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Original article

Novel epidemiological data on Balkan Endemic Nephropathy in endemic foci of Federation of Bosnia and Herzegovina

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Abstract

Introduction: While Balkan endemic nephropathy (BEN) remains one of the leading causes of renal failure in Federation of Bosnia and Herzegovina (FB&H), valid epidemiological data on BEN in FB&H after 1989 do not exist. We conducted a study to determine the prevalence of BEN, and analyse epidemiological and relevant clinical data of patients in different phases of BEN.
Materials and methods: A cross-sectional study which surveyed inhabitants of eight endemic villages in FB&H was performed in 2013. For every participant, physical examination, urinalysis on albuminuria presence and urine creatinine measurement were performed. A specially designed questionnaire was filled in for every participant. Patients classified in a group with intermittent proteinuria (IP), according to Danilovic's criteria, were referred for further diagnostics followed by definite classification.
Results: We examined a total of 486 patients. Prevalence of diseased was 0.7%, suspect 2.7%, and patients with IP 17.4%. Females were dominant in group with IP while sex ratio was 1:1 in the suspect group; diseased patients were all males. Age category 60-79 is dominant in all three BEN groups. Statistically significant difference in mean/median values of diastolic blood pressure, albumin/creatinine ratio, erythrocyte count, haemoglobin, blood urea nitrogen and blood creatinine levels, between BEN groups, was found to exist.
Conclusions: BEN will continue to be the leading cause of renal failure in endemic areas of FB&H, as prevalence indicates that there still are cases of BEN and the endemicity continues.

Keywords: Balkan endemic nephropathy, epidemiology, Federation of Bosnia and Herzegovina

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Introduction

At the time of its discovery (in 1957), Balkan endemic nephropathy (BEN) was found in six municipalities of former socialistic republic of Bosnia and Herzegovina (SR B&H): Bijeljina, Brčko, Bosanski Šamac, Modriča, Odžak, and Orašje, representing 4.12% of the total SR B&H territory (1). Northern boundary was Sava river, east boundary was Drina river, west boundary was partially created by Bosna river and neighbouring municipalities of Bosanski Brod, Derventa and Doboj, while on the south boundary neighbouring municipalities were Gradačac, Lopare, Ugljevik and Zvornik (1). From the above named six municipalities, on today's territory of FB&H remained only two (Odžak and Orašje), as well as a newly formed Domaljevac municipality (formerly part of municipality of Bosanski Šamac). Research performed by, among others, epidemiologists from Faculty of Medicine University of Sarajevo, in the period 1984-1989 found 10 endemic villages in Odžak municipality and 12 in Orašje municipality; village of Domaljevac (situated on the territory of today's Domaljevac municipality) was also endemic (2).

Research performed during 1975 and 1976 on over 200,000 inhabitants of endemic villages in B&H cite the prevalence of suspect cases of 13.5%, being smallest in Odžak municipality (3.2%) and largest in Bijeljina municipality (57.0%), while prevalence of confirmed cases was 5.9% (ranging from 17.7% in Bijeljina municipality to 2.4% in Odžak municipality) (3). Researches performed during the period of 1984-1989 cite the total number of diseased of 1,435, meaning prevalence of 4.82 per 1,000 population, ranging from 2.16 per 1,000 population (Modriča) to 7.24 per 1,000 population (Bosanski Šamac) (2). Same researches cite the prevalence among females 1.3 times higher than in males (56.1% of all cases were women), without difference through age categories; only in the period of 1957-1958, morbidity rate was higher

among men (gender ratio 6.4‰ to 5.3‰), with this difference decreasing significantly in the period of 1984-1985 (4.2‰ to 3.7‰) (2). Same researches cite that the highest prevalence of the disease was in the age group 40-59, followed by the age group 25-39; four cases among those aged 15 and younger were identified (1, 2). Over 95% of cases were farmers and, rarely, pensioners, teachers or clerks, who lived in the endemic areas for longer period of time and performed field work in their free time (2).

Today, BEN remains one of the leading causes of renal failure in B&H; in endemic regions of our country prevalence of patients receiving dialysis treatment due to BEN is 3.5 times higher than those receiving treatment due to other causes (14).

On the other hand, although research on BEN on the territory of B&H is being performed until today, by (almost exclusively) clinicians – nephrologists, data collected earlier in the late 1980s were the last methodologically correctly collected data on epidemiological characteristics of BEN in B&H.

