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## Cross-Sectional Study in the Balkan Endemic Nephropathy Village of Vreoci (Serbia)

I. Maric<sup>1</sup>, D. Bukvic<sup>1</sup>, M. Bogdanovic<sup>1</sup>, M. Milic<sup>1</sup>, M. Ajdacic<sup>1</sup>, G. Popovic<sup>1</sup>, V. Toskovic<sup>1</sup>, A. Arsenovic<sup>1</sup>, S. Vitas<sup>1</sup>, V. Pakic<sup>1</sup>, V. Pejovic<sup>1</sup>, A. Dimitrijevic<sup>1</sup>, D. Lojovic<sup>1</sup>, D. Stefanovic<sup>1</sup> and Lj. Djukanovic<sup>2</sup>

<sup>1</sup>Institute of Endemic Nephropathy, Lazarevac, <sup>2</sup>Academy of Medical Science, Serbian Medical Society, Belgrade, Serbia

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### Abstract

**Background.** The municipality of Lazarevac is the most affected region with the Balkan endemic nephropathy (BEN) on the Balkan Peninsula. The present cross-sectional study was carried out in Vreoci, one of the most affected villages of municipality of Lazarevac. The aim of the study was to determine the prevalence of BEN in the village and to examine clinical characteristics of patients in different phases of BEN.

**Methods.** The study was carried out in three phases based of the field study method, and the diagnosis of BEN was established according to Danilović's criteria.

**Results.** The cross-sectional study included 2009 inhabitants of the village of Vreoci (82% of the total population above 18 years of age). BEN was diagnosed in 19 patients and suspected BEN in 23 patients. The prevalence of the disease was 2.09%. All the patients diagnosed with BEN had positive epidemiological criteria, but azotemia and proteinuria were the most common among the other criteria while low specific gravity of the urine was the least commonly evidenced criterion. In addition to patients diagnosed with BEN and suspected BEN, 30 individuals with positive epidemiological criteria and proteinuria were identified in the BEN families. None of the remaining Danilović's criteria was found in these persons but multiple kidney function disorders not included in existing criteria were revealed.

**Conclusions.** The cross-sectional study carried out in the village of Vreoci evidenced that the prevalence of the disease was 2.09% indicating that the intensity of the endemic process in this village remained unchanged throughout decades.

**Key words:** Balkan endemic nephropathy, cross-sectional study, prevalence

### Introduction

After describing Balkan endemic nephropathy (BEN) in the village of Šopić near Lazarevac (Serbia) (1), Danilović and collaborators carried out the first field study in the region of Kolubara. The municipality of Lazarevac was found to be the most affected, and the villages of Petka, Šopić and Vreoci were described as three most seriously affected villages in the municipality (2).

Over the last decade, controversial results on the prevalence and incidence of BEN have been reported. The authors from

South Serbia and Bulgaria described a decreasing incidence of BEN (3,4), however, unchanged prevalence of BEN was described in the region of Kolubara (5), high specific BEN mortality in endemic foci of Croatia (6) and a high prevalence of chronic dialysis patients with BEN in North-Eastern Bosnia (7).

The present cross-section study, carried out in the village of Vreoci in the period 2002 – 2003, was undertaken with an aim to determine prevalence of BEN in Vreoci and to examine clinical characteristics of patients in different phases of BEN.

### Patients and methods

The study was carried out in the following three phases according to the previously developed method for BEN field study (8).

*The first phase of the study* was performed in the field. All inhabitants of the village of Vreoci above 18 years of age were invited to participate based on the electoral roll, and the first phase included epidemiological questionnaire as well as personal and family history of the subjects, blood pressure measurements and analysis of proteinuria in the morning urine using sulfosalicylic acid.

*The second phase of the study* was carried out at the Institute of Endemic Nephropathy, when history data were supplemented, detailed objective examinations were performed, body weight and body height measurements were obtained as well as laboratory analyses of blood count, glycemia, urea, creatinine and qualitative examination of the morning urine. Arterial hypertension was diagnosed when systolic blood pressure was >140 mmHg and/or diastolic pressure was >90 mmHg or if an antihypertensive treatment was prescribed. All persons with any detected disorder were summoned for the following phase.

