

## DAMAGE TO KIDNEY IN BALKAN ENDEMIC NEPHROPATHY: INITIAL LESION, TARGET STRUCTURES AND PATHOMORPHOGENESIS

Vojin Savić<sup>1</sup>, Rade Čukuranović<sup>1</sup>, Natalija Stefanović<sup>2</sup>, Vladisav Stefanović<sup>1</sup>

<sup>1</sup>Institute of Nephrology and Hemodialysis, Faculty of Medicine, Niš, Serbia

<sup>2</sup>Institute of Anatomy, Faculty of Medicine, Niš, Serbia

E-mail: stefan@ni.ac.yu

**Summary.** *Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial kidney disease of unknown etiology. Not only the etiology of the disease but even the initial lesion, target nephron structure and morphogenesis remain unresolved. We have previously performed quantitative morphometric analysis on kidney biopsy specimens of patients in different stages of BEN (without renal failure, with initial and advanced renal failure). In the initial stage of the disease, when glomerular filtration rate is normal, the increase of cortical interstitium and a reduction of glomerular volume are demonstrated. A significant reduction of the tubular epithelium volume density is characteristic of the advanced stage. The number of renal interstitial capillaries per 0.1 mm<sup>2</sup>, and the length density expressed per mm<sup>2</sup>, both significantly decreased in BEN patients with normal kidney function ( $p < 0.01$ ) and with renal failure ( $p < 0.001$ ).*

*We have also investigated the immunolocalization of laminin, cytokeratin and vimentin in the kidney of patients in different stages of BEN. In the early stages of BEN a marked overexpression of laminin in renal interstitial capillaries was observed with a moderately increased expression in tubules. Later stages were characterized by intensive expression of laminin in atrophic tubules, much more in proximal than in distal ones. The coexpression of vimentin and cytokeratin in proximal tubular cells was also demonstrated.*

*Evidence is presented that renal vascular changes occur early in BEN. A significant role of tubular injury in the pathogenesis of BEN was supported by the finding of vimentin and cytokeratin coexpression in the tubular epithelial cells. Interstitial sclerosis could result from the overproduction of extracellular matrix by injured proximal tubular epithelium and interstitial capillary endothelial cells. The changes described, particularly those taking place at the level of interstitium, bear the key responsibility for the BEN progression.*

**Key words:** *Balkan endemic nephropathy, cortical interstitium, interstitial capillaries, interstitial sclerosis, glomeruli, proximal tubules*

### Introduction

Balkan endemic nephropathy (BEN) is a progressive tubulointerstitial nephritis of unknown etiology (1). In fact, in most studies tubulointerstitial lesions were described as primary, and glomerular and extraglomerular vascular changes were considered as the secondary consequences of the disease.

The morphological studies in BEN place an emphasis on the earliest and the predominant tubulointerstitial changes (2-5). However, some authors consider BEN to be a glomerulopathy (6-8). Earlier morphologic (2-5) as well as recent immunohistochemical studies (9,10) point to the renal extraglomerular vascular changes as early kidney lesions in BEN.

### Qualitative analysis of renal changes

Tubulointerstitial changes were the most prevalent findings in several well conducted studies of BEN patients in the early stage of disease (2-5). In a study by

Ferluga et al. (5) on 52 BEN patients, interstitial sclerosis was found in 98%, tubular atrophy in 98%. In 92% of cases tubular atrophy was characterized by narrowing or complete disappearance of the lumens and pronounced thickening of the tubular basement membrane. Interstitial sclerosis was always multifocal, and surrounding atrophic tubules. Global glomerular sclerosis with microvascular hyalinosis/sclerosis was found in 80% of cases, segmental glomerular sclerosis in 10% of cases. Renal vascular changes occurred in 80% of biopsies. The renal vascular involvement was mostly multifocal, expressed as hyalinosis of arterioles and small interlobular arteries in 70% of cases. All these vascular changes were found to be only slightly more frequent and intensive among the 10 BEN patients with low labile arterial hypertension than among 40 patients who were normotensive.