Therefore, we performed a study to determine prevalence of BEN in analysed study sample of FB&H's endemic villages' inhabitants and to examine epidemiological and clinical characteristics of patients in different phases of BEN.

Materials and Methods

This study was performed on inhabitants of seven endemic villages in Odžak municipality: Donja Dubica, Gornja Dubica, Prud, Novo Selo, Vojskova, Osječak, and Zorice, and village of Domaljevac (in Domaljevac municipality). Participants younger than 18 as well as those with confirmed chronic kidney disease (including endemic nephropathy), were excluded from the study.

Field work was conducted in two time periods (5-9 November 2012, and 15-16 March 2013).

For every village the study period and place were scheduled and announced upfront. Endemic villages inhabitants – volunteers, who reported to the study site were firstly asked to read, agree upon and sign the informed consent, followed by filling in the specially designed questionnaire (by trained research staff). At the same time, participants were asked to provide a fresh urine sample which was subsequently analysed on albuminuria presence and the value of creatinine in urine was measured, using SIEMENS Microalbustix™ reagent strips, following by the manufacturer prescribed method (in a fresh urine sample, dip in the reagent strip and take out immediately afterwards; after 50 seconds the results were read visually by comparing the colour on the strip to a colour chart on the label (for albumin concentration) and the same process was repeated after 60 seconds (for creatinine concentration)). Afterwards albumin-to-creatinine ratio was calculated and NKF (*the National Kidney Foundation*) KDOQI (*Kidney Disease Outcomes Quality Initiative*) guidelines were used for interpretation of the results (for women: <25 mg/g – normal, 25-355 mg/g – microalbuminuria, >355 mg/g – albuminuria; for men: <17 mg/g – normal, 17-250 mg/g – microalbuminuria, >250 mg/g – albuminuria) (4).

Subsequently physical examination was performed for every participant, and blood pressure was measured using a standard sphygmomanometer (Conrad Germany), following the standard procedure.

Using Danilovic's criteria (5-10), participants who were classified as having intermittent proteinuria (IP) were referred to further clinical (diagnostic) testing: laboratory analyses of complete blood count, serum urea (BUN) and serum creatinine, performed in the laboratory of Health Center Odžak (for participants coming from Odžak municipality) / laboratory of Health Center Domaljevac (for participants coming from Domaljevac municipality). Following these results, participants were definitely classified into one of four groups: diseased, suspect, having IP, or healthy (5-10).

Data analysis was performed using MS Excel 2010 for Windows, and SPSS Statistics v. 20.0, by descriptive and inferential biostatistical methods. Normally distributed numerical variables were described by arithmetic mean (and standard deviation), while those non-normally distributed were described using the median (and interquartile range) (Kolmogorov-Smirnov, and Shapiro-Wilk test, where appropriate, was used to test normality of the distribution). Qualitative variables were described using absolute and relative frequencies. Hypothesis testing was performed using the appropriate test with significance level set to 0.05. Results were also presented using tables and graphs.

Results

We examined total of 448 participants, 164 males and 284 females. Out of 114 (25.4%) participants who were referred to further clinical (diagnostic) testing, 93 (81.6%) responded (two participants died and 19 refused the testing). After obtaining laboratory tests results, 3 participants were classified as diseased (fulfilled the following criteria: being farmers from endemic settlements, having positive family history on BEN, proteinuria, anaemia, and increased BUN and serum creatinine), 12 as being suspect to have BEN (fulfilled the following criteria: being farmers from endemic settlements, having positive family history on BEN, proteinuria, and either anaemia or increased BUN and serum creatinine), and 78 as having IP (fulfilling only

the first three criteria as described above), indicating the prevalence of the disease is 3.3% (Table 1).

Structure of participants in BEN groups by age and gender is shown on Graphs 1 and 2, and Graph 3 shows their structure by occupation.

Table 2 shows values of participants' clinical and laboratory findings, by BEN groups (data for 21 participants who did not come for clinical examination and therefore remained unclassified are not presented).

