Thin basement membranes in minimally abnormal glomeruli

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Abstract

The light microscopic, immunofluorescence, and electron microscopic appearances of renal biopsy specimens were reviewed and correlated with the clinical and laboratory findings in 61 patients in whom the findings were initially considered to be either normal or to show only minor non-specific abnormalities. In all cases this reassessment included quantitative measurement of glomerular basement membrane thickness by an orthogonal intercept technique. On the basis of the indication for biopsy, patients were classified into three groups: those with haematuria (group I, n = 41); those with a minor degree of proteinuria (group II, n = 16); and those without any urinary abnormality but in whom possible renal disease as a result of systemic disease was suspected (group III, n = 6). About half of the patients with haematuria had significantly thinner glomerular basement membranes than those in the other two groups, irrespective of the variable selected for assessment, and in three this was confirmed in follow up biopsy specimens. Follow up for up to eight years showed that in patients either with or without thin basement membranes haematuria commonly persisted, but the long term outlook in all three groups was otherwise good and no patient developed impaired renal function.

Renal biopsy specimens from patients who are being investigated for asymptomatic haematuria or proteinuria are often either normal or show only mild non-specific morphological changes, and immunofluorescence fails to show specific abnormal protein deposits. Although there are many patients with these minor lesions at renal biopsy there have been few systematic studies of them. It has been recognised, however, that in some patients with minor abnormalities at light microscopy who also have a history of haematuria an abnormally thin glomerular basement membrane is evident by electron microscopy. In some cases the abnormalities have been focal^{1,2} but in others there has been generalised attenuation of the glomerular basement membrane.³⁻¹² This was first noted in children^{3-6,8} but several adult cases have also been reported.^{7,9-12} There is often a family history of haematuria; a few of these patients have had features of Alport's syndrome^{8,10} but

most have been classified as cases of benign familial haematuria, although morphologically identical basement membrane thinning has been described in the absence of a family history of renal disease.^{5,7-12} The condition seems to have a benign prognosis, although in a few cases some impairment of renal function has been observed on follow up.⁹

In this study a series of patients was studied in whom renal biopsy specimens, when first examined, were classified as being either normal or as showing only "minor non-specific abnormalities". The histology, results of immunofluorescence, and the ultrastructural appearances were all reviewed and, in particular, glomerular basement membrane thickness was determined by a morphometric technique. These findings have been correlated with the clinical features and laboratory data at the time of presentation and during subsequent follow up.

Methods

Sixty one patients were selected from the biopsy files of the department of anatomical pathology at this hospital. Of these, 10 were classified as being within normal limits on the basis of both light microscopical and immunofluorescence appearances while the remaining 51 were considered to have minor glomerular abnormalities, which, in some cases, were associated with minor tubulointerstitial lesions. In all of these patients the case histories were reviewed to obtain the clinical and family histories in addition to results of routine urinalysis and renal function tests both at the time of the initial biopsy and during follow up.

On the basis of the indication for renal biopsy these 61 patients were subdivided into three groups.

Group I comprised 41 patients with recurrent or persisting haematuria. This was macroscopic in eight but detected only on routine urinalysis in the remaining 33.

Group II comprised 14 patients with recurrent or persistent proteinuria below the nephrotic range (3.5 g/24 hours).¹³ This excluded all patients in the files in whom a diagnosis of "minimal change" glomerulonephritis (lipoid nephrosis) had been made mainly on the basis of electron microscopical appearances when the biopsy specimen was first examined.

Group III comprised six patients in whom renal biopsy had been done to investigate the

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possibility of renal disease in various systemic diseases and in whom there was no abnormality on urinalysis.

LIGHT MICROSCOPY

Tissue was fixed for one hour in formol sublimate (900 ml saturated mercuric chloride, 100 ml 10% neutral buffered formalin). After embedding in paraffin wax, 2 μ m sections were cut at multiple levels through the needle core. Sections from at least three levels were stained with haematoxylin and eosin and additional sections were stained by periodic acid Schiff, periodic acid-silver methanamine with a Masson counter-stain, aldehyde fuchsin Gomori, Lendrum's stain for fibrin, Perls's method for haemosiderin, and methyl green pylonin and Congo red or Sirius red B for amyloid. In all cases the original biopsy material was reviewed and the degree of any abnormality was scored on a standard form.

