Prognostic significance of the number of renal glomeruli in reflux nephropathy

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OBJECTIVE

To monitor the decrease in the number of glomeruli in reflux nephropathy (RN) and to investigate its association with glomerular hypertrophy or clinical data, as some cases of RN progress to end-stage renal failure, although the mechanism of progression remains unknown and is generally thought to depend on remnant normal renal tissue mass, i.e. the number of remnant renal glomeruli (functional nephrons).

PATIENTS AND METHODS

From 1987 onward, a renal biopsy was taken in 71 patients (mean age 8.08 years) in two institutions to estimate the prognosis of patients with RN. The number of glomeruli per unit area and glomerular size in grossly normal renal tissue specimens were determined to explore the potential association between these variables and the total renal scars, renal function (glomerular filtration rate, GFR), or daily urinary protein excretion.

RESULTS

The number of glomeruli was closely correlated with the actual size of glomeruli ($y = 14.783 - 0.052x$, $R = 0.782$). To a lesser extent, the number of glomeruli was also closely correlated with the size of glomeruli expressed in SDs ($y = 6.264 - 0.832x$, $R = 0.630$). There was a good correlation between the number of glomeruli and the extent of renal scarring, renal function, or daily urinary protein excretion, although its association with renal function (GFR) was least evident.

CONCLUSION

There was an association between the decrease in the number of glomeruli and glomerular hypertrophy, decreased renal function, or increased proteinuria. A renal biopsy taken from radiographically and macroscopically normal regions can be useful for assessing RN, and the size and number of glomeruli in the specimens might provide an important measure for estimating the prognosis of RN.

KEYWORDS

vesico-ureteric reflux, reflux nephropathy, renal biopsy, number of glomeruli, glomerular hypertrophy

INTRODUCTION

Currently, reflux nephropathy (RN) is widely interpreted as renal parenchymal damage associated with VUR [1–3]. One of the major concerns with RN is that this type of nephropathy progresses to end-stage renal failure (ESRF) in some patients [4,5], but the mechanisms of disease progression to ESRF remain unclear.

RN is characterized by the occurrence of localized damage, e.g. local renal scar formation. Our previous histopathological examination of renal tissues obtained by open renal biopsy showed the progression of tissue damage in radiographically normal regions with no scars in patients with RN, the extent of which was closely associated with proteinuria, renal function, or other clinical findings. In addition, quantitative analysis of radiographically identified renal damage in both the left and right kidneys showed that glomerular size and the glomerulosclerosis index (percentage of overall sclerosis) increased steeply in radiographically normal renal tissues with total renal scars above a certain threshold. This steep change was associated with total renal scars of grade a + c (presented by a combination of bilateral renal scarring grades according to Smellie's classification); therefore, this was presumably a threshold of irreversible renal damage. The degree of glomerular hypertrophy is thus expressed as a threshold glomerular size of two SD above the age-adjusted normal values, and is thus expressed in SD 'units' in this report. The threshold was also associated with a glomerulosclerosis index of 18%, urinary protein excretion of 105 mg/day and a DTPA-estimated GFR of 70 mL/min; we previously reported these calculations as threshold values [6].

As suggested by these findings, the primary factor in the progression of RN is generally thought to depend on remnant normal renal tissue mass, i.e. the number of remnant renal glomeruli (number of nephrons). As a mechanism of progression to renal failure, the functional overload on remnant glomeruli associated with fewer normal glomeruli (hyperfiltration) was proposed [7,8], which might induce glomerular hypertrophy, glomerulosclerosis and finally renal failure [6,9,10].

Thus the present study was designed to monitor the decrease in the number of glomeruli and to assess its association with glomerular hypertrophy and clinical data on renal function and proteinuria, in patients with RN.

PATIENTS AND METHODS

The study included 92 patients who had a renal biopsy taken at the authors' institutions from 1987 onward, to assess the degree of RN and the prognosis. Of these, 91 patients had a renal biopsy taken almost concurrently with

TABLE 1 The characteristics of the patients in whom the number of glomeruli was determined

Characteristic	Male	Female
Total number of patients	31	40
Number of patients with:		
reflux grade (biopsy side)*		
low (I, II)	2	6
moderate (III)	5	15
high (IV, V)	24	19
scarring grade†		
none or local (a, b)	11	34
diffuse (c, d)	20	6

*$P < 0.032$; †$P < 0.001$.

antireflux surgery, and only one had antireflux surgery before 1987 and renal biopsy during the follow-up after 1987.