### Quantitative analysis of renal changes

A recent quantitative analysis by means of the stereologic method has established the importance of

interstitial sclerosis, proximal tubular, glomerular, and extraglomerular vascular changes in different stage of BEN (11). Eighteen patients were classified into three groups with regard to the clearance of  $^{99m}\text{Tc}$ -DTPA (normal kidney function, initial and advanced renal failure). The initial stage of the disease was characterized by an outstanding increase of the cortical interstitial volume (50% over control volume) and a significant reduction of the glomerular volume (45% decrease) (Table 1). The volume of tubular epithelium was not significantly changed. The stages with a reduced glomerular filtration rate are characterized by a further increase of the interstitium (almost twofold), and a decrease of the glomerular volume to one third of the control value. Volume density of tubular epithelium was also significantly decreased. The number of renal interstitial capillaries per  $0.1\text{ mm}^2$ , and the length density expressed per  $\text{mm}^2$ , both significantly decreased in BEN patients with normal kidney function ( $p < 0.01$ ) and with renal failure ( $p < 0.001$ ) (12).

### Immunolocalization of laminin, vimentin and cytokeratin in renal lesions

We have studied expression of laminin, the major noncollagenous basement membrane protein, and expression of two intermediate filament proteins, vimentin and cytokeratin, in the kidney of patients in different stages of BEN (9,10). Renal biopsy specimens were obtained from BEN patients having  $^{99m}\text{Tc}$ -DTPA clearance in the range from 28-122 ml/min.

In the early stage of Balkan nephropathy, a marked overexpression of laminin in renal interstitial capillaries was observed with a moderately increased expression in tubules (9). Later stages were characterized by the intensive expression of laminin in atrophic tubules, much more in proximal than in distal ones. Increased expression of laminin in glomerular capillaries was also demonstrated, sometimes it was segmental. The pattern of laminin staining in glomeruli corresponded to focal and segmental glomerular sclerosis present in the advanced stages of Balkan nephropathy. This study confirms that major changes in Balkan nephropathy are localized in interstitial capillaries, occurring in the early stages (9). Markedly increased laminin expression in interstitial capillaries was demonstrated in patients with

normal blood pressure. Marked tubular atrophy, especially in proximal tubules, was associated with an overexpression of laminin. The observed changes were much pronounced in the outer cortex.

Cytokeratin was overexpressed in tubular epithelium, most intense on atrophic proximal tubules of the outer cortex, where also light microscopic changes were the most pronounced (10). Vimentin expression in glomeruli was slightly increased, segmental and mostly in mesangium. Extraglomerular localization was marked, especially in atrophic tubules. Proximal tubular overexpression of vimentin corresponded to the degree of proximal tubule damage. Some peritubular blood vessels were also vimentin positive. Very intense vimentin staining was found in the areas of marked interstitial sclerosis. The observed overexpression of vimentin was much pronounced in the outer cortex. Distal tubules were vimentin negative. Damage to the tubular epithelium has been followed by the coexpression of vimentin and cytokeratin. In this present study, further evidence on the tubulointerstitial lesions in the early stages of Balkan nephropathy is presented (10).

### Discussion

Previous studies in BEN have described multifocal, sclerotic and atrophic lesions of renal cortex involving all nephron structures, irrespective of the stage of disease. The predominant pathology is that of a chronic, multifocal interstitial nephritis (2-5). The findings are non-specific and similar to those associated with aging (13-15). While the most prominent histopathologic features seen in BEN patients are those of aging, other factors are not consistent with such a pathogenesis (5). Similar nonspecific findings are associated with nephrotoxic metals such as lead (16), cadmium (17), and lithium (18). Histologic changes in BEN share similarities with renal damage caused also by cyclosporine and some other toxic substances. In the first place ochratoxin A (19), aristolochia toxins (20), and polycyclic aromatic hydrocarbons (21) should be considered, as they are both nephrotoxic and cancerogenic. BEN is associated with high incidence of urothelial cancer (22).