*The third phase of the study* included more detailed laboratory analyses that involved determination of peripheral blood cell counts, serum and urine levels of urea, sodium and phosphate measured with a commercially available kit. Serum creatinine was determined by the modified Jaffe rate method. Urine glucose was measured as a "spot test" with a dipstick containing a color-sensitive pad and urine alpha1-microglobulin by immunoturbidimetric assay (Turbitex  $\alpha_1$ -microglobulin; Roche/Hitachi 902) (normal value <1.5 mg/mmol creatinine). Morning urine specimens were used for all the above-mentioned analyses.

In the sample of urine collected over 24 hours, protein was measured by a biuret method and proteinuria greater than 200 mg per day was considered to be a pathological finding. Urine creatinine was determined by the Jaffe method and 24h urinary creatinine clearance was calculated. Fractional sodium excretion (FENa) was calculated using the following formula:  $(\text{urine}_{\text{Na}} \times \text{serum}_{\text{creatinine}} / \text{serum}_{\text{Na}} \times \text{urine}_{\text{creatinine}}) \times 100$ . Percentage tubular phosphate reabsorption (TRP) was calculated according to the following formula:  $[1 - (\text{urine}_{\text{PO}_4} \times \text{serum}_{\text{creatinine}} / \text{serum}_{\text{PO}_4} \times \text{urine}_{\text{creatinine}})] \times 100$ . Urine specific gravity was measured in a morning specimen after 8-hour water deprivation and the values below 1005 were considered to be pathological ones.

Sonographic examinations were performed by highly skilled physician on a Vivid 3-General Electric ultrasound machine with 3.5 MHz sector probe. Lengths of the left and right kidneys were measured in each subject, and the length smaller than 10 cm was considered to be a reduced one.

BEN diagnosis was established according to the criteria proposed by Danilović: (1) farmers from the affected villages, (2) family history of the renal diseases, (3) proteinuria, (4) low specific gravity of the urine, (5) anemia, (6) increased blood urea, (7) symmetrically shrunken kidneys. Based on Danilović's recommendation, the subjects were allocated to the following groups: (1) individuals with proteinuria and positive family history (subjects meeting the initial three criteria) (2) patients with suspected BEN (meeting in addition to the first three criteria, one of the remaining criteria), (3) patients with BEN (meeting at least five criteria) (9).

Detailed clinical studies were carried out in all the patients suspected as having BEN in order to rule out other renal diseases. Individuals from the non-endemic families with suspected kidney diseases were subjected to a detailed diagnostic evaluation aimed at establishment of the final diagnosis. These groups of patients were not involved in the present study.

*Statistical analysis.* Results are reported as frequencies of individual diagnostic criteria. According to Danilović, prevalence of BEN is defined as the sum of BEN and sBEN patients expressed in percentage of the total number of examined inhabitants. Results of clinical and laboratory examinations are reported as frequency for categorical data and as mean and SD for continuous data. The variables of the three groups were compared with one-way analysis of variance (ANOVA) and the statistical significance of the differences between the groups was evaluated using the  $\chi^2$ -test and unpaired t-test as appropriate.

**Results**

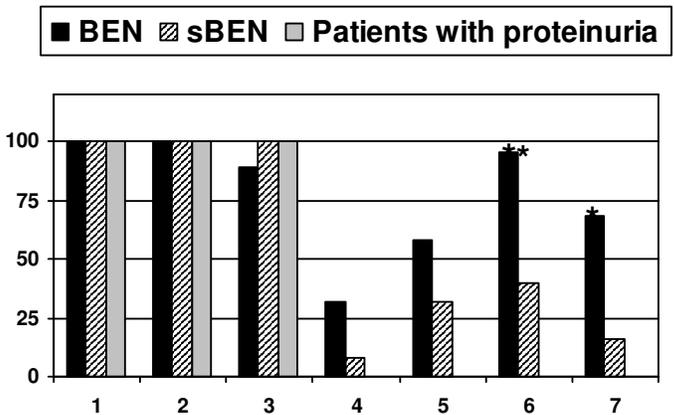
During the first phase of the study, 2,462 inhabitants of the village of Vreoci above 18 years of age were invited. The totals of 2009 individuals responded to the invitation and were examined within the first phase. Based on the examinations involved in the first phase, it was evidenced that 536 individuals had a positive family or personal history of the kidney diseases and proteinuria. These individuals were invited to participate in the second phase of the study. Out of 536 invited individuals, 388 responded while in 151 of them pathologic findings were detected either by objective or laboratory investigations. These subjects were thereafter