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Tissue was snap frozen in a liquid nitrogen/isopentane mixture. Cryostat sections, 6 μ m thick, were routinely stained by a standard direct immunofluorescent technique for whole globulin, IgM, IgG, IgA, C3, Clq and albumin.

ELECTRON MICROSCOPY

Tissue blocks, 1 mm³, were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer and post fixed in 1% osmium tetroxide. After dehydration in ethanol the tissue was embedded in Epon-Araldite. Glomeruli were selected by light microscopical examination of 0.5 μ m sections stained with toluidine blue, then ultrathin sections (gold) were stained for electron microscopy, with 5% uranyl acetate in

50% methanol in distilled water followed by lead citrate (final concentration 0.04 M lead 0.08 M sodium hydroxide).¹⁴

ULTRASTRUCTURAL MORPHOMETRY

Measurements were made from electron micrographs which covered the entire cross sectional area of up to three glomeruli in each case. In all cases the final print magnification used was 23 300, determined by the use of a standard carbon replica grating. Sites for measurement of basement membrane thickness were selected by a strictly randomised procedure.¹⁵ Each electron micrograph was overlaid with a sheet of plastic on which had been drawn a sampling squared line grid with a line distance of 2.3 cm (fig 1). Where the grid intersected with the plasma membrane of an endothelial cell in a peripheral capillary loop, at a site where endothelial and epithelial cytoplasmic membranes were clearly visible, the shortest distance perpendicular from the intercept between the grid and the endothelial aspect of the basement membrane to the epithelial membrane was measured to within 0.1 μ m with a pointed leg caliper. This distance has been defined as the length of the orthogonal intercept.¹⁶ About 100 such measurements were taken for each case.

The means and the maximum and minimum values of basement membrane thickness of each patient in each of the three groups of patients were plotted as histograms at intervals of 100 nm and the significance of the difference of mean values of basement membrane thickness among the groups was assessed by the non-paired Student's *t* test and by the non-parametric Mann-Whitney and Kolmogorov-Smirnov tests.

Figure 1 Electron micrograph of glomerulus with transparent sampling line grid superimposed. The original line distance of grid was 23 mm. (a) Actual sample size; (b) reduction of original to show a representative standard micrograph used for morphometry.

Table 1 Clinical and laboratory features in patients with normal glomeruli or with minimal glomerular lesions

	Mean age (years)	Sex M:F	Diastolic blood pressure > 90 mm	Loin pain	Blood urea mmol/l (mean)	Serum creatinine mmol/l (mean)	GFR ml/seconds (mean)	Serum protein g/l (mean)	Serum albumin g/l (mean)	Serum C3 g/l (mean)
Haematuria: Group I (n = 41)	33.3	1:2.5	4/41	7/41	5.4	0.08	1.66	70.1	42.2	1.16
Proteinuria: Group II (n = 14)	32.8	1:3	3/14	—	5.6	0.15	1.32	67.0	44.8	1.08
Normal urine: Controls (n = 6)	40.5	2:1	—	—	4.8	0.07	1.28	74.6	38.8	0.95

GFR: glomerular filtration rate.

