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ORIGINAL PAPER

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Early Screening of Balkan Endemic Nephropathy

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ABSTRACT

Introduction: Balkan endemic nephropathy (BEN) is a chronic irreversible interstitial sclerosis, for which over the last 25 years, chronic exposure to aristolochic acid from the contaminated cereal seeds has been considered the most likely cause. The aim of our research is to reevaluate trends of disease and to try to obtain new information about practical implementing of in-field screening of BEN, and to find indicators or a reliable biomarker for an early detection of the disease, especially for in field conditions. **Patients and methods:** Study was conducted in two phases (two groups of respondents). The first group related to respondents with BEN and microalbuminuria in the family. After filling out the questionnaire and following the consent of the respondents, their medical records were taken, and they were subjected to clinical examination and laboratory tests as well as to abdominal ultrasound and urinary tract examination. **Results:** For a long time, the disease is asymptomatic, with no hypertension, anemia or disturbed glucoregulation. Only A1M values were increased in the second group (16.22 mg), whereas A1M/CrUrine value was normal in both groups. Renal function in form of creatinine clearance and size of kidneys were in their referent values. **Conclusion:** The early stages of the disease are nonspecific, with no hypertension and disturbed glucoregulation, with normal renal function and blood count. The kidney size was in referent values. The only reliable symptom in the early stage of the renal disease was microalbuminuria.

Keywords: balkan endemic nephropathy, renal function, microalbuminuria.

clematitis seeds was most likely the cause of the disease (1, 2, 3). There haven't been any new epidemiological studies since 1978, which could provide conclusions as to the disease trends. Until the war, which in Bosnia and Herzegovina broke out in 1992, BEN was discovered in field conditions and was properly and systematically diagnosed, treated and followed-up in special institutions so called departments of endemic nephropathy. Based on daily records and correspondence, majority of BEN experts are of the opinion that the overall number of patients has decreased but that the number of hemodialysis patients is approximately the same and it relates to patients over 50 years of age (3). Histopathological events in the early stages of BEN have been tested and generally accepted (4). The clinical picture has also been reliably established and tested, but it has significantly changed since the first description of the disease (3, 5). For all the above mentioned, the early stages of BEN are important, and based on the opinion of a majority of scientists, they are difficult to detect, they are long lasting and occur in later life (3). Patients with no clinical symptoms and with normal renal function make early stages of BEN (3).

There is no reliable biomarker for field research, and the disease is associated with renal pelvis and ureter transitional cell cancer (6, 7, 8). Over 30% of patients in the North-East Bosnia are included in the hemodialysis program due to BEN and 11% of patients in the entire country of Bosnia and Herzegovina (B&H) (9). Therefore, in addition to being a scientific-medical problem, BEN also represents an economic and organizational problem given that it requires detection, treatment and construction of a Hemodialysis and Transplantation Center. The aim of the study was to examine clinical symptoms, laboratory tests, and the size of kidneys in the early stages of BEN in indigenous peoples in the hyper-endemic territory of the North-East of B&H.

1. INTRODUCTION

Balkan endemic nephropathy (BEN) was described in 1957 but its etiology was unknown for a long time. Serious studies carried out in the past 25 years gathered evidence and showed that toxin, aristolochic acid (AA) from *Aristolochia*

2. AIM

The aim of our research is to reevaluate trends of disease and to try to obtain new information about practical implementing of in-field screening of BEN, and to find indicators or a reliable biomarker for an early detection of the disease, especially for in field conditions.