invited to participate in the third phase of the study. Out of the 151 invited individuals 146 complied with the invitation. Based on the analyses performed within the third phase and using the criteria for diagnosis of BEN established by Danilović, 19 subjects were diagnosed as having BEN, while 23 individuals as suspected BEN and 30 individuals had proteinuria and positive epidemiological and family history (group 1 according to Danilovic). All these 72 individuals with the signs of kidney disease were detected in 41 previously known BEN families. It was concluded that among the studied population of 2,009 inhabitants of the village of Vreoci, there were 42 individuals who met the criteria for BEN and suspected BEN, which gave the prevalence of the disease of 2.09% (Table 1). None individual from non-BEN families met the criteria for BEN.

**Table 1.** Prevalence of Balkan endemic nephropathy in the village of Vreoci found in a cross sectional study conducted in 2002-2003

Number of invited persons	Number of examined persons	Number of persons With BEN suspected BEN		Prevalence
2462	2009 (81.6%)	19	23	2.09%

Figure 1 presents the prevalence of the diagnostic criteria defined by Danilovic in patients with BEN, suspected BEN and those with proteinuria. All the examined individuals were from the BEN-affected village of Vreoci and they belonged to BEN families and all of them, except one BEN patient, had proteinuria at the time of study. Beside these first three diagnostic criteria, none of the remaining criteria was found in the group of patients with proteinuria. The prevalence of anemia and low specific gravity differed insignificantly between BEN and suspected BEN, while azotemia and reduced kidney length were found to be significantly more frequent in BEN than in the suspected BEN patients (Fig. 1).



**Figure 1.** Prevalence of the diagnostic criteria defined by Danilovic in the three examined groups. Criteria: (1) farmer families in the endemic villages, (2) familial history positive for BEN (3) proteinuria, (4) low urine specific gravity, (5) anemia, (6) azotemia, (7) symmetrically shrunken kidneys (1) \*p<0.05 as compared to all other groups ( $\chi^2$ -test)

Table 2 presents the demographic, clinical, laboratory and ultrasound data on three patient groups detected in BEN families during cross sectional study in the village of Vreoci. The results presented show that patients with proteinuria

were significantly younger, had lower systolic blood pressure and serum urea and creatinine levels as compared to BEN and BEN suspected patients. However, no significant difference in frequency of hypertension and glucosuria was found between patients with proteinuria and BEN and BEN suspected patients. Significant difference in creatinine clearance, alfa1- microglobulinuria and kidney length was found among the three examined groups. Patients with proteinuria had normal mean creatinine clearance and kidney length and increased alfa1-microglobulinuria appeared less frequently than in BEN and BEN suspected patients. BEN patients had the lowest and subnormal creatinine clearance and kidney length and the most excessively increased alfa1-microglobulinuria. The later group also had significantly lower hemoglobin value as compared to the other two groups.

**Table 2.** Demographic, clinical, laboratory and ultrasound data on the three groups from BEN families

Characteristic	BEN	BEN suspected	BEN members with proteinuria
Sex, F/M	10/9	11/12	13/17
Age, year	68.3 ± 13.9	59.2 ± 13.5*	53.5 ± 11.5*#
Systolic BP, mmHg	176 ± 23	159 ± 25*	146 ± 20.*#
Diastolic BP, mmHg	100 ± 9	97 ± 15	93 ± 10.6*
No of pts with hypertension	16/19 (84%)	15/23 (65%)	16/30 (53%)
u-protein, g/24h	354 ± 259	331 ± 158	322 ± 151.8
Specific gravity	1015 ± 5	1017 ± 6	1017 ± 5
Hemoglobin, g/l	129 ± 21	137 ± 15	144 ± 11*
s- Urea, mmol/l	10.0 ± 4.2	8.0 ± 2.8	5.0 ± 1.0*#
s- Creatinine, umol/l	125 ± 45	88 ± 31	64 ± 14*#
Ccr ml/min	64.8 ± 29.5**	90.0 ± 24.6**	143.8 ± 34.8**
No of pts with glucosuria	8/19 (42%)	11/23 (48%)	8/30 (27%)
u- alfa1-MG, mg/mmolCr	6.94 ± 6.75**	2.85 ± 4.77**	0.89 ± 1.2**
No of pts with ↑ u- alfa1-MG	14/19 (74%)	15/23 (65%)	5/30 (17%)*#
FENa, %	1.03 ± 0.45	1.16 ± 1.50	0.61 ± 0.22
TRP, %	82 ± 7	81 ± 31	87 ± 7
Kidney length, cm	9.6 ± 0.6**	10.3 ± 0.6**	11.0 ± 0.8**