Table 2 Summary of renal biopsy findings in minimal glomerular lesions

	Light microscopy					Immunofluorescence							Electron microscopy		
	Normal	Mesangial widening	Glomerular sclerosis	Tubulo inter-stitial change	Arterio-sclerosis	Negative	IgG	IgM	IgA	C3	Clq	Fibrosis	Loss of foot processes	Mesangial widening	Deposits
Haematuria: Group I (n = 41)	13 (31%)	24 (58%)	4 (9%)	7 (17%)	5 (12%)	28 (68%)		15 (36%)		13 (31%)			5 (12%)	24 (58%)	
Proteinuria: Group II (n = 14)	5 (35%)	7 (50%)	2 (14%)	3 (21%)	2 (14%)	7 (50%)		5 (35%)		7 (50%)	2 (14%)		5 (35%)	7 (50%)	
Normal value: Controls (n = 6)	4 (66%)	2 (33%)												2 (33%)	

Results

CLINICAL FEATURES AND LABORATORY INVESTIGATIONS

Data collected when the initial biopsy was performed are summarised in table 1. Of the patients with haematuria (group I), seven complained of loin pain. In group II patients in whom biopsy was done for proteinuria, total serum protein was slightly less than in the other two groups but this could not be attributed to a low serum albumin. Serum creatinine was slightly increased in these patients but there was no associated reduction in glomerular filtration rate as measured by creatinine clearance.

LIGHT MICROSCOPY

The principal morphological changes evident on review of the sections from these patients are summarised in table 2. In some the appearance of glomeruli was within normal limits (fig 2), while in others there was a minor degree of thickening of the mesangial matrix in at least some glomerular lobules (fig 3), and an occasional globally sclerosed glomerulus was present (fig 4). The tubulointerstitial changes consisted of focal atrophy of tubules, usually with some narrowing of the lumens, associated with some mild fibrous thickening of the interstitial tissue (fig 4). Changes classified as "arteriosclerosis" consisted of mild eosinophilic thickening of arterioles, or distal interlobular arteries, or some thickening of the intima in proximal interlobular and arcuate arteries. There was no difference in the prevalence of any of these minor abnormalities in the three different groups of patients.

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The data in table 2 summarise the initial biopsy findings. Staining, when present, was always of low intensity, usually with a granular pattern, and was distributed irregularly among glomeruli. Results in patients with haematuria and with proteinuria were the same.

ELECTRON MICROSCOPY

The principal abnormality, which was found in many patients with haematuria (group I) but not in any other group, was a generalised reduction in the thickness of the glomerular basement membrane. When this was pronounced the thickness of the basement membrane was less than half the height of overlying foot processes. This change seemed to be due mainly to a reduction in the thickness of the lamina densa, which in places seemed to consist of two parallel strands of dense material separated by a narrow lucent zone.

In all groups of patients thickening of the mesangial matrix seen by light microscopy was confirmed, but abnormal deposits were not detected either within the expanded mesangium or in glomerular capillary walls. An occasional focus of effacement of foot processes was present in some of the group II patients with proteinuria (fig 5c).

Figure 2 Glomerulus classified histologically as "normal"; mesangial area is slender and seldom contains more than two nuclei. (Haematoxylin and eosin.)

Figure 3 Glomerulus classified histologically as "minimally abnormal". There is some thickening of the mesangial matrix and more than two nuclei are present in some lobules. (Haematoxylin and eosin.)

ULTRASTRUCTURAL MORPHOMETRY

These results are summarised in table 3. Across all three groups distribution of basement membrane thickness, as indicated by the length of orthogonal intercepts, had a unimodal distribution irrespective of whether the variable used to assess it was the arithmetical mean per case or the minimum or maximum value of basement membrane thickness. There was very little difference between the value in each patient group of the arithmetical mean and the mode (table 3), suggesting that the data were not greatly skewed. When mean values of glomerular basement membrane thickness of individual patients in the three groups were compared, 27 of the 41 group I patients with haematuria gave a value of less than 313 nm, the smallest mean value in any of the group II patients with proteinuria. When pooled mean values of basement thickness from the three different groups were compared a significant difference was evident between the value for the group I patients (haematuria) and group II (proteinuria) or group III (control), but the differences between mean values for group II (proteinuria) and group II (control) patients were not significant.

When minimum or maximum basement membrane thickness were used as the basis of comparison between the three groups it was still evident that thinner basement membranes were present in group I patients with

Figure 4 Minimal abnormality. One glomerulus has undergone global sclerosis and there is an associated focus of tubular atrophy and interstitial fibrosis. (Periodic acid-silver methenamine.)

haematuria, but as with mean basement membrane thickness, there was considerable overlap among the three groups. No detailed statistical analysis, however, was made using these two variables.