3. PATIENTS AND METHODS

Since the end of the war in 1995 not a single reliable BEN related study has been carried out in B&H. Based on the project of the Academy of Sciences and Arts of Bosnia and Herzegovina and recent instructions of the 2013 Consensus, we conducted a study in a the hyper-endemic village of Domaljevac in the North-East of B&H (3). The study was approved by the Ethics Committee and Managing Board of the University Clinical Center Tuzla. The first phase involved invitation of all participants over 18 years of age and the urine microalbuminuria test (MA). As per strict instructions of the Consensus, urine samples were collected before breakfast and examined with urine test strips (3). Patients with positive protein in urine and positive microalbuminuria test were scheduled for the next day. The next day their anamnestic data were taken, they signed the questionnaire and were subsequently examined on the same day. Afterwards, their blood samples were taken for laboratory testing. Following the examination and recording of all clinical-epidemiological criteria and blood sample analysis, each respondent was subjected to detailed abdominal and urinary tract ultrasound examination (3, 10). Patients in the early stages of BEN are those with normal eGFR values according to the MDRD. Subsequent to the analysis of all anamnestic data and after clinical examination, the following laboratory tests were performed: red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), serum iron concentration (SI), glycemia, serum lipid levels, creatinine, glomerular filtration rate (GFR) according to the MDRD eGFR Equation, urine creatinine (CrUrine), Alpha 1-microglobulin (A1M), A1M/CrUrine ratio. The size of the right kidney was measured in the supine position in the mediosagittal maximum front section. For the left kidney the size was measured in the mediosagittal section on the right hip, given that as a rule the left kidney cannot be properly examined in the supine position. Ultrasound schools recommend mandatory renal examinations from the posterior surface of the stomach. However, in the continuous ultrasound practice these positions have been increasingly abandoned.

Maximum renal dimensions vary by race, gender, age and region. Normal length values are considered to be from 90 to 130 mm, and cortical thickness from 10 to 14 mm. The thickness of the kidney is around 40-50 mm and width around 40 mm. Variations in the length may vary up to 15 mm and are considered normal. In addition to length, width, appearance and size of renal parenchyma are the key factors in the functional state of the kidney. The span was measured at the junction of the middle and outer thirds of the kidney in maximal longitudinal mediosagittal front section (10). Both kidney size parameters vary with age. The length of a normal kidney is already 100 and over 100 mm at the age of 12-13, but real size is achieved at the age of 20. From the age of 70 the length of kidneys, if mutually symmetrical, at even 90 mm is considered normal and each parenchyma around 11 mm

width is still considered normal (5, 10).

Basic inclusion criteria were: permanent residence (over 15 years) in the hyper-endemic area of the village of Domaljevac, presence of MA and positive and negative family history of BEN, respectively. Exclusion criteria were: the existence of already diagnosed diabetes mellitus, hypertension, microhematuria, as well as the presence of any other diseases which could act as confounder and influence the results. Out of the total of 360 respondents examined in the village of Domaljevac in the first phase of the study, we selected 131 respondents with positive MA. Out of 131 respondents, 72 had to be excluded from the study due to hypertension and diabetes mellitus which resulted in 59 respondents remaining. Based on the presence and absence of BEN in the family history the respondents were divided into two groups: the first group included respondents with positive family history of BEN (36) and the second group respondents with negative family history (23). In the course of statistical data processing, we calculated the mean value, standard deviation, minimum and maximum value of the examined parameters and statistical significance.

4. RESULTS

In the first group comprising 36 respondents, 44.4% related to women and 55.6% to men. In the second group comprising 23 respondents, 60.9% related to women and 39.1% to men (Table 1). The mean age of women and men in

Group	Variable	N	Min	Max	mean
1	Female age (years)	16	31	75	49.2
	Male age (years)	20	18	72	46.3
	RBC (x10 ¹² /L)	36	3.06	5.59	4.60
	Hgb (g/L)	36	96.00	169.00	137.59
	Hct (%)	36	29.00	49.00	40.97
	SI (mcmol/L)	36	2.60	47.50	19.24
	Creatinine (mcmol/L)	36	55.00	132.00	80.36
	eGFR (mL/min)	36	48.00	126.00	91.14
	A1M (mg/L)	36	5.22	40.00	10.80
	CrUrine (mg/L)	36	2.21	40.00	15.38
	A1M/CrUrine (ratio)	36	.20	3.90	1.13
2	Female age (years)	14	26	77	54.3
	Male age (years)	9	28	73	52.3
	RBC (x10 ¹² /L)	23	3.22	5.08	4.39
	Hgb (g/L)	23	102.00	162.00	134.59
	Hct (%)	23	31.00	46.00	39.73
	SI (mcmol/L)	23	4.20	31.60	16.17
	Creatinine (mcmol/L)	23	49.00	119.00	77.39
	eGFR (mL/min)	23	39.00	130.00	86.70
	A1M (mg/L)	23	5.22	66.70	16.22
	CrUrine (mg/L)	23	2.65	34.70	13.98
	A1M/CrUrine (ratio)	23	.20	10.30	1.53

