Virchows Arch. A Path. Anat. and Histol. 383, 265–270 (1979)

Virchows Archiv A

Interstitial Fibrosis of the Renal Cortex in Minimal Change Lesion and Its Correlation With Renal Function

A Quantitative Study

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Summary. Morphometric investigations were performed in 18 biopsies from 16 patients with the nephrotic syndrome and 10 biopsies from 10 patients with mild proteinuria not associated with oedema. All biopsies showed normal glomeruli on light microscopy. The interstitial fibrosis was significantly increased in both patient groups compared with controls, and it was greater in the patients with mild proteinuria than in the group with nephrotic syndrome. A significant negative correlation was present between the degree of fibrosis and renal function in both groups of patients.

Key words: Interstitial fibrosis – Minimal change lesion – Renal function – Nephrotic syndrome – Quantitative.

Introduction

Nephrotic syndrome without light microscopical glomerular changes is a condition which has been known for more than 60 years. Initially, it was called lipoid nephrosis by Munk (1913), a term used by some authors up to the present time (Heptinstall, 1974). The disease is a common cause of the nephrotic syndrome in children but occurs also in adults (Hopper, 1970). In children it will often remit on steroid therapy while the proportion of steroid resistent cases is greater in adults. Although initially defined morphologically by the absence of light microscopical glomerular lesions, more recent reports suggest, either implicitly or explicitly, that negative immunofluorescence and absence of deposits on electron microscopy are prerequisite for the diagnosis.

As the aetiology and pathogenesis are presently unkown some investigators have searched for morphological lesions too slight to be revealed by simple subjective evaluation. Slight glomerular hypercellularity, mesangial (Wehner,

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0340-1227/79/0383/0265/$01.20

1974) or endothelial (Ludwigsen, 1977) has thus been demonstrated by quantitative techniques. Recently, it has been reported that an increase of the relative interstitial volume is present in the kidney in several patients with minimal change lesion and nephrotic syndrome, and that this increase is positively correlated to the serum creatinine concentration at the time of biopsy (Bohle, 1977).

The present study was undertaken in order to test whether these results could be reproduced and whether interstitial fibrosis is also present in cases of minimal change lesion with proteinuria but no nephrotic syndrome.

Material and Methods

The Patients. All percutaneous renal biopsies with normal glomerular light microscopy from patients suffering from nephrotic syndrome[1] or proteinuria without oedema[2] were selected from our files from the period of 1964 to 1977. Only biopsies containing at least 3 glomeruli were included (most had more than 10) and orthostatic proteinuria, cases with Hodgkins disease or other secondary proteinurias, were excluded. The total material thus defined consist of 28 biopsies. 18 biopsies were from 16 patients with nephrotic syndrome, 10 biopsies were from 10 patients suffering from proteinuria without oedema. Electron microscopy was performed on 9 biopsies which all showed fusion of epithelial foot processes but no other significant alterations and specifically no deposits. 19 biopsies were investigated by immunofluorescent microscopy with antisera against IgG, IgM, IgE, IgA, IgD, C3 and fibrinogen by a technique previously described (Olsen, 1974). All were negative.

Biopsy specimens from 18 patients served as controls. They were obtained either from kidneys designated for transplantation but not used for this purpose for technical reasons, or from kidneys obtained at autopsy within a few hours after death in patients who died suddenly without evidence of renal disease. Patients and controls were comparable with regard to age and sex (see Table 1). All had normal blood pressure.

Table 1

	n	Male/ female	Age	Interstitial volume (%)	Serum-creatinine[a] (mg/100 ml)	Creatinine clearence[a] (ml/min)	Protein-uria[b] (g/day)
Group I			$\bar{x}$ 32.7	15.6	1.20	100.3	10.9
Nephrotic syndrome	18	1.6	*s* 16.8	6.7	0.40	39.7	7.4
			range 9–62	5.1–29.1	0.5–2.5	17.5–155	3.0–31.7
Group II			$\bar{x}$ 23.2	19.1	0.97	108.7	0.9
Proteinuria without oedema	10	1.5	*s* 9.4	3.2	0.18	24.8	0.6
			range 13–44	14.6–24.4	0.75–1.35	80–165	0.1–2.3
Group III			$\bar{x}$ 41.2	9.4			
Controls	18	2.6	*s* 14.9	2.6			
			range 17–61	5.3–14.5			

[a] Measured within 3 days before biopsy

[b] The average of the last 3 measurements before the biopsy

[1] All had proteinuria > 3g/day and oedema

[2] Proteinuria < 3g/day

Methods. The biopsy specimens were fixed in Carnoy's solution (32 specimens), Helly's solution (9) or 4% buffered aqueous formaldehyde (5). Following paraffin embedding 2 to 3 μm sections were cut and stained with haematoxylin and eosin and by Picro-Sirius staining for connective tissue (Sweat, 1964). Biopsies from patients and controls were randomly mixed and coded and the quantitative examination was performed as a blind study by a method previously described (Hestbech et al., 1977).