Data over a period from three to eight years were available from 16 patients with haematuria from group I and from six patients with proteinuria from group II. Fourteen of the 16 patients in group I had persistent haematuria; of these, 12 had a mean glomerular basement membrane thickness of 313 nm or less but the values in the other four were well within the range found in group II and group III patients, with mean values of basement membrane thickness ranging from 391 to 485 nm. No deterioration in renal function occurred in any of the group I patients, but one developed renal vein thrombosis, and transitional cell carcinoma of the bladder developed

Table 3 Glomerular basement membrane thickness (in nm) in minimal glomerular lesions

	Mean (SD)	Mode (SD)	Range	
			Mean minimum thickness (SD)	Mean maximum thickness (SD)
Haematuria:				
Group I (n = 41)	296 (82)*	272 (79)	156 (59)	513 (137)
Proteinuria:				
Group II (n = 14)	430 (47)*	395 (55)	270 (55)	716 (125)
Normal:				
Controls (n = 6)	418 (48)*	391 (57)	313 (78)	671 (69)

*Student's *t* test results:
Group I v controls *t* = 3.61, df = 45, *p* < 0.001
Group I v group II *t* = 5.10, df = 53, *p* < 0.001
Group II v controls *t* = 0.145, df = 45, *p* = 0.56.

Figure 5 Electron micrograph showing comparative thickness of glomerular basement membrane in (a) haematuria and thin basement membrane (group I); (b) control—normal urine (group III); (c) proteinuria (group II).

in a second. All six patients in group II had persistent proteinuria; cryoglobulinuria developed in one but there was no impairment of renal function in any of them.

Repeat renal biopsies at intervals of between three months and five years were performed in three patients with haematuria. No differences were evident by light microscopy, immunofluorescence, and electron microscopy, and there was no significant difference in the mean value for glomerular basement membrane thickness (table 4).

Discussion

This investigation confirms previous observations³⁻¹² that a thin glomerular basement is frequently associated with otherwise unexplained haematuria, and this study has shown that this is a relatively common occurrence in renal biopsy specimens which are categorised by light microscopy either as normal or as showing only minor abnormalities. When the change is well developed it is easily recognised by an inspection of the electron microscope image or micrograph. A useful guide to its recognition is that the affected basement membrane has a thickness that is less than half the height of overlying foot processes

and, in addition, there may be a narrow central zone of low electron density. Because thinning of the basement membrane is usually generalised, so that clearly demarcated segments of normal basement membrane are not available for comparison, for certain diagnosis of this condition, particularly of lesser degrees, objective measurement of basement membrane thickness is required.

There are some difficulties in deciding what constitutes an abnormally thin basement membrane and it should be recognised that most available methods¹⁰⁻¹⁷ will provide only an approximate indication of absolute true basement membrane thickness.

Different methods of fixation can influence the apparent thickness of the glomerular basement membrane,¹⁸ but the same method of processing tissue has been used throughout this investigation and is unlikely to be responsible for the differences in glomerular basement membrane thickness among the different groups. A second cause of error in the absolute measurement of basement membrane thickness arises from the fact that the length of the orthogonal intercepts, as measured in the plane of the electron micrograph, will not represent the true thickness of the basement membrane because this structure, being extensively folded at different intercepts, will be orientated at varying angles to the plane of the micrograph. This problem has been subjected to detailed mathematical study.¹⁶ Attempts were made to correct the data from selected cases in this study by the "unfolding" and "parametric" methods¹⁶ but both produced considerable distortion of the results and it was decided that it was more satisfactory to present the data as a summary of the measured orthogonal intercepts without attempting any transformation.

