The significance of apoptosis for early diagnosis of Balkan nephropathy

M. Savin¹, V. Bumbaširević², L. Djukanović¹ and V. Petronić¹

¹Institute of Urology and Nephrology, Institute of Histology and ²Human Embryology, School of Medicine, University of Belgrade, Yugoslavia

Introduction

Balkan nephropathy—a toxic nephropathy

Balkan nephropathy is a non-destructive, low inflammatory, progressive tubulointerstitial disease. It frequently and exclusively occurs in inhabitants of some rural areas along tributaries of large rivers in ex Yugoslavia, Bulgaria and Romania. Focal changes of kidney parenchyma are characterized by atrophy and segmental disappearance of tubuli with considerable (acellular) interstitial fibrosis contrasting with relatively preserved glomeruli. The renal insufficiency aggravates gradually, 5–30% of these patients from different districts had to undergo regular haemodialysis [1]. Severe anaemia with insufficient synthesis of erythropoietin is more pronounced as that seen in other kidney diseases [2]. The other disease affecting the population of endemic regions is (upper) urothelial cancer, occurring with a 100 times greater incidence than in the general population [3]. In spite of scientific efforts, the aetiology of Balkan nephropathy and associated urothelial carcinomas have not been fully understood.

Two almost regular microscopical findings in kidney tissue obtained by biopsy in the initial phase of Balkan nephropathy may help to elucidate the nature of these lesions. These are discrete foci of tubular atrophy and/or mild vascular lesions of intertubular capillaries and afferent arterioles [4]. These lesions apparently lead to capillarosclerosis and arteriolar hyalinosis in distinct areas of the parenchyma with tubular atrophy, starting from the superficial part of the cortex. Based on the observation that the kidneys in Balkan nephropathy show similarities with other numerous toxic nephropathies (due to analgesic abuse, mycotoxins ochratoxin A, aristolochic acid, cyclosporine A), a toxic aetiology of Balkan nephropathy is plausible, numerous factors are likely to act synergistically in the tubuli. Toxic agents or their metabolites directly affect the vasculature and tubuli [1] while resulting ischaemia may assist in the development of focal tubular lesions [2]. Mycotoxin ochratoxin A highly contaminates food in several investigated endemic villages [5]. Ochratoxin A is a nephrotoxin with carcinogenic potential [6].

Tubular defects

Tubular defects appear early in Balkan nephropathy [7]. Proteinuria is mild, not in excess of 1.5 g/day. Pronounced enzymuria was observed in both the diseased and healthy population from endemic villages (alanine-amino peptidase of brush border membrane of proximal tubular cell, acetate dehydrogenase of cytoplasm, N-acetyl-D-glycosaminidase of lysosome). Transitory (reversible) tubular injury observed among diseased and healthy people in endemic villages is associated with high beta-2 microglobulin concentrations in the urine in comparison to controls living outside endemic areas [8–10]. It would seem, therefore, that enzymuria or beta-2 microglobulin may not be reliable biomarkers of the early phase of Balkan nephropathy. High concentrations and/or activity of these molecules in urine rather suggest that some part of the population has been exposed to an agent that damages the tubuli. The amount of toxin probably varies. Individuals who are more susceptible to that agent will develop kidney disease and/or urothelial cancer. This questions the importance of genetic factors contributing to the pathogenesis of both diseases. Based on epidemiological investigations of patients and relatives in endemic regions, who had initial morphological changes compatible with Balkan nephropathy [11] the inherited predisposing genes for Balkan nephropathy are possibly localized in a region between 3q25–3q26. The opposite fact is that Balkan nephropathy and associated urothelial carcinomas are not hereditary diseases, although they may affect many individuals in the same household. Maximally a third of patients suffer from both diseases [3].
Apoptosis—a biomarker of the early phase of Balkan nephropathy

Our investigation is in favour of the theory that an increase in apoptosis (in tubuli) occurs in the early phase of Balkan nephropathy, and much before the osmolar overload of the remnant nephrons is observed [12,13].

Research on 12 large tissue biopsy samples obtained by lumbotomy (n = 5) and at the moment of surgery for urothelial tumours in the ureter (n = 2) or renal pelvis was able to identify early tubular apoptosis. We confirmed the presence of apoptosis by TUNEL assay following short exposure of the kidney tissue to low activity of proteinase K (5–6.2 μg/ml). In a TUNEL, strand breaks from DNA fragmentation during apoptosis are perceived by end labelling in situ. The presence of nuclear p85, the caspase 3 cleaved fragment of poly(ADP-ribose) polymerase (PARP) was detected by immunohistochemistry. The caspase 3 is an effector molecule in apoptosis (middle phase—substrate cleavage). Activation of downstream proteases should contribute to internucleosomal degradation of DNA in apoptosis (late phase—nuclear disintegration). Because these two markers recognize different phases of apoptosis, it is not obligatory that dual cell positivity refers to apoptosis. A nephrotoxin, such as ochratoxin A in a low dose may lead to increased caspase activity in human proximal tubular cells in culture. Exposure to higher dose is followed by DNA fragmentation in cells [14].

In our patients, serial tissue sections displayed tubular cells, which are mostly either TUNEL + or PARP p85+, although most of them belonged to tubuli in the same tissue area. By light microscopy of the biopsy specimens, the patients were in the initial phase of Balkan nephropathy. They were middle-aged; the clearance of creatinine was over 60 ml/min at biopsy. Nine out of 10 patients had increased apoptosis in some tubuli in comparison to that seen in the control kidney biopsies of individuals from the same endemic regions. Controls included healthy persons or patients suffering from urothelial cancer with no obstruction or Balkan nephropathy. We then calculated the sum of the mean IxTUNEL and maximal IxPARPp85 for tubuli in each biopsy, referred to as the estimation of apoptosis (EsApop). In Balkan nephropathy, this parameter (in a logarithmic model) significantly exceeds the tubular IxPCNA, which is the proliferation marker (Figure 1).

- It is possible that when some nephrotoxin affects tubular cell function, it might trigger ‘inappropriate’ apoptosis of tubular cells.
- Resulting ischaemia from decreased blood flow and structural changes of intertubular capillaries and afferent arterioles within foci of Balkan nephropathy (the early stage) may trigger apoptosis (oxidative stress).
- The injury of tubular cells that results in apoptosis is probably of multifactorial origin. Genetic predisposition could influence insufficient or aberrant metabolism of a toxin. Cytogenetic investigation showed an increased frequency of folate sensitive Fra sites in these patients [15]. Infection by slow viruses (Corona) could induce additional (genetic) alterations that facilitate apoptosis under the same conditions.

Conclusion

We suggest that the estimated apoptosis to proliferation ratio for tubuli is a new marker for early diagnosis of Balkan nephropathy. It would be of interest to investigate whether PARP p85 fragment is a marker of progression of Balkan nephropathy in the initial phase of the disease. Besides in tubular epithelial cells, apoptosis may occur in other cells in kidney parenchyma (mesangial cells, podocytes, vascular endothelial cells and interstitial cells) and this should be the subject of its determination, in tissue or urine samples of patients with Balkan nephropathy.

References