We took a renal biopsy in patients who had grade ≥ III VUR, according to the international grading system, or radiographically confirmed renal scars. However, from 1992 onward patients were eligible only if they had VUR and renal scars. Moreover, a renal biopsy was taken only in those who were fully informed and provided consent. Patients and their parents (if the patient was a child) were informed that the invasive examination was not a treatment, that the objective was to determine the degree of renal damage caused by VUR, and that they would possibly know the prognosis of RN.

Of the 92 patients, 10 were excluded because they had causes of secondary VUR (phimosis in one, urethral valve in one, urethral ring in two, uninhibited bladder in three, tethered-cord syndrome in one, and prune-belly syndrome in two), leaving 82 patients. As described below, patients who were not evaluable for the number of glomeruli were also excluded. Consequently, 71 patients were included in the following analysis. There were 31 males (mean age 7.29 years, range 0.33–18) and 40 females (mean age 8.69 years, range 0.58–40); the genders were of similar age (no significant difference), although males had more severe reflux and scars in the kidney that was biopsied (Table 1).

Renal scars were assessed primarily by IVU and ^{99m}Tc-DMSA renal scintigraphy, and CT was also used as an adjunct. The degree of scarring was graded according to a modified Smellie classification [11], in which 'N' was added to the original system to establish a five-point scale (N, normal with no scars; a, one or two scars; b, three or more scars or scars covering either extremity of the kidney; c, generalized scars, parenchymal thinning, or a small kidney; d, an atrophic kidney with markedly decreased renal function). To quantitatively assess bilateral renal scars, the total renal scar score for each patient was obtained as a sum of numerical ratings, where N = 0, a = 1, b = 2, c = 3 and d = 4. To avoid the potential influence of UTI, scars were assessed 3–6 months after the last infection.

For the renal biopsy, an open approach was used to access the lower pole of the kidney with the greater renal damage, as shown by more significant renal scars and atrophy, and a wedge resection was used on normal-appearing renal parenchyma, rather than regions with grossly identifiable scars, atrophy or poor blood circulation. Methods for specimen processing, staining and measuring glomerular size were reported previously [6]. Indices of glomerular size included the absolute measurement of the maximum glomerular diameter, and the difference between the maximum glomerular diameter and age-adjusted normal control, as expressed by the SD.

For normal values of glomerular size by age, our previously reported results were used [6]. For control values of adult glomerular size, the size of glomeruli in non-tumoral renal tissues excised in our department from eight young patients with T1 RCC was measured and served as normal values for Japanese adults; the mean (SD) was 213.6 (15.86) μm, and very similar to previously used adult normal values reported by Zollinger *et al.* [12].

Open renal biopsy specimens from the surface of the kidney were examined microscopically at ×100 to count the glomeruli. The number of glomeruli per unit area was obtained by dividing the number of glomeruli on the monitor by the area of a visual field ($0.72 \times 0.95 = 0.684$ mm^2). However, analysis was limited to patients for whom at least five non-overlapping fields of view were available, and the mean served as the mean number of glomeruli for each patient. Because patients were excluded if they had fewer than five fields of view available, 71 patients were included in the subsequent analyses (Table 1). To assess proteinuria, the total protein in a 24-h urine specimen was measured by the sulphosalicylic acid method. The GFR was determined with ^{99m}Tc-DTPA [13].

Regression analysis was used to assess the potential association between the mean number of glomeruli in specimens and each variable (actual measurement of glomerular size, age-adjusted SD of glomerular size, daily urinary protein excretion, renal function, i.e. GFR, and total renal scar score). Linear regression lines and correlation coefficients were obtained, and the significance was tested by ANOVA.