Table 1. Prevalence of balkan endemic nephropathy in endemic villages of FB&H (2012-2013)

Total number of participants		448
Number of participants	With BEN	3
	With suspected BEN	12
Prevalence	Diseased	0.7%
	Suspect	2.7%
Overall prevalence		3.3%

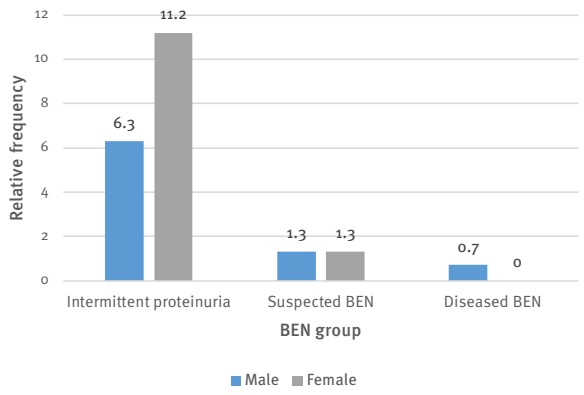

Figure 1. Structure of participants in BEN groups by gender

Discussion

Comparing the prevalence of BEN obtained in this study to prevalence obtained in the previous researches of the disease within six municipalities of SR B&H (taking into account only the number in a diseased group), we conclude that, with respect to year 1976,

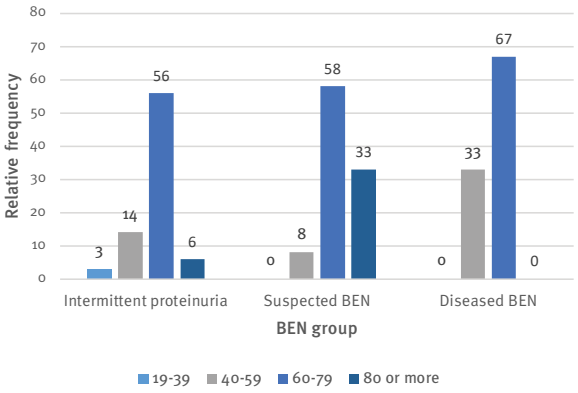

Figure 2. Distribution of participants in BEN groups by age

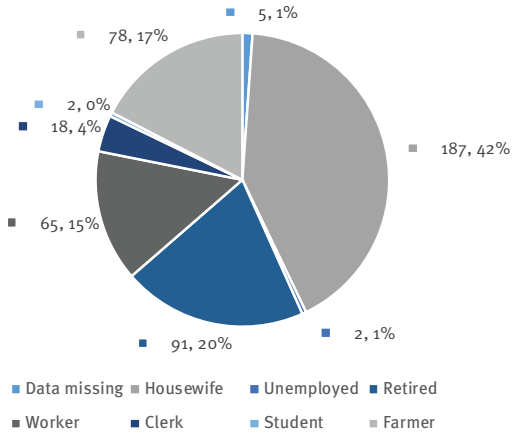

Figure 3. Structure of participants by occupation

Table 2. Descriptive data on participants' clinical and laboratory findings, by BEN groups

Variable	BEN group (Danilovic's criteria)			
	Healthy (with respect to BEN) (n=334)	With IP (n=78)	Suspect (n=12)	Diseased (n=3)
Systolic blood pressure (mmHg)	145 (130-165)	145 (130-160)	140 (125-165)	120 (104-160)
Diastolic blood pressure (mmHg)#	90 (80-95)	90 (80-95)	85 (80-90)	80 (62-90)
Percent of hypertensive subjects	55.0%	52.1%	50.0%	33.3%
Albumin/creatinine #	19.0 (10.0-60.0)	50.0 (33.0-100.0)	100.0 (63.8-381.8)	100.0 (50.0-266.7)
Erythrocyte count (x 10^9) *#	N/A	4.2 (±0.4)	3.6 (±0.3)	3.3 (±0.7)
Haemoglobin (g/L) *#	N/A	131.4 (±11.9)	99.5 (±13.8)	94.3 (±18.5)
BUN (mg/dL) #	N/A	19.8 (15.9-25.8)	48.8 (32.3-57.6)	88.5 (59.0-103.1)
Serum creatinine (mg/dL) #	N/A	1.0 (0.7-1.2)	2.0 (1.6-4.3)	8.4 (7.4-8.4)