Table 1. Descriptive statistics of age, gender and laboratory

Parameter	Student t test	
	t	df
RBC	1.552	50
Hgb	.734	49
Hct	1.117	49
SI	1.595	56
Creatinine	.656	57
eGFR	.851	57
A1M	-1.524	57
CrUrine	.561	57
A1M/CrUrine	-1.001	56

Table 2. Results of statistical significance testing between the two examined groups

Group	Size (mm)	N	Min	Max	Mean
1	Right kidney length (mm)	36	78.00	120.00	103.94
	Right kidney cortical thickness (mm)	36	8.00	19.00	14.29
	Right kidney width (mm)	36	32.00	60.00	47.31
	Left kidney length (mm)	36	88.00	124.00	106.19
	Left kidney cortical thickness (mm)	36	9.00	19.00	14.83
	Left kidney width (mm)	36	38.00	55.00	47.64
2	Right kidney length (mm)	23	86.00	116.00	103.78
	Right kidney cortical thickness (mm)	23	10.00	18.00	13.91
	Right kidney width (mm)	23	37.00	58.00	45.65
	Left kidney length (mm)	23	83.00	121.00	103.87
	Left kidney cortical thickness (mm)	23	8.00	20.00	13.87
	Left kidney width (mm)	23	35.00	55.00	46.22

Table 3. Descriptive statistics of ultrasound kidney measurements in mm

the first group was 49.2 (range 31-75) and 46.3 (range 18-72) respectively. The average age in the second group was 54.3 (range 26-77) for women and 52.3 (28-73) for men. Comparison of the groups did not show any significant difference in gender and age values, $p>0.05$. Demographic and laboratory values are shown in Table 1 which contains RBC, Hgb, Hct, SI in their referent values. Creatinine, eGFR, A1M, CrUrine, A1M/CrUrine were also in their referent values. Only A1M values were increased in the second group (16.22 mg, range 5.22-66.70), whereas A1M/CrUrine value was normal in both groups. Statistical data is presented in Table 2 and 4 and it is visible that there are no significant differences in the mean values between the groups. Table 3 presents size of kidneys, maximum longitudinal and transverse diameter and kidney parenchyma width. Results of ultrasound imaging did not show a statistically significant difference in respect to nor-

Anatomical part	Student t test	
	t	df
Right kidney length (mm)	.065	56
Right kidney cortical thickness (mm)	.548	56
Right kidney width (mm)	1.046	56
Left kidney length (mm)	.925	57
Left kidney cortical thickness (mm)	1.437	57
Left kidney width (mm)	1.090	57

Table 4. Results of statistical significance testing between two examined

mal kidney size values or statistical differences between the groups regarding the obtained results.

5. DISCUSSION

In the first group of patients with family history of BEN, as well as in the second group with no family history of BEN, there was no difference in BEN prevalence between men and women, or in age difference, ($p>0.05$). Earlier, the prevalence of the disease was associated with women due to the fact that they spent more time at home (3, 5, 9). The disease does not have acute stages and may last for years, asymptomatic-lingering. The recent data of the B&H Renal Register (2015) shows that over 60% of BEN hemodialysis patients is over 65 years of age and that manifestation phases start in persons over 50 years of age (9). In our study 60% of respondents were over 50 years of age. For a long time aside from microalbuminuria and proteinuria there were no clear indicators for BEN among other biomarkers (5, 9). A study conducted a few decades ago pointed to anemia as an early symptom of the disease which was wrong, given that the creatinine clearance was not strictly determined in the test samples. Hence, the results were wrong since with normal serum creatinine values creatinine clearance may be reduced up to 50 ml/min (5). Not a single respondent from our groups suffered from anemia. All mean values of the serum creatinine and eGFR average values according to the MDRD equation were in their referent values. In the early stages of the disease in the endemic area, renal functions in both groups of respondents were in their referent values (5, 8).