A Zeiss microscope with Zeiss Neofluar 40/0,75 objective and an X10 ocular with a 36-point grid was used. Counting was performed on varying number of fields in a row through the total length of the cortex in the biopsy. There was no significant difference between the number of fields counted in the specimens from patients and in controls. The average number of fields counted was 23 (range 7 to 50) in patients with nephrotic syndrome, 20 (range 3 to 42) in patients without oedema and 25 (range 8 to 57) in controls. Fibrosis of interstitial tissue was determined by the point counting technique as the number of points in "fibrous tissue" compared to the total number of points. The areas which appeared red in the Sirius-staining were counted as interstitial "fibrous tissue". Points covering glomeruli and the great vessels were discarded.

Statistical Methods. A non-parametric rank correlation method (Mann-Withny) was used for sample comparison. Spearman's correlation coefficient r was used for correlation analysis (Snedecor, 1968).

Results

The results of the morphometrical determination of interstitial fibrous tissue and the variables for renal function and proteinuria appear in Table 1 and Figs. 1 to 4.

Interstitial fibrosis is significantly increased ($P=0.05$) in both patient groups when compared with controls. Furthermore the fibrosis is significantly greater ($P=0.05$) in group II (proteinuria without oedema) than in group I (nephrotic syndrome). A significant, positive correlation is present between the degree of interstitial fibrosis and the serum-creatinine value in group I as well as in group II. In group I (but not in group II) there is a significant, negative correlation between the degree of interstitial fibrosis and creatinine-clearence.

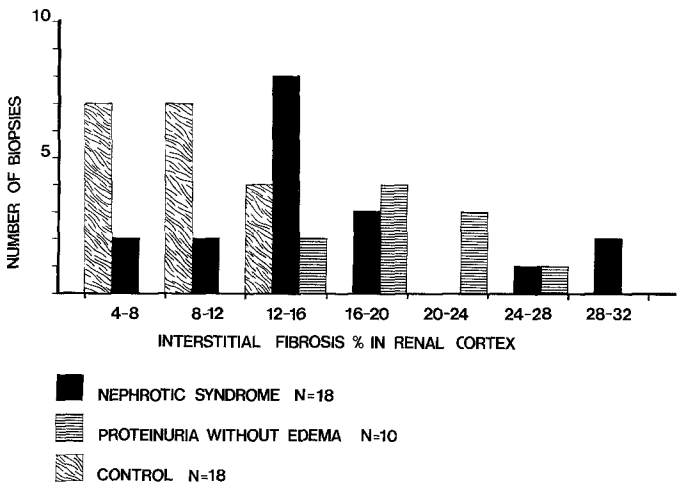

Fig. 1. Number of biopsies and the degree of interstitial fibrosis in the different groups

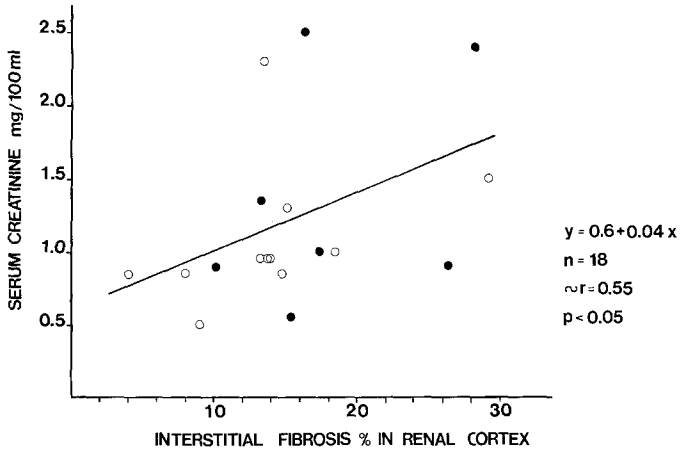

Fig. 2. Patients with nephrotic syndrome. Correlation between the degree of interstitial fibrosis and the renal function at the time of biopsy as measured by serum-creatinine concentration. ● = ♀, ○ = ♂

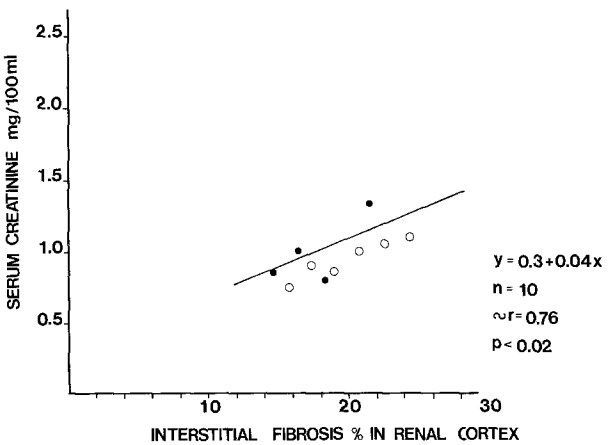

Fig. 3. Patients with mild proteinuria not associated with oedema. Correlation between the degree of interstitial fibrosis and the renal function at the time of biopsy as measured by serum creatinine concentration. ● = ♀, ○ = ♂