IP: intermittent proteinuria; BUN: blood urea nitrogen
* Normally distributed variable; # Statistically significant difference in mean/median value between groups ($p<0.05$)

it is in a decline (3) (5.9% comparing to 0.7%), while, comparing to data from 1980s it is slightly higher (0.7% to 0.5%) (1, 2). If we analyse the data from single municipalities – taking, for example, Odžak, this difference in prevalence is somewhat smaller: in 1976 it was 2.4% (3) comparing to 0.7% today and 0.3% in the 1980s. The same trend of prevalence decrease is evident in the group of suspect cases (13.5% in 1976 (3) to 2.7% today). While interpreting the true meaning of these results, we should bear in mind the fact that territorial division of B&H today is not the same as it was in 1970s and 1980s. Some villages that are part of Odžak municipality today were in the past part of other municipalities (e.g. village of Prud was in the past part of Bosanski Šamac municipality). The same goes when interpreting results obtained in village of Domaljevac: prevalence of the diseased in former Bosanski Šamac municipality in 1976 was 12.3% and 0.7% in 1980s (firstly, our study did not identify any cases of BEN in this village, the percentage of suspect cases is 2.0%, while 25.0% of inhabitants of this village referred to clinical examination remained unclassified; secondly, village of Domaljevac today is a part of Domaljevac municipality while in the past it belonged to Bosanski Šamac municipality). Also, it should be considered that the research conducted in 1970s and 1980s encompassed over 90% of all endemic villages' inhabitants (1-3), while our study was performed on endemic villages' inhabitants – volunteers, so we can assume that the obtained prevalence is probably overestimation of the real proportion of diseased / suspect cases in the total endemic villages' population. Nevertheless, this study's results imply that the endemic process in B&H is still on-going.

Results of the study on BEN prevalence in Vreoci village, one of the most affected villages of municipality of Lazarevac, Serbia (traditionally the most affected BEN region in Serbia), conducted in 2002-2003, cite the prevalence of suspect cases to be 1.14% and diseased 0.95% (12). This study's results, which used Danilovic's criteria for BEN diagnosis, are consistent with our study's results as they cite a similar percentage of the diseased as identified by our study. On the other hand, it cites the prevalence of intermittent proteinuria of only 1.5% (12), which is considerably smaller than that identified by our study. A study performed in Croatia's three endemic villages during 2005-2010, which used WHO's criteria for BEN diagnosis (6, 13), cite the prevalence of diseased to be 1.3% and suspect 3.6% (6), which is also consistent with our results.

As with the overall structure of participants by sex, our sample included 60.2% of females and 39.8% of males. It is interesting that we did not identify any females among the diseased, meaning our results are contradicting the 1980s study's results which cite higher proportion of females among the diseased: out of total number of diseased in Odžak municipality, 56.3% were females, and almost as many in Domaljevac (55.0%). On the other hand, the same research identified twice as many diseased males in the village of Novo Selo (Odžak municipality), and equal number of diseased males and females in the village of Prud (Bosanski Šamac municipality) (1, 2). Similarly, if we compare the proportion of women identified by our study in groups of diseased and suspect together, sex ratio is still in favour of males (1.5 to 1), although in the suspect group there is the same number of females and males. On the other hand, in the group with intermittent proteinuria, females are considerably dominant (64.1% to 35.9%).

With respect to participants' age, age category 60-79 is dominant in all three BEN groups. Among diseased there are no participants younger than 40 and older than 80 years of age; 66.7% are aged 60-79 and 33.3% are 40-59. These results confirm hypothesis that manifest disease is the most prevalent in older age categories, proven by many authors (5, 11). Comparing to the researches results in B&H from 1980s, when the disease was most prevalent in age category 40-59 (2), the same hypothesis is confirmed. One of the possible explanations for this age distribution is that those identified as diseased in 1980s (assuming they are still alive) are the same people who are now aged 60-79 and/or 80 or more, but since the incidence of the disease is unknown, as well as BEN specific mortality rates, this explanation is not based on scientific facts.

Data on demographic characteristics of participants classified in different BEN groups from studies of Croatian, Serbian and Bulgarian authors: more females being affected by the disease than males, most of the diseased being in the sixth decade of life (5, 6, 12), are also consistent with our study's results, except for that our study did not identify any diseased females and equal number of females and males in the suspect group.