RESULTS

Of the patients with primary VUR who had a renal biopsy for assessing RN, the number of glomeruli per unit area was calculated in 71 who had at least five non-overlapping fields of view of the renal surface of open renal biopsy specimens, and for which glomeruli were reliably counted within the renal tissue specimens. Attempts were made to correlate the number of glomeruli with glomerular size or other clinical data. First, any association of the number of glomeruli with glomerular size was evaluated. For this purpose the actual measurements of glomerular size and the SD corrected by age-adjusted paediatric normal values were used for each patient; the regression equation for the first is shown in Fig. 1a and for the second in Fig. 1b. For both variables there was an association, suggesting that glomeruli increased in size as the number of glomeruli decreased. Most patients were children, and the SD for the difference from age-adjusted normal values was used to allow age difference-adjusted comparisons, as reported previously. However, the number of glomeruli was more closely correlated with the actual measurement of glomerular size.

The regression equation for renal function (GFR), which had been expected to be most strongly associated with the number of glomeruli, is shown in Fig. 1c. Although there was an association it was not as strong as that with glomerular size, and less evident than with proteinuria or renal scars (below). There was a close association between the number of glomeruli and daily total urinary protein excretion (Fig. 1d), indicating that urinary protein excretion increased exponentially as the number of glomeruli decreased. The renal biopsy was taken from macroscopically normal renal tissues rather than those with renal scars. Thus the number

of glomeruli in renal biopsy specimens (density) was expected to be least associated with radiographically determined total renal scars, but there was a close association (Fig. 1e), clearly indicating that the number of glomeruli in macroscopically normal regions decreased as total renal scarring increased.

DISCUSSION

There is no doubt that the prognosis of RN depends on remnant normal renal tissue mass, i.e. the number of remnant normal glomeruli. As with the mechanisms of progression of many other conditions to ESRF, it is understood that overload on remnant glomeruli (hyperfiltration) associated with fewer functional glomeruli [7,8] can induce the morphological changes of glomerular hypertrophy, which progress to glomerulosclerosis and ultimately renal failure [3,6,9,10,14].

The pathological characteristics of renal damage associated with RN include tubulo-interstitial damage, and glomerular damage that can occur secondarily to ischaemia or hyperfiltration. However, remnant glomeruli might be relevant in elucidating the pathological feature of renal damage in progressive RN, because renal dysfunction in RN eventually involves glomerular injury.

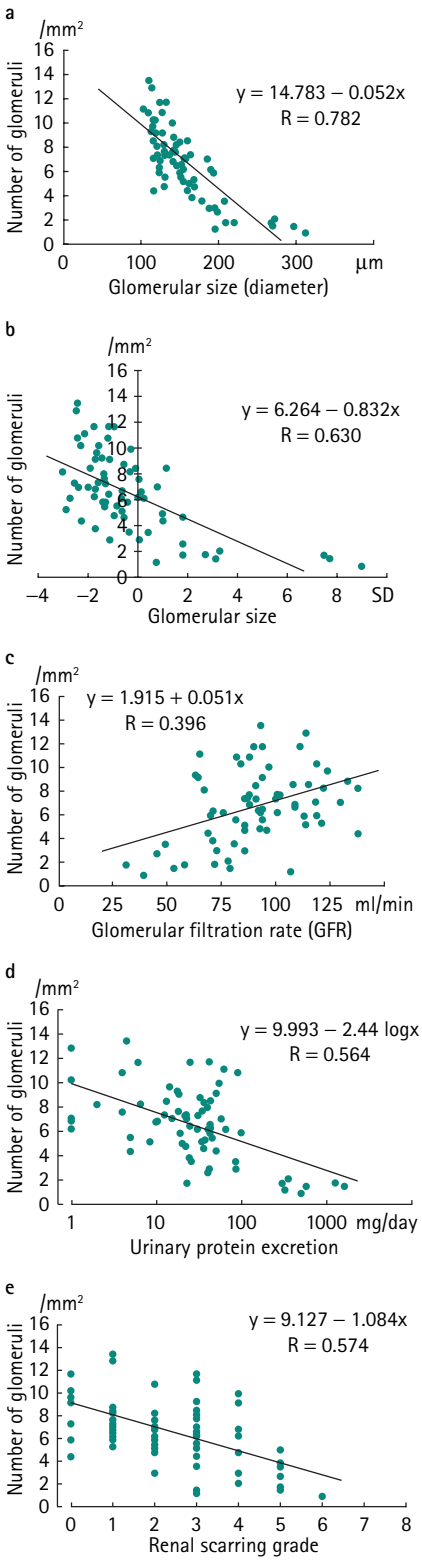
Since 1987, to determine the factors for progression of RN and its threshold, we have taken renal biopsies in patients with RN and clinicopathologically examined them. Our previously reported results in 48 patients were: (i) in RN, glomerular injury can occur not only in regions with scintigraphically identifiable scars, but also in radiographically and macroscopically normal regions; (ii) the threshold for progressive RN is probably associated with notable glomerular hypertrophy, as indicated by a glomerular size that is two or more SD above the age-adjusted normal values; and (iii) the threshold is probably associated with relatively mild clinical abnormalities, with a GFR of 70 mL/min and a urinary protein excretion of 105 mg/day [6]. Thus, we reported that the occurrence of glomerular hypertrophy in the remnant renal region is an important indicator for progressive RN. We thought it necessary to monitor the decrease in the number of glomeruli (a probable cause of the occurrence) and to investigate its association with glomerular hypertrophy, and clinical data on renal function and proteinuria in patients with RN.

