Mini-review

Balkan endemic nephropathy: a need for novel aetiological approaches

V. STEFANOVIC

From the Institute of Nephrology and Haemodialysis, Faculty of Medicine, Niš, Serbia, Yugoslavia

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Summary

Aetiology remains the main unanswered problem in Balkan endemic nephropathy (BEN) despite investigations into the roles of genetic factors, environmental agents and immune mechanisms. Evidence has accumulated that BEN is an environmentally-induced disease. Weathering of low-rank coals near to the villages where BEN is endemic produces water-soluble polycyclic aromatic hydrocarbons and aromatic amines, similar to metabolic products of acetaminophen that cause analgesic nephropathy. Many of these compounds are known to be carcinogenic and could also cause urothelial cancer. Genetic studies have supported genetic predisposition to BEN. The candidate genes have been localized to a region between 3q25 and 3q26, the 3q BEN marker being detected in both BEN patients and in some healthy relatives with initial morphological changes peculiar to BEN. Three bands with increased frequencies of spontaneous and induced aberrations contain oncogenes. The frequent association of BEN and urinary tract tumours (UTT) can be explained by the chromosomal hypothesis of oncogenesis. The results of molecular biological investigations will allow the identification of genetic markers of BEN, permitting early detection of BEN-predisposing mutations and identification of susceptible individuals who may be at risk of exposure to the environmental agents. An increased incidence of tumours of renal pelvis and ureter in patients with BEN and in population from endemic settlements has been observed. Familial clustering of the UTT was also reported. The frequency of urinary bladder tumours in BEN-endemic settlements is also increased compared with the non-endemic villages and cities. The geographic correlation between BEN and UTT supports the speculation that these diseases share a common aetiology.

Introduction

Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial kidney disease with a slow progressive course, ultimately leading to end-stage renal failure. Described 40 years ago in Serbia by Danilović et al., this unusual renal disease is prevalent among the population of settlements along the tributaries of the Danube River in Serbia, Bosnia, Croatia, Bulgaria and Rumania. The disease is limited to a relatively small region north and south of the Danubian Iron Gates and located in a few areas along the tributaries of this river in the plains and low hills at an altitude of 150 to 500 m above sea level. In Serbia, 73 villages with BEN were found: in the Kolubara valley, in the flood areas of Podrinje and Mačva, and around the Morava River. The endemic region includes 54 villages in Bosnia, near Bijeljina, and 14 villages in Croatia, near Slavonski Brod. In Bulgaria, the endemic region lies in the

Address correspondence to Dr V. Stefanović, Institute of Nephrology and Haemodialysis, B. Taskovića 48, 18000 Niš, Yugoslavia
foothills of the Balkan mountains, with 33 endemic settlements in the Vratsa District and seven in the Mikhaylovgrad District. In Rumania, the endemic area, with 41 affected settlements, is situated on both sides of the southern Carpathians. Not all villages in the endemic area are affected; however, those affected are sometimes only a few kilometres from unaffected ones.

The familial character of the disease was described in 1957. Within the affected village there are many unaffected households living close by affected families.

An increased incidence of tumours of the renal pelvis and ureter has been described in the population of endemic settlements.1-3 BEN is usually manifested in the fifth decade of life. The disease progresses slowly, asymptomatic, unaccompanied by salt retention and hypertension. Anaemia is a constant feature of BEN, and can be demonstrated in early stages. Renal oedema does not occur, and lumbar or back pain is not a clinical feature. Small or shrinking kidneys are found in patients with renal failure. They are usually bilaterally small with a smooth outline.

Relatively minor and intermittent proteinuria has been described, increasing to approximately 1g/l or less in advanced renal failure. Urinary samples contain a few red and white blood cells per high-power field.

Abnormal renal tubular functions have been documented in BEN patients with normal glomerular filtration rate. Estimation of urinary \( \beta_2 \)-microglobulin, as a marker of proximal tubular dysfunction, has been used in epidemiological surveys of endemic areas. Accompanying tubular proteinuria are defects in acidification, ammonia and uric acid excretion. Renal salt wasting has been observed early in the disease. Glomerular filtration rate decreases slowly and progressively over the years.

