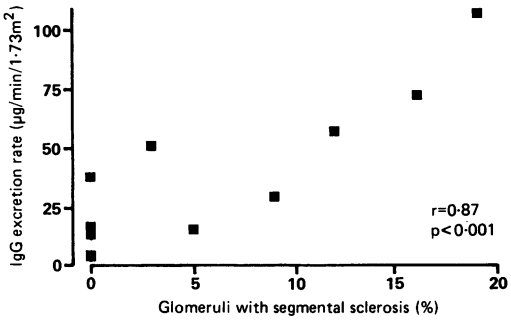

Figure 3 Correlation between the IgG excretion rate and the percentage of glomeruli with segmental sclerosis.

of sclerotic glomeruli and urinary excretion of albumin and IgG, which is illustrated in table 3 and fig 3.

The patients with tubulointerstitial changes in the biopsy specimen (fibrosis and/or inflammation), had a lower GFR than those with no interstitial changes. GFR (table 2) and ERPF declined with advancing fibrosis, while fractional sodium excretion increased. Fractional sodium excretion was significantly higher (2·03 (0·22)%, p<0·05) in patients with modest (+) interstitial fibrosis than in those with no (1·22 (0·10)%) or slight (±, 1·31 (0·17)%) fibrosis. There were no significant differences in UPE between the interstitial groups.

Sixteen of the 24 patients (67%) had urinary albumin >30 mg/l (mean 684 (433) mg/l), five patients (21%) were Albustix positive (urinary albumin >150 mg/l, mean 2040 (1256) mg/l), and three (9%) were nephrotic with urinary excretion rates of albumin of >40 mg/h/m^2, mean 3072 (1974) mg/l). Twelve patients (50%) had urinary IgG >10 mg/l (mean 59·8 (31·2) mg/l), and nine patients (39%) had urinary β_2 microglobulin of >100 µg/l (mean 200 (41) µg/l). Only one patient had a urinary β_2 microglobulin concentration above the upper reference limits (<375 µg/l).

The mean GFR (110 (4) ml/min/1·73 m^2) and ERPF (571 (24) ml/min/1·73 m^2) in the patient group were significantly reduced compared with controls (119 (2) and 627 (14) ml/min/1·73 m^2 respectively, p<0·05) but the filtration fraction did not discriminate (19·5 (0·7)% and 19·2 (0·4)% respectively).

No patient was hypertensive (mean systolic blood pressure 114 (2), diastolic 67 (2) mm Hg), but one boy with albuminuria and a reduced GFR was on antihypertensive treatment.

The albumin excretion rate was determined in 23 patients (one girl missed the urine collection). GFR in those without albuminuria (124 (5) ml/min/1·73 m^2) did not differ from controls, but those with microalbuminuria (104 (6) ml/min/1·73 m^2) and with albuminuria (95 (9) ml/min/1·73 m^2) had significantly (p<0·05) lower GFRs compared with non-albuminurics as well as with controls. The urinary excretion rate of IgG increased with rising urinary albumin excretion rate. The blood pressure tended to rise with increasing albumin excretion, and the albuminuric patients (mean blood pressure 118 (5)/74 (2) had significantly higher diastolic blood pressure (p<0·05) than the non-albuminurics (mean blood pressure 109 (3)/65 (3)).

The patients with rates of urinary excretion of IgG >10 µg/min/1·73 m^2 had a lower GFR (95 (6) ml/min/1·73 m^2, p<0·01) than those with excretion rates <10 µg/min/1·73 m^2 (119 (4) ml/min/1·73 m^2) but there were no further significant differences in renal haemodynamics or UPE between the two groups, nor in the groups based on excretion rates of β_2 microglobulin.

Six patients had GFRs below 2SD of the controls (<100 ml/min/1·73 m^2). A comparison of this group of patients using the χ^2 test to those with GFR >100 ml/min/1·73 m^2 revealed significantly more patients with overt albuminuria (4/6, p<0·05) and raised urinary excretion rates of IgG (6/6, p<0·01) and more patients with interstitial fibrosis (6/6, p<0·01), >± mesangial cell proliferation (6/6, p<0·05), >± mesangial volume expansion (5/6, p>0·05), and more global sclerosis (5/6, p<0·05).

One patient (a 13 year old boy) developed end stage renal failure within seven months of the kidney biopsy, which had demonstrated a slight mesangial volume expansion and cell proliferation, slight interstitial fibrosis, 19% glomeruli with segmental and 10% with global sclerosis. At the time of biopsy he was nephrotic with a reduced GFR (93 ml/min/1·73 m^2) and ERPF (466 ml/min/1·73 m^2).

Discussion

Although far from negligible, the morphological lesions of our patients were less prominent than those generally described in studies of adult IgA nephropathy patients.[2 3 20] Still the mean GFR and ERPF values at the time of biopsy were significantly lower than in controls, and one fourth of the patients exhibited a reduced GFR (below 2SD). Thus the severity of an IgA nephropathy, even in childhood, must not be underestimated.