However, there are few reports showing the decrease in the number of glomeruli in renal tissues of patients with RN, most of which addressed resected renal tissues. Furthermore, increased scarring and developmental abnormalities, e.g. hypoplasia and dysplasia in renal morphogenesis, might also be involved in the decrease in functional renal tissues in RN [15].

In the present study we focused on the number of functional glomeruli in RN to elucidate these issues. We took open renal biopsies of grossly normal regions rather than those with renal scars or poor blood circulation, and collected wedge specimens, as we think it is essential to evaluate the size and number of glomeruli in normal remnant renal regions. The study showed that the decrease in the number of glomeruli was closely correlated with an increase in glomerular size (Fig. 1), that the number of glomeruli was inversely proportional to the degree of radiographically identifiable total renal scarring, and was proportional to GFR, a measure of renal function. In addition, the decrease in the number of glomeruli was exponentially correlated with the increase in urinary protein. These results show clearly that the decrease in the number of glomeruli is associated with the increase in glomerular size, i.e. glomerular hypertrophy in RN. Most of the patients were children, and the SD for the difference from age-adjusted normal values was used to allow age difference-adjusted comparisons. However, the number of glomeruli was more closely correlated with the actual measurement of glomerular size; moreover, the close correlation with decreased renal function or increased urinary protein shows its clinical involvement in the progression of renal damage.

Glomerular hypertrophy occurs with the decrease in the number of remnant functional glomeruli [16]. The hypertrophy is thought to be induced by mechanisms (e.g. cytokines and other mediators) to compensate for haemodynamic changes or decreased glomerular function. Thus, glomerular hypertrophy may be a change that occurs diffusely in the kidney. Therefore, we still think that glomerular hypertrophy is the most important finding in estimating the prognosis of RN from renal biopsy specimens. However, we initially thought that the number of

FIG. 1. The relationship between the number of glomeruli and: ***a,*** *glomerular size (actual measurement);* ***b,*** *glomerular size (as SD);* ***c,*** *renal function (GFR);* ***d,*** *daily total urinary protein excretion; and* ***e,*** *total renal scar score.*



glomeruli would be unlikely to be the best indicator, because the renal biopsy was taken from radiographically and macroscopically normal renal tissues rather than those with renal scars.

Renal hypoplasia or dysplasia might be involved in RN [17]; hypoplasia during nephrogenesis can often occur locally or predominantly in either kidney. We therefore thought that assessing the number of glomeruli by renal biopsy, a highly restricted tissue sampling method, would have limitations. Unexpectedly, the method yielded a good correlation (Fig. 1e), clearly indicating that the number of glomeruli in macroscopically normal regions decreases as overall renal scarring increases. This might be because reflux has some influence on regions with no noticeable scars. Conceivably, increased scarring may enhance the influence of ischaemia and other events on unscarred regions, which possibly results in glomerular injury, glomerulosclerosis, and ultimately loss of glomeruli. Alternatively, kidneys susceptible to renal scarring may be complicated by marked hypoplasia; therefore, those kidneys intrinsically have fewer glomeruli in regions with no scars. All these factors might be involved.