In the last 40 years, much progress has been made in the understanding of this disease. The results of these studies have been reviewed elsewhere.2-4

I would like to survey the studies performed in the last decade, and the prospects for future research. Major emphasis will be given to the aetiology, associated urothelial cancer and prevention of BEN.

Environmentally-induced disease

Evidence has accumulated that BEN is an environmentally induced disease. Trace elements such as cadmium, lead, manganese and copper are known to have a nephrotoxic effect, however, it is unlikely that there is a causal relationship of one of these elements or a combination of them with BEN.2,3 There is also the possibility that a deficiency of some essential trace element, e.g. selenium, might be involved in the aetiology of BEN.5 Selenium has been demonstrated as an integral part of glutathione peroxidase (GSH-Px), an enzyme which catalyses the reduction of lipid hydroperoxides and hydrogen peroxide. GSH-Px activity in BEN patients was significantly decreased, comparing to healthy controls, but was similar to that in some other renal failure patients. In healthy relatives of BEN patients, GSH-Px activity was similar to that in the control group.6 Selenium deficiency has been associated with cardiac disease in China, but there has been no association with renal disease. Selenium-deficient areas are found in many countries, and it is unlikely that selenium plays a role in the pathogenesis of BEN.

The effect of environmental factors is important for the developing kidney, therefore a study of the possible effect of these factors was performed in children from endemic settlements, non-endemic villages and large cities.7 The effect of environmental factors was estimated by determining several markers of glomerular and tubular functions.8 As the effect of environmental factors is associated with seasonal variations, this study was performed in different seasons (autumn, winter and spring) during a three-year period. The study showed a significant environmental effect on the kidneys of schoolchildren in endemic settlements, and especially in families with BEN. The effect of environmental factors was found in all seasons investigated, and particularly in autumn (October). In children, urinary \( \beta_2 \)-microglobulin, and N-acetyl glucosaminidase (NAGA) are sensitive indicators of kidney damage induced by toxic factors in the environment.

Feder et al. studied the geochemistry of the areas where the disease is endemic.9 They found that endemic settlements are located near weathered coal deposits. Most endemic foci have Pliocene-age liginites in their vicinity. Pliocene-age coals are 1.6 to 5.3 million years old, and are the youngest coals in the Balkans. These low-rank coals in the Balkans still retain many of the complex organic compounds contained in the decaying plant precursors of the coal. Weathering of the low-rank coals could generate complex mixtures of water-soluble hydrocarbons, which would appear in the drinking water of shallow farm wells. Preliminary results from qualitative chemical analyses of drinking water from shallow farm wells in endemic settlements indicate the presence of soluble polar polycyclic aromatic hydrocarbons and aromatic amines, for example naphthylamine, aniline, antracene and pyrene. Many of these compounds are known to be carcinogenic and could also cause urothelial cancer. This natural contamination may combine with numerous sources of man-
made pollution common in both the endemic and control villages to provide the factor or co-factors responsible for the disease.

Numerous low-rank Pliocene coals also occur in Turkey, Greece, Italy and Burma.Interstitial nephropathy similar to BEN has been described in Greece. It will be important to determine if a disease similar to BEN occurs in association with the Pliocene coals in other countries, and if not, to understand how these areas differ from BEN foci.