\*p<0.05 in comparison with BEN; # p<0.05 in comparison with BEN suspected;

\*\* p<0.05 in comparison with all other groups

BP- blood pressure; CCR- creatinine clearance; AP – alkaline phosphatase

## Discussion

By the end of nineties and at the beginning of the new century, some authors reported vanishing of BEN in some endemic regions (2,3,10,11). As opposed to such information, studies carried out in the endemic region of the municipality of Lazarevac indicated the stable incidence of the disease or even increase of BEN incidence (12,13). The present study was carried out in order to verify the prevalence of BEN in the village of Vreoci.

The cross-section study included 2,009 residents of the BEN affected village of Vreoci, which accounted for 82% of the

total number of inhabitants above 18 years of age. The investigation was carried out in the three previously described phases (8) and the patients were classified according to Danilovic's criteria (9) given above in the introductory section. BEN was evidenced in 19 cases while it was suspected in 23 individuals, and thus prevalence of the disease was calculated to be 2.09%. Danilović and collaborators reported within the first field study carried out in the villages in the municipality of Lazarevac a prevalence of BEN of 1.66% in the same village (2), which indicates that the endemic process is still present in this village with the unchanged intensity.

Major characteristic of this cross-sectional study is that it included in all three phases the individuals originating from the families burdened with BEN as well as those not burdened with BEN. Within the second phase of the study, signs of the disease were evidenced in 151 individuals out of whom 146 (97%) were examined within the third phase. This phase included detailed diagnostic methods routinely used in differential diagnostics of the renal diseases. Persons that fulfilled diagnostic criteria for BEN and suspected BEN were found only in 41 families already proven to be burdened with BEN, but in none of the members of 89 non-BEN families.

Analysis of the prevalence of criteria proposed by Danilovic showed that almost all BEN patients had azotemia and its prevalence was significantly higher in BEN than in patients with suspected BEN. The former explained significantly more frequently reduced kidney length in BEN than in suspected BEN patients (Figure 1). This analysis as well as the comparison of the mean values for other parameters relating to kidney function and size (Table 2) confirmed the fact that the current criteria enable diagnosis of BEN only in the late stages of the disease. These patients had chronic kidney failure and therefore exhibited anemia, significantly higher values for urea and creatinine, lower creatinine clearance and lower kidney length in comparison with BEN suspected patients and patients with proteinuria (Table 2). Namely, in addition to patients who could have been diagnosed as BEN and suspected BEN based on the existing criteria, 30 individuals with proteinuria were identified in the BEN families, as well. Although some of them had additional pathological findings, these individuals could not have been classified in BEN group or suspected BEN group. Moreover, they were not diagnosed with other diseases. These individuals may be categorized as group 1, based on Danilović's classification. Analysis of individual results of these patients revealed increased alpha1-microglobulinuria in 5, glucosuria in 8 cases, decreased % TRP in 5 and hypertension in 16 individuals. Although tubular disorders are less frequent in patients with proteinuria than in BEN and BEN suspected patients, the presence of different tubular disorders in BEN family members with proteinuria suggest that they might be considered as patients in the early phase of the disease and the definition of this group requires revision.

## Conclusion

The cross-sectional study carried out in the village of Vreoci included 82% of all residents above 18 years of age. Using Danilović's criteria and ruling out of other renal diseases led to diagnosis of BEN in 19 patients and suspected BEN in 23 individuals and the prevalence of the disease was calculated to be 2.09%. Diagnosis of BEN based on existing criteria

was established in the late stage of the disease. Individuals designated as patients with proteinuria with insufficient criteria for diagnosis BEN and suspected BEN had multiple disorders of kidney function and required repeated and close controls

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