No correlation between the degree of proteinuria and interstitial fibrosis is present in the two patient groups. In seven patients from group I there was no remission of the proteinuria following treatment with steroid and these patients had significantly more fibrous tissue than patients with remission. The group without remission embraced three patients in whom rebiopsy 14, 16, and 24 months later showed focal segmental sclerosis.

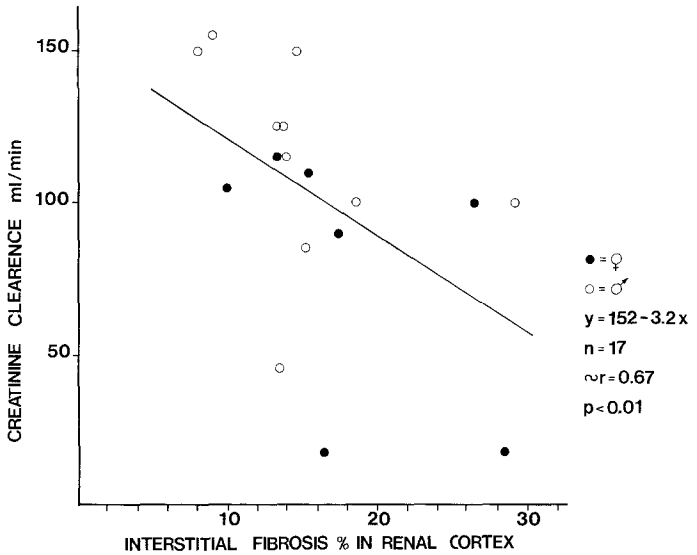

Fig. 4. Patients with nephrotic syndrome. Correlation between the degree of interstitial fibrosis and the renal function at the time of biopsy as measured by creatinine clearence. $N=17$, in one patient creatinine clearence was not available.

Discussion

Some cases of minimal change lesion are associated with interstitial fibrosis, an observation which has been suspected since Jao (1973) reported this finding in 11 of 37 patients with this lesion, basing his conclusions on semiquantitative investigations.

The present quantitative morphometric study demonstrates a significant increase in relative interstitial fibrosis in patients with nephrotic syndrome and minimal change on light microscopy compared with controls without renal disease and confirm the report of Bohle (1977) of a significant correlation between serum-creatinine and relative interstitial volume in patients with this lesion.

In addition a significant increase in interstitial fibrosis has been demonstrated in patients with proteinuria *without* oedema and the interstitial fibrosis is significantly greater in this group than in patients with nephrotic syndrome.

The delimitation of the concept of minimal change lesion is not easy. Glomerulonephritis with slight glomerular hypercellularity may not be detectable on light microsopy, and negative immunofluorescence microscopy and electron microscopy without evidence of deposits should ideally be included in the criteria. Exclusion of focal segmental sclerosis, which has a poor prognosis, is another important difficulty which cannot always be resolved by IF and EM. The focal character of this disease may give rise to an erroneous diagnosis of minimal change lesion, following failure to go into remission a later biopsy may give the correct diagnosis (as in our series). It cannot, however, be excluded

a priori that series of biopsies showing minimal change lesion contains several from patients suffering from focal sclerosis. Interstitial fibrosis is greater in this disease than in nephrotic syndrome with minimal change, as demonstrated by Bohle and his group (1977) and the augmented amount of interstitial tissue in a group of patients with minimal change lesion might thus depend on an admixture with unidentified cases of focal segmental sclerosis.

The aetiology and pathogenesis of minimal change lesion is still an enigma. The demonstration of increased interstitial fibrosis in this condition gives no clue to pathogenesis. It is clear, however, that the condition is associated with lesions other than glomerular foot process fusion, which so far has been the most impressive structural alteration. It is not easy to see in what way fibrosis per se could have a causal relationship to proteinuria. A more probable explanation might be that interstitial fibrosis is secondary to persistent proteinuria just as the lipid degeneration of the tubular cells in this lesion is regarded as secondary to the increased concentration of lipids in the serum of patients with nephrotic syndrome. We cannot explain why interstitial fibrosis is greater in patients with proteinuria alone than in those with nephrotic syndrome.

The evidence available at the moment does not permit us to determine whether the increased interstitial fibrosis is progressive or stationary, because the time of onset of proteinuria is almost always unknown in these patients.

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Received April 8, 1979