Some 25 years ago aristolochic acid was suggested as the aetiological agent of BEN. Ivic has found aristolochic acid in flour obtained from wheat contaminated with seeds of Aristolochia clematis in endemic region. He conducted a survey of the geographical distribution of the plant, Aristolochia clematis, in the endemic area. This plant has both nephrotoxic and carcinogenic action. Focal tubulointerstitial changes were observed in rabbits poisoned by giving them orally various amounts of flour made from ground dried Aristolochia seeds. The most pronounced changes were found in the superficial part of the kidney. Tubules were replaced by connective tissue, usually without cellular infiltration. Glomeruli were either preserved or, in advanced stages, presented various degrees of changes in the form of axial or concentric sclerosis, and even sclerosis of the whole glomerulus. These changes corresponded completely to the changes characteristic of BEN. Several cases of end-stage interstitial kidney disease have been recently reported in young women who have been on slimming regimens including Chinese herbs. Aristolochic acid was isolated from several batches of pills, accidentally delivered as powder of Aristolochia fangchi, in place of the non-toxic Stephania tetrandra. Several characteristics of this nephropathy have been described: development of end-stage kidney disease within 1–2 years, normal arterial blood pressure, extensive interstitial fibrosis, LMW proteinuria, urothelial atypias and malignancy. The similarity of the morphology and clinical features of Chinese herbs nephropathy and BEN has raised the possibility of a common aetiologic agent, aristolochic acid. However, there are many different features of these two nephropathies.

Ochratoxin A is a mycotoxin demonstrated to be responsible for porcine nephropathy in northern Europe. Porcine nephropathy is primarily a tubulo-interstitial disease similar to BEN in many ways, suggesting a common causal relationship. The most pronounced food-born exposure to ochratoxin has been found in the area in Croatia where BEN is prevalent. The associated nephrotoxicity and carcinogenicity of ochratoxin A recently described make this hypothesis particularly attractive. However, the fact that non-endemic as well as endemic regions have ochratoxin contamination raises doubts about the primary role of this mycotoxin in the aetiology of BEN. Indeed, to date there is no evidence that ochratoxin is responsible for any kidney disease in humans.

Results presented by the same group studying BEN in the Croatian villages around Slavonski Brod point to environmental rather than genetic factors as the key determinants in an individual’s susceptibility to BEN. Čeovic et al. have analysed a natural experiment induced by the immigration of people from the Ukraine to the endemic and non-endemic areas near Slavonski Brod. Approximately the same incidences of BEN were found among the population of the Croatian nationals and the Ukrainian immigrants and their offspring.

**Genetic predisposition to BEN**

A familial aggregation of BEN was described 40 years ago. Development of BEN in emigrants from the endemic region, who left their native villages in early childhood and settled hundreds, sometimes thousands of miles away, supports the role of inheritance in the development of BEN. A study in the region of Slavonski Brod, Croatia, comprising 143 families with 4501 persons from 3–5 generations has estimated the heritability of BEN as 24.5 ± 5.5%. Čukuranovic et al. have speculated that predisposition to BEN is inherited polygenetically, and the manifestations of the genotype are modified by the environment. The frequencies of 20 randomly-selected morphophysiological properties controlled by one or a few genes with alternative dominant or recessive mode of expression were compared in BEN patients and healthy controls. Statistically significant differences were found in 5/20 morphophysiological properties analysed, and it was concluded that these two groups differ approximately in 25% of alleles.

In Bulgaria, Mihailov has found that BEN is confined to definite families and is transmitted as an autosomal dominant trait. However, the pattern of BEN transmission in those families is not typical of a single-gene disorder. Extensive epidemiological and genetic studies show characteristics of polygenic BEN inheritance.

A specific chromosome marker has been established on chromosome 3. It was suggested that the genetic factor is located in 3q25, and that there is an increased instability in the long arm of the chromosome 3. An analysis of spontaneous aberration and chromosome breakages induced by X-rays and folic acid deficiency has revealed that in BEN
patients the 3q25 band was most frequently involved in the aberrations. Three of the additional five bands with increased frequencies of lesions in BEN patients were found to contain oncogenes: 1q36-csrc, 3p25-raf-1, and 6q23-myb.

To clarify the genetic mechanisms of BEN, chromosomal analysis was done of healthy relatives of BEN patients, who were born in non-endemic areas and lived in a BEN-endemic environment. This study showed that certain BEN relatives carry chromosomal anomalies that have already been described in BEN patients, and it is proposed that they are at high risk for developing the disease. Epidemiological data for patients and their healthy relatives are lacking, however, follow-up studies of healthy relatives could test the hypothesis that a genetic mechanism is involved in the development of the disease even in the absence of the exposure to a BEN environment.