The number of glomeruli was more closely correlated with actual measurements of glomerular size than with age-adjusted glomerular size, possibly because glomeruli do not enlarge beyond a certain limit, regardless of age. Because glomerular epithelial cells (podocytes) are terminally differentiated, non-regenerative cells, glomerular hypertrophy induces podocyte injury and may progress to glomerulosclerosis when glomeruli enlarge to a certain limit of size [18,19]. These changes are also likely to occur in children. Tada *et al.* [20] examined renal biopsy specimens taken concurrently with anti-VUR surgery in patients with RN, and assessed renal function 10 years later for comparison. They reported that the diameter of glomeruli did not change and that cystic dilatation and marked lengthening of glomerular capillaries, adhesion of the tuft to Bowman's capsule, and podocyte detachment all predicted progressive renal dysfunction.

Renal function (GFR), which had been expected to show the closest association, actually showed the least association with the number of glomeruli. This might be explained by a renal functional reserve [21], which possibly precludes an accurate assessment of the degree of renal dysfunction at a given stage. More specifically, a numerical decrease in GFR might not directly represent impaired renal function. We therefore think that the increase in glomerular size rather than GFR provides a relatively accurate representation of the decrease in the total number of glomeruli, a manifestation of advanced RN, because the decrease in the number of glomeruli was most closely correlated with the increase in glomerular size.

We previously reported the number of glomeruli per unit area corresponding to various indicators of progressive RN; a glomerular size of two SD, a GFR of 70 mL/min and a urinary protein excretion of 105 mg/day were associated with 4.60, 5.49 and 5.06 glomeruli per mm^2, respectively. The threshold for progressive RN is probably associated with ≈5.0 glomeruli/mm^2 of renal biopsy specimen. The present data represent two-dimensional information on small renal biopsy specimens. Further studies are warranted to investigate the association of glomerular hypertrophy or clinical data with the number of glomeruli estimated in the whole kidney, as shown in renal grafts by Fulladosa *et al.* [22]. However, this requires accurate three-dimensional information on regions with no scars, which is not easy to obtain in patients with RN.

Many questions await further studies, e.g. the limit of glomerular enlargement and the relationship between actual progression of RN and glomerular hypertrophy or the decrease in the number of glomeruli. It is some time since we started our histological study on RN and we will continue the study, considering temporal factors. However, our method for renal biopsy can be criticised as invasive; although we think that gross examination of the renal surface is important to collect appropriate wedge specimens, we have established stricter criteria for the indication, as we have better knowledge of the thresholds. We are now using the procedure in patients with bilateral multiple renal scars and at least one small kidney. We will use a less invasive procedure such as laparoscopy-guided biopsy.

Finally, renal lesions associated with RN are characterized by tubulo-interstitial lesions; there is a strong relationship between the degree of interstitial lesions and that of renal damage [23]. Furthermore, Nath [24] suggested the possibility that tubulo-interstitial damage might progress independently as glomerulosclerosis progresses, because of nephron loss-induced glomerular hypertension and hyperfiltration. It is therefore necessary to elucidate the mechanism and indicators of progression of RN from the viewpoint of tubulo-interstitial damage. In the present study we did not analyse subgroups of gender; it is deemed appropriate to analyse data in the population of males and females combined, because progressive RN might ultimately occur when normal renal tissue mass decreases to the threshold level. However, male patients had significantly more severe renal scars; consequently, glomerular hypertrophy and the decrease in the number of glomeruli appeared to be more evident in males. In view of these findings, more attention should be paid to male patients, and further investigation is warranted.

In conclusion, the decrease in the number of glomeruli is associated with glomerular hypertrophy, decreased renal function, and increased proteinuria in RN. A renal biopsy taken from radiographically and macroscopically normal regions is important in RN, and assessing the size and number of glomeruli in the specimens can provide an important indicator for the prognosis of RN. The threshold for progressive RN is probably associated with ≈5.0 glomeruli/mm^2 in a renal biopsy specimen.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **RN**, reflux nephropathy; **ESRF**, end-stage renal failure.