Our studies on immunolocalization in early stages of BEN have demonstrated marked overexpression of laminin in renal interstitial capillaries with a moderately increased expression in tubules, and have pointed that

Table 1. Relative volume densities of cortical interstitium, tubular epithelium and glomeruli in BEN patients [adapted from Čukuranović et al. (11)]

Group	Cortical interstitium	Tubular epithelium	Glomeruli
Control	0.137 ± 0.005 (14%)	0.502 ± 0.015 (50%)	0.217 ± 0.040 (22%)
BEN without RF	0.182 ± 0.129 <sup>a</sup> (18%)	0.494 ± 0.005 (50%)	0.151 ± 0.050 <sup>i</sup> (15%)
BEN with initial RF	0.253 ± 0.022 <sup>bc</sup> (25%)	0.472 ± 0.009 <sup>g</sup> (47%)	0.142 ± 0.014 <sup>k</sup> (14%)
BEN with advanced RF	0.303 ± 0.015 <sup>def</sup> (30%)	0.438 ± 0.003 <sup>hi</sup> (44%)	0.1322 ± 0.013 <sup>k</sup> (13%)

Values are means ± SD; percentage in parenthesis. RF-Renal failure.

<sup>a</sup> vs. group 1 ( $p < 0.05$ ), <sup>b</sup> vs. group 2 ( $p < 0.05$ ), <sup>c</sup> vs. group 1 ( $p < 0.01$ ),

<sup>d</sup> vs. group 3 ( $p < 0.05$ ), <sup>e</sup> vs. group 2 ( $p < 0.01$ ), <sup>f</sup> vs. group 1 ( $p < 0.001$ ),

<sup>g</sup> vs. groups 1 and 2 ( $p < 0.05$ ), <sup>h</sup> vs. groups 1 and 2 ( $p < 0.001$ ),

<sup>i</sup> vs. group 3 ( $p < 0.01$ ), <sup>j</sup> vs. group 1 ( $p < 0.05$ ), <sup>k</sup> vs. group 1 ( $p < 0.01$ ).

renal vascular changes are the primary event in BEN. A significant role of tubular injury in the pathogenesis of BEN was supported by the finding of vimentin and cytokeratin coexpression in the tubular epithelial cells. Interstitial sclerosis could result from the overproduction of extracellular matrix by injured proximal tubular epithelium and interstitial capillary endothelial cells.

The changes described, particularly those taking place at the level of interstitium, bear the key responsibility for the BEN progression. The increase of the cortical interstitial volume results in resistance of the post-

glomerular capillary network with impairment of the glomerular flow (23). This impairment leads to a chronic rise in hydrostatic pressure. The increase of the cortical interstitium additionally leads to an increase in the length of diffusion between the tubules and the intertubular and peritubular capillaries (24). This increase in the length of diffusion subsequently results in atrophy of the tubules, reduction of reabsorption, and therefore impairment of the effective filtration pressure (23).

Further studies are needed in BEN to explain the primary event in the kidney and pathomorphogenesis.