Evidence for an inherited metabolic susceptibility to BEN has been obtained by Ritchie et al., using the drug debrisoquine as a probe of variable metabolism. The ability to metabolize debrisoquine to its metabolite 4–hydroxy-debrisoquine is known to be highly variable between individuals and is controlled by a single autosomal gene locus. The group of BEN patients contained a greater proportion of subjects with enhanced oxidative ability compared to the control group. This is consistent with the view that some individuals, because of their genetically-determined metabolic status, may be more likely to develop BEN and UTT, due to their sensitivity to a chemical in their environment.

Preliminary results of Pavlović et al. indicate that lecithin-cholesterol-acyltransferase (LCAT) partial deficiency may play a part in the pathogenesis of BEN. LCAT deficiency is a genetic disease. Renal damage in LCAT deficiency is characterized by renal tubular abnormalities. These findings are an argument with tumours of the urothelium. BEN. LCAT deficiency is a genetic disease. Sporadic cases of urinary bladder tumours in the renal pelvis and/or ureter were five times more frequent in families of patients with BEN, but a less common association of UTT and BEN was established in both Yugoslavia and Bulgaria. An increased incidence of UTT was observed in families of patients with BEN. In endemic settlements, tumours of the renal pelvis and ureter were five times more frequent in families with BEN than in those without, and up to 224 times more frequent than in large city families.

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Urothelial cancer

The increased frequency of UTT in the population of endemic villages was described in the first reports on BEN, both in Bulgaria and Yugoslavia. In 1965, Petrinška-Venkovska described UTT in 20/53 autopsy cases of BEN, but a less common association of UTT with BEN was found in Yugoslavia. However, a geographic correlation between UTT and BEN was established in both Yugoslavia and Bulgaria. An increased incidence of UTT was observed in families of patients with BEN. In endemic settlements, tumours of the renal pelvis and ureter were five times more frequent in families with BEN than in those without, and up to 224 times more frequent than in large city families.

Sporadic cases of urinary bladder tumours in patients with BEN have been previously described. In a survey of BEN in Bulgaria, Puchlev et al. confirmed that kidney disease is very often associated with tumours of the urothelium. In 90/218 patients with BEN, (41.3%) UTT was demonstrated. Twelve cases (5.5%) of urinary bladder tumours were found in these 218 patients, four combined with tumours of the renal pelvis and/or ureter. In Yugoslavia, urinary bladder tumours were found equally frequently in endemic and non-endemic regions until 1980. In the last ten years, however, they are becoming increasingly more frequent in advanced stages of BEN, especially with longer survival of patients on maintenance haemodialysis. A recent survey of UTT in the South Morava River basin and its tributaries where BEN is endemic revealed increased frequency of not only tumours of the renal pelvis and/or ureter. For this purpose, 659 surgery and autopsy records of patients with UTT were reviewed. Upper-tract renal pelvis and ureter urothelial tumours were 57 and 61.8 times more frequent in the BEN-endemic population than in control rural and city populations.
The frequency of urinary bladder tumours in endemic settlements was also increased compared with the non-endemic villages and large cities, up to 11.9 and 8.5 times, respectively. Familial clustering was also noted. Urinary bladder tumours were seven times more frequent in families with BEN than in those without, and up to 22.5 times more frequent than in the city families.\(^\text{38}\)