Many investigations have been made to evaluate clinical factors predicting progressive renal disease in IgA nephropathy.[8 10] The type of glomerular lesions observed in the first renal biopsy in childhood IgA nephropathy has been claimed to be predictive of the clinical course of the disease.[13 11] Already at the time of biopsy our patients with more advanced morphological lesions (glomerular and/or tubulointerstitial) had lower GFRs than those with no or less pronounced changes in their biopsy specimens.

Mesangial volume expansion and mesangial cell proliferation coincided except in four cases with more pronounced mesangial expansion than cell proliferation (fig 1). These results differ somewhat from those of Yoshikawa *et al* who found predominant mesangial hypercellularity to be characteristic of early childhood IgA nephropathy, while the mesangial matrix increased later, suggesting progressive disease.[21 22] Their children with prominent hypercellularity had the shortest interval between onset of the disease and biopsy (mean (SD) 4·1 (4·0) months). All Japanese schoolchildren are screened yearly for urinary abnormalities and renal diseases are therefore often diagnosed at an early age in Japan. Our biopsies were performed with a median interval of 2·6 years from onset of disease, thus corresponding to the children studied by Yoshikawa *et al* with waning hypercellularity. Consequently the difference in our results mainly consists in our findings of persisting mesangial cell proliferation.

Half of our patients (54%) exhibited sclerotic glomeruli, and these patients had reduced renal function and higher IgG excretion, though no patient had more than 19% glomeruli with segmental sclerosis. These findings agree with the assumption that glomerular obsolescence and segmental glomerulosclerosis are associated with an increased risk of renal failure.[2 3 5–9] Vascular lesions are unusual in children,[12 22] and we recorded no vascular changes. Thus the glomerular sclerosis could not be attributed to ischaemic damage,[2 23] which also agrees with previous findings by D'Amico *et al*.[9]

Interstitial lesions, especially interstitial fibrosis, have been claimed to be associated with an increased risk of developing renal failure.[2 8] Although interstitial findings were sparse, the patients with interstitial changes had reduced GFR. The fact that interstitial lesions did not correlate with the extent of glomerular changes suggest that at least partly different mechanisms are responsible for glomerular and tubulointerstitial damage, which has also been observed previously by D'Amico *et al*.[9]

Heavy proteinuria is another marker of severe disease.[4 5 6 8 9] The reduced GFRs found in albuminuric as well as in microalbuminuric patients correspond to our previous findings[24] that microalbuminuria may also be a marker of more severe IgA nephropathy.[24] A rising IgG excretion rate seems to indicate both a reduced GFR and increasing segmental glomerulosclerosis. As the extent of segmental sclerosis is considered to be associated with an increased risk of developing renal failure,[9] raised IgG excretion may be predictive of progressive renal disease.

GFR was reduced in patients with comparatively modest mesangial, sclerotic, or interstitial changes and deteriorated further with more severe histological damage. An exact GFR determination is therefore of great importance in the follow up of IgA nephropathy. Although GFR was reduced even in patients with minor morphological changes, urinary albumin and IgG excretions distinguished the patients with more advanced renal damage and mainly sclerotic glomerular lesions.

In summary, our patients with reduced renal function and raised albumin and IgG excretions had more pronounced histological renal lesions. We suggest that an accurate determination of renal haemodynamics and UPE at the time of biopsy may give an indication of the extent of renal damage. The fact that the IgG excretion rate increased in the patients with more extensive segmental glomerular sclerosis may make it a valuable analysis in the follow up of IgA nephropathy. Increasing IgG excretion may be an early marker of progressive disease.

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Early diet and eczema

The association between early feeding and the later development of atopic diseases is a hardy perennial of a topic which has generated many an article but so far little of real practical importance. The role of breast feeding in this respect is still not clear. In a recent study from New Zealand, Ferguson and his colleagues (*Pediatrics* 1990;86:541–6) have examined the association between early solid feeding and later eczema.

They collected data on a cohort of 1265 children born in Christchurch in 1977 who were seen at birth, at 4 months, and at annual intervals to the age of 10 years. At the 4 month visit the amount and types of solid food given to the child were recorded, the foods being classified into six types: dairy products, egg or related products, cereals, vegetables, meat products, and fruit.

This report concerns the incidence of recurrent or chronic eczema. The diagnosis was accepted from the mother's report provided that the child had seen the family doctor at least three times for eczema, the condition had been present for at least three consecutive years, and the child was receiving regular treatment for eczema.

The overall incidence of recurrent or chronic eczema by the age of 10 years was 7·5%. The association between individual components of the early diet and later eczema was weak and not statistically significant. In comparison with those who had been given no solids up to the age of 4 months the risk of later eczema was increased by a factor of 2·35 in those who had received four or more types of food and by 1·4 in those who had received between one and three types. The findings were still significant after allowing for possible confounding variables such as family atopy, early breast feeding, or social background.

The authors therefore conclude that the development of eczema is related to the diversity of early diet rather than to individual dietary components.

Presumably for each individual child there may be a small number of dietary antigens which are important but the risk of contact with those antigens increases with greater dietary diversity. If you're playing Russian roulette the fewer live bullets in the chamber the greater are your chances of telling the tale.

These results need to be confirmed, but perhaps too much diversity in the diet of young babies is not a good thing, especially when there is a close family history of atopy.

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