## References

- Hall PW, Dammin GJ. Endemic Balkan nephropathy. In: Tisher G, Brenner BM (eds) *Renal Pathology with Clinical and Functional Correlations*, JB Lippincott Co, Philadelphia, 1989: 913.
- Dojčinov D, Strahinjić S, Stefanović V. Pathohistology of the kidney in the early phases of endemic (Balkan) nephropathy. In: Strahinjić S, Stefanović V (eds), *Endemic (Balkan) Nephropathy*. University Press, Niš, 1979: 91-104.
- Mandal AK, Sindjić M, Sommers SC. Kidney pathology in endemic nephropathy. *Clin Nephrol* 1987; 27: 304-308.
- Savić V, Ferluga D, Hvala A, et al. Histopathology of early phase of endemic (Balkan) nephropathy. Two case reports. In: Strahinjić S, Stefanović V (eds), *Etiology of Endemic (Balkan) Nephropathy*. University Press, Niš, 1987: 171-179.
- Ferluga D, Hvala A, Vizjak A, et al. Renal function, protein excretion and pathology of Balkan endemic nephropathy. III. Light and electron microscopic studies. *Kidney Int* 1991; (suppl 34): S57-67.
- Craciun EC, Georgescu L, Rosculescu I, et al. Morphological observations concerning endemic nephropathy. In: Puchlev A, Popov N, Astrug A, Dotchev D, Dinev I (eds), *Proceedings of the International Symposium on Endemic Nephropathy*. Bulgarian Academy of Sciences Press, Sofia 1965, 87-92.
- Suša S, Pantić V, Dumović B. Immune aspects in endemic nephropathy. In: Hrisoho D, Masin G, Polenaković M, Zafirovska K, Grozdanovski R (eds), *Proceedings of the 1st Yugoslav Scientific Meeting of Nephrology*. Galenika, Belgrade, 1977: 333-342.
- Macanović M. Immune mechanisms in the pathogenesis of endemic nephropathy. In: Danilović V (ed), *Proceedings of the 2nd Symposium sur la Nephropathie Endémique*. Academie Serbe des Sciences et des Arts, Belgrade, 1979: 129-136.
- Čukuranović R, Savić V, Dojčinov D, Stefanović V. Immunohistochemical localization of laminin in renal lesions of Balkan nephropathy. *Nephron* 1995; 70: 504-505.
- Stefanović V, Čukuranović R, Dojčinov D, Savić V. Coexpression of vimentin and cytokeratin in damaged tubular epithelia of kidney in Balkan nephropathy. *Nephron* 1996; 72: 119-120.
- Čukuranović R, Stefanović N, Savić V, Stefanović V. Quantitative analysis of the renal changes in Balkan endemic nephropathy. *Int Urol Nephrol* 1998; 30: 229-236.
- Stefanović N, Čukuranović R, Savić V, et al. Some possibilities of the quantitative analysis of the capillaries network of cortical renal interstitium. *Folia Anatomica*, Beograd 1998; 26 (suppl 1): 49-51.
- Kasiske BL. Relationship between vascular disease and age associated changes in the human kidney. *Kidney Int* 1987; 31: 153-1159.
- Darmady EM, Offer J, Woodhouse MA. The parameters of the ageing kidney. *J Pathol* 1973; 109: 195-207.
- Kaplan C, Pasternack B, Shah H, Gallo G. Age-related incidence of sclerotic glomeruli in human kidney. *Am J Pathol* 1975; 80: 227-234.
- Schreiner GE, Maher JF. Toxic nephropathy. *Am J Med* 1965; 38: 409-449.
- Kazanizis G. Cadmium nephropathy. *Contr Nephrol* 1979; 16:161-166.
- Hestbech J, Hanse HE, Amidisen A, Olsen S. Chronic renal lesions following long-term treatment with lithium. *Kidney Int* 1977; 12: 205-213.
- Pfohl- Leszkowicz A, Grosse Y, Castegnaro M et al. Ochratoxin A related DNA adducts in urinary tract tumours of Bulgarian subjects. *IARC Sci Publ* 1993(124): 141-148.
- Cosyns JP, Jadoul M, Squifflet JP, et al. Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? *Kidney Int* 1994; 45: 1680-1688.
- Feder GL, Radovanović Z, Finkelman RB. Relationship between weathered coal deposits and the etiology of Balkan endemic nephropathy. *Kidney Int* 1991; 40 (Suppl 34): S9-S11.
- Čukuranović R, Ignjatović M, Stefanović V. Urinary tract tumors and Balkan nephropathy in the South Morava River basin. *Kidney Int* 1991; 40 (suppl 34): S80-84.
- Bohle A, Gise H, Mackensen-Haen S, Stark-Jakob B. The obliteration of postglomerular capillaries and its influence upon the function of both glomeruli and tubuli. *Klin Wochenschr* 1981; 59: 1043-1051.
- Sindić M. Interpretation of morphologic renal changes in endemic nephropathy. In: Petković S (ed), *Proceedings of the 3rd Symposium sur la Nephropathie Endémique*. Academie Serbe des Sciences et des Arts, Belgrade, 1982: 159-185.