As with participants' occupation, most are housewives (42%), followed by pensioners (20%) and farmers (17%). Rest are workers, clerks, students and unemployed, while for 1% of participants occupation is unknown. It is interesting that, when occupation is analysed by BEN groups, 66.6% of diseased participants are workers, and for the rest the occupation is unknown; all of them work on the land in their free time. In the group of suspects, equal percentage of participants are pensioners and housewives (33.3%), followed by farmers (25.0%); one participant is a worker. In the intermittent proteinuria group, the distribution of participants follows the overall distribution by participants' occupation, meaning that most participants are housewives (43.6%), followed by pensioners

(28.2%), farmers (17.9%), workers (9.0%) and clerks (1.3%). All participants, whose primary occupation is not farming, work on the land in their free time.

Analysing the values of relevant (laboratory) parameters obtained from participants in this study, we found no statistical significance in the difference in median values of systolic blood pressure between participants in different BEN groups (p=0.342). This opposes to the results of a recent study on BEN conducted in the region which proved the existence of this significance among diseased and suspect, as well as among diseased and those in the IP group (12); results of another study, which used WHO criteria (6, 13) for classification of participants into BEN groups cite the existence of this significance among suspect and those under risk for BEN, and suspect and others (6).

Difference in median values of diastolic blood pressure between participants in different BEN groups, was found to be statistically significant (p<0.001), which is consistent with other studies' results (6, 12). It is interesting that the largest percentage of participants with hypertension is found within the healthy group (55.0%), but is also high among those in the IP group (52.1%) (which might possibly explain their pathological urinalysis results, i.e. (micro)albuminuria due to another kidney disease – possibly hypertensive nephropathy). The smallest percentage of participants with hypertension is found among the diseased (33.3%), confirming the hypothesis that people with BEN are usually normotensive (9, 10) and that BEN diseased in terminal stage show systolic blood pressure around 200 mmHg and diastolic blood pressure around 110 mmHg in only 30-40% of cases (9).

Proteinuria was assessed by calculating and interpreting the values of albumin to creatinine ratio. Median value of albumin/creatinine ratio in the diseased group was 100.0 mg/g (IQR 50.0-266.0 mg/g), as well as in the suspect group (IQR 63.8-381.8 mg/g); in the IP group it was 50.0 mg/g (IQR 33.0-100.0 mg/g). Healthy participants had median value of albumin/creatinine ratio of 19.0 mg/g (IQR 10.0-60.0 mg/g). We found statistically significant difference in median values of albumin/creatinine ratio between BEN groups (p<0.001).

When analysing parameters of blood count (measured only for participants originally classified in the IP group requiring further clinical testing), we found the difference in mean values of number of erythrocytes between participants in different BEN groups to be statistically significant (p<0.001), as well as in haemoglobin level (p<0.001). This is expected, as these parameters' values are taken into account when the classification in BEN groups using Danilovic's criteria is performed (5). These results are consistent with a recent study's results performed in Croatia (6) (which used *WHO* criteria (6, 13) for classification of participants into BEN groups), as well as with other studies from the region (6, 12).

Blood urea nitrogen (BUN) value is also a diagnostic criterion when diagnosing BEN using Danilovic's criteria; the upper limit of the normal range is 50 mg/% (5-10). We found the difference in median values of BUN levels between participants in different BEN groups to be statistically significant (p<0.001), which could be expected as for the above named reason. These results are consistent with a recent study's results performed in Serbia which cite the existence of this significance among participants in different BEN groups (12).

Blood creatinine values, along with BUN levels, are used to assess the existence of uraemia and to diagnose BEN following Danilovic's criteria (5-10); values of 1.5 mg/% or less are considered normal. Statistical significance of the difference in median values of creatinine levels between participants in different BEN groups was proved to exist (p<0.001), which could also be expected as for the above named reason. These results are consistent with the recent studies' results performed in the region which cite the existence of this significance between participants in different BEN groups (6, 12).

Conclusion

Balkan endemic nephropathy will, according to this study's results, remain one of the leading causes of kidney failure in north-eastern part of FB&H, where endemic settlements are. Although the incidence of the disease is unknown, its prevalence – identified within this study, is not to be disregarded: 3.3%, accounting for those classified as diseased (0.7%) and suspect (2.7%), as it shows that there are still people suffering from BEN and that endemic process is still on-going.

Declaration of interest

The authors declare no conflicts of interest.

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