Table 4 Follow up measurements of glomerular basement membrane thickness in three group I patients with haematuria

Case No	Year	Thickness mean (SD) (nm)
1	1981	136 (48)
	1983	217 (66)
	1986	164 (39)
2	1981	167 (46)
	1986	136 (51)
	1984	158 (55)
3	1986	228 (65)

The values presented probably do not indicate absolute basement membrane thickness in any patient but in samples of sufficient size errors from this cause will probably affect each group equally and are unlikely to have seriously affected comparisons among the three patient groups. Furthermore, it is not certain that methods of data correction applicable to normal glomeruli could be used for basement membranes affected by a pathological process.

Because the data in all three groups have a continuous unimodal distribution and there is considerable variation in measurements of basement membrane thickness in different regions of an individual glomerulus, it is difficult to decide what constitutes a "thin" basement membrane. For purposes of comparison the lowest mean value in the patients with proteinuria (group II) of 313 nm was arbitrarily selected as a cut off point. Another perhaps more valid option would be to select values either 2 or 3 standard deviations below the mean value in either the group II proteinuria or group III control patients (table 3). Further study with more extensive measurements of basement membrane thickness by computer based image analysis may help to resolve this problem. Despite the difficulty of deciding what is the most appropriate cut off point for the lower limit of normal basement membrane thickness, it is evident that, using the most stringent of the above criteria—basement membrane thickness of 273 nm—at least 20 of the 41 patients with haematuria have abnormally thin basement membranes. Interestingly, this cut off value agrees closely with the value of 264 nm selected by Tiebosch *et al* in their recent prospective study of the incidence of thin basement membrane nephropathy in adults with persistent haematuria.¹²

This basement membrane change, referred to as "thin basement membrane syndrome" in the World Health Organisation classification of glomerular disease¹⁹ has been reported in several series of patients with idiopathic haematuria,³⁻¹¹ particularly in childhood,^{3-6,8} but also in adults.^{5,7-11} In many cases a strong family history of renal symptoms, particularly haematuria prevailed. Only a few cases have features of the hereditary nephritis of Alport's syndrome^{8,10} and most are considered to be cases of benign familial haematuria. There was, however, no recorded family history of renal disease in the present series but relatives were not tested for microscopic haematuria. Others, however, have reported cases of non-familial haematuria associated with thin basement membranes which are ultrastructurally indistinguishable from those of benign familial haematuria, although in the study by Tina *et al* the mean basement membrane thickness was considerably less in children with benign familial haematuria than in those with non-familial idiopathic recurrent haematuria.⁶ A benign prognosis has generally been reported in both familial and non-familial cases of thin basement syndrome but impaired renal function was noted in three of 12 patients in one series.⁹ Impairment of renal function was not

found in any patient in the present series but further follow up is needed for accurate assessment of the long term outcome.

In contrast to patients with persistent haematuria, no clinically important basement membrane abnormalities were found in patients in whom persistent proteinuria was associated with minor glomerular lesions. The number of patients in this category was small, but the long term prognosis seems to be good and glomerulonephritis had not developed in any of these proteinuric patients with a diagnosis of "minor lesions" at the time of the initial biopsy.

Although the association between thin glomerular basement membrane and persistent haematuria is strong, our study shows that the association is not absolute. No differences were evident either clinically or pathologically between those patients in whom persistent haematuria was associated with a thin basement membrane and the smaller number of patients with persistent haematuria in whom basement membrane thickness was within normal limits. While a thin glomerular basement membrane may be associated with increased fragility of the glomerular capillary wall, resulting in haematuria, there is no direct evidence of a cause and effect relation. Despite many observations erythrocytes were not observed in transit through the walls of capillaries with attenuated basement membranes in this study or in many others, although Rogers *et al* described several ultrastructural changes which they considered to be indicative of increased capillary wall fragility.³ Thin basement membrane and persistent haematuria, rather than being causally related, may both be reflections of a more fundamental, perhaps biochemical, basement membrane defect. Although this condition is apparently common, its aetiology remains obscure. The absence of a family history in many cases suggests that factors other than heredity may be involved but, at present, there is no positive evidence to implicate any infectious, toxic, or nutritional environmental factors in the production of thin glomerular basement membranes.

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