The changing pattern of urothelial tumours in BEN is similar to that seen in analgesic nephropathy, another tubulointerstitial kidney disease associated with urothelial cancer. The early reports pointed out an increased incidence of upper-tract urothelial tumours in analgesic nephropathy.\(^\text{39}\) An analysis of urothelial cancer related to phenacetin abuse in Basel, Switzerland, 1963–1977, has revealed that carcinomas of renal pelvis were 77 times more common, carcinoma of the ureter 89 times more common, and those of the urinary bladder seven times more frequent in analgesic abusers than in non-abusers.\(^\text{40}\) In a 25-year autopsy study, 1953–1977, Mihatsch and Knusli compared two periods of observation, one from 1953 to 1966 and the other from 1967 to 1977. During the 1953 to 1966 period, urinary bladder tumours were equally frequent among abusers and non-abusers, however, during the period 1967 to 1977, the overall incidence of urinary bladder tumours increased 7.5 times, and these tumours were 5.8 times more frequent in abusers than in non-abusers.\(^\text{41}\) One explanation for the recently increased incidence of the urinary bladder tumours could be the longer induction time for their emergence. The induction time for tumours of the renal pelvis was found to be about 20 years, and about 27 years for tumours of the urinary bladder.

In the regions where BEN is endemic, maximal incidence of upper-tract urothelial tumours was in the sixth decade of life, and that of urinary bladder tumours over 70 years pointing to a longer induction time for bladder tumours.\(^\text{38}\) With this prolonged induction time increased incidence of urinary bladder tumours in endemic settlements would be expected. BEN patients on haemodialysis with a longer survival are at increased risk of urothelial and other malignancies.

The strong association of BEN and UTT supports the speculation of a common aetiology for both nephropathogenic and carcinogenic processes. In UTT occurring in regions where BEN is endemic, genetic predisposition may interact with environmental determinants to produce the tumours. Analysis of familial and geographic aggregation by laboratory and epidemiological investigations may be helpful in identifying the carcinogenic agents and mechanisms in urothelial cancer. These studies may farther help elucidate the aetiology and mechanisms of both renal disease and cancer in endemic villages.

**Prevention and treatment of BEN**

Major advances have been made in understanding of the aetiology and pathogenesis of BEN. The migration of affected families outside of the endemic region could have an impact on the disease, through the avoidance of the endemic environment and marriage with healthy partners. Since well-conducted studies have suggested the presence of aetiologic factor(s) in water, tap-water supply from distant, healthy regions could change the disease profile of endemic settlements.

BEN is a long-lasting, slowly advancing disease. Treatment is planned according to the stage of the disease. The prevention of an excessive loss of salt and water is of major importance, especially associated with overzealous use of diuretics, vomiting and diarrhoea in the hot summer time. In renal failure, a low-protein diet could reduce the loss of residual nephrons and postpone renal replacement treatment.\(^\text{42}\) Detection and treatment of potentially reversible factors, such as infection, metabolic disturbances, hypertension and others, and avoidance of nephrotoxic drugs, could add several months of life off dialysis.

End-stage kidney disease patients are treated by maintenance haemodialysis and peritoneal dialysis. Hypertension is not a major problem in BEN patients, and these patients did better on haemodialysis than did patients with chronic glomerulonephritis.\(^\text{43}\) With longer survival on dialysis, many patients develop urothelial cancer. Painless haematuria is of great diagnostic significance. Preventive diagnostic check-up should be instituted, especially in patients with no urine output.

Several patients with BEN have received a kidney graft. No special problems related to BEN have been reported.\(^\text{44}\) The recurrence of BEN in a grafted kidney has not been described to date.\(^\text{45}\)

**Future research**

Aetiology remains the major problem for research in BEN. Besides genetic predisposition, only a few described environmental factors should be considered. The water-soluble polycyclic hydrocarbons and aromatic amines from weathered coal deposits nearby endemic settlements seem to play a role similar to the acetaminophen reactive metabolites in analgesic nephropathy. Many of these compounds are carcinogenic and could also cause urothelial cancer.

The results of molecular biological investigations will allow the discovery of genetic markers of BEN, permitting early detection of BEN-predisposing muta-
tions and identification of susceptible individuals who may be at risk.

Strict preventive measures should follow identification of the aetiological agents of BEN. This could enable eradication of BEN, one of the last environmental diseases with considerable morbidity and mortality.

References