By means of Sephadex electrophoresis one study revealed the existence of BEN in 60% of urine samples, increased values of Beta-2 microglobulin, but also emphasized the increased values of A1M in the same urine sample in 100% of respondents (6).

It is known that A1M is tubular injury marker. In our first group values of A1M in urine were referent, with mean value 10.8 mg/L, and it was obvious that average concentration was higher in the second group (16.22 mg/L), but with no statistical significance, ($p>0.05$), (Table 2). Also, the A1M/CrUrine ratio was undisturbed and in referent values. Taking into consideration the average age and reduction of the kidney function with age, the second group was somewhat older, but again with no statistical significance, ($p>0.05$). All residents of the endemic areas were exposed to the AA. It was considered that A1M values and A1M/CrUrine ratio should be higher in the first group given that due to family history the respondents

had predisposition for the disease. However, this study did not find any evidence in that regard. We believe that respondents from both groups were in the same stages of the disease, and their common features were that they lived in the endemic area and had elevated values of microalbuminuria. For the time being, microalbuminuria is probably the only reliable parameter and early marker of early renal injury, in other words the marker of the early stages of BEN(7). The average elevated A1M values were not elevated in the early stages, and recent studies consider it as a non-reliable biomarker (8). When elevated A1M values are found in residents, that almost certainly means that they have a proximal tubule injury and advancing BEN. Given that its calculated values were similar between the groups, we believe that only the amount of ingested toxin influences the dynamic of BEN development. We believe that it takes some time for a sufficient amount of toxin to entered through contaminated flour (Aristolohioic acid), and subsequent development of BEN. Due to the required duration of exposure to poison the disease has not been detected in childhood and adolescence (3). Also, mosaic and irregular layout of BEN on the terrain explains the significance of the duration and intensity of exposure to AA.

Because of the consequences of the 1992-1995 war in Bosnia and Herzegovina-residents who have moved from BEN territories inhabited cities and it remains an open question how will the disease develop in population displaced from endemic territories to other countries all over the world.

All average kidney ultrasound measurements were in their referent values and there was no significant difference between the groups, ($p>0.05$), (Table 3 and 4).

Reduces number of BEN patients (there are no new patients and new focal points of BEN in the countries of ex-Yugoslavia (B&H, Croatia, Serbia, Macedonia), Romania and Bulgaria) is the result of fact that life conditions and cultivation of the land have improved and that strict use of own flour and its products has been reduced. Flour is also imported from other regions. It is also generally known that herbicides are used in the production of crops (3, 9). Early stages of the disease are associated with older age. There are no differences in prevalence of the disease by gender. The disease progression is asymptomatic, mild, without hypertension, anemia and diabetes mellitus. Renal functions, A1M, A1M/CrUrine, and ultrasound size of the kidney in the early stages of BEN are all in their referent values.

6. CONCLUSION

Our study has shown that the only reliable early biomarker of the disease, which could be used in the field screening, is elevated microalbuminuria. If there were respondents with elevated microalbuminuria in the endemic area, regardless

of their family history, they should be taken for further planning, monitoring and eventual renal biopsy.

We met our study aim, and reevaluated trends of the disease and obtained new information about practical implementing of in-field screening of BEN, and also have found early indicator in form of a reliable biomarker for an early detection of the disease, especially for in field condition.

The small sample could be a possible limitation of this study.

• Conflict of interest: None

• **Author contribution:** : S.T., E.N., E.T., E.T., make substantial contributions to conception and design of study, and and interpretation of data. S.T., E.N., E.T.,E.T.. participate in drafting the article or revising it critically for important intellectual content. Authors give final approval of the version to be submitted.

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