ORIGINAL ARTICLE

Kidney length in healthy members of Balkan endemic nephropathy families

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Abstract

Background: Kidney size may differ between healthy members of Balkan endemic nephropathy (BEN) and non-BEN families. The present study was designed to elucidate this, in comparison with values for BEN patients.

Methods: A total of 71 BEN patients (34 males, 64.4 ± 12.0 years), 74 healthy BEN family members (39 males, 49.1 ± 12.2 years), and 59 non-BEN family members (19 males, 49.2 ± 12.3 years) were involved. We measured the longest craniocaudal length and minimal parenchymal thickness on each kidney of all examined subjects using ultrasound.

Results: No significant difference was found between the kidney length of healthy subjects from BEN (11.0 ± 0.8 cm) and non-BEN families (10.9 ± 0.8 cm), but kidneys were significantly longer than in BEN patients (9.9 ± 1.3 cm). Minimal parenchymal thickness was similar in all three groups. When subjects from each group were divided according to estimated glomerular filtration rate (eGFR), kidney length of the healthy groups was significantly longer than in BEN patients both in stage 1 ($p = 0.039$) and stage 2 ($p = 0.044$) of chronic kidney disease. The parental history of BEN was not associated with kidney dimensions, eGFR, or urinary excretion of albumin and alpha1-microglobulin.

Conclusion: Kidneys of BEN patients were significantly shorter than in healthy members of both BEN and non-BEN families, but no difference was found in kidney length and parenchymal thickness between healthy members of BEN and non-BEN families. No significant association was found between parental history of BEN and kidney size and function either in BEN patients or in healthy members from BEN families. Hippokratia 2015; 19 (4): 304-308.

Keywords: Balkan endemic nephropathy**,** kidney length, ultrasound

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Introduction

Symmetrically shrunken kidneys have been considered as a characteristic of Balkan endemic nephropathy (BEN) since the very beginning of investigation[1]. Therefore, reduced kidney size was included among the first definitions of diagnostic criteria for BEN[2,3]. However, in the recent proposals for screening and diagnosis of BEN, kidney size is no longer one of the elements for establishing BEN diagnosis[4,5], even though it is still paid considerable attention as an important characteristic of BEN.

The introduction of diagnostic ultrasound facilitated investigations of kidney dimensions in BEN, but there are some inconsistencies in the reported results arising mostly from differences in the studied groups. Nevertheless, several author groups agree that kidney size is shorter in BEN patients, even in those with normal kidney function than in healthy persons[6-9]. On the other hand, there is disagreement about kidney dimensions in members of BEN families without confirmed diagnosis of the disease. While Dimitrov et al[9] found significantly smaller kidney length and minimal cortex width in BEN offspring than in non-BEN offspring, Aleckovic et al[10] reported no difference in kidney length between persons from BEN and non-BEN families. In both studies, some of the examined members of BEN families had microalbuminuria and/or low-molecular weight proteinuria. The question remains about kidney size of BEN family members without any signs of kidney disease.

In the present study, we measured kidney length and parenchymal thickness in healthy members of BEN and non BEN families, and in patients with BEN. The aim of the study was to determine whether kidney size of BEN family members without any laboratory sign of BEN differed from those in healthy members of non-BEN families and to compare kidney size of these two groups with those in BEN patients.

Material and Methods

The study involved 204 persons selected during screening for chronic kidney disease in adult inhabitants of BEN villages in Bijeljina municipality (Bosnia and Herzegovina). The first phase of the screening involved

1938 persons over 18 years old and consisted of an interview, blood pressure measurement and urine dipstick test. The 324 persons with a history of more than three family members suffering from BEN, a personal history of kidney disease or pathological urinary findings (microalbuminuria - Micral-test, ACCU-CHEK products, Roche Diagnostics, Rotkreuz, Switzerland; proteinuria, hematuria, leukocyturia - urine dipstick test) were invited to the second phase of the examination. Also, 178 subjects randomly selected from the remaining persons with negative family and personal histories of kidney disease and normal urinary findings were also invited to the second phase of the examination. A total of 363 individuals responded and were subjected to an objective examination, blood pressure measurement, laboratory analysis of serum and urine and kidney ultrasound. Based on the results of these examinations 204 persons were included in the present study, while those with detected kidney diseases other than BEN or any other chronic disease were excluded.

The selected participants were allocated to three groups: one group of 74 healthy persons (39 males, aged 30-76 years) from BEN families and the second of 59 healthy persons (19 males, aged 31-84 years) from families without BEN, all with normal objective findings, normal blood pressure and normal laboratory results. The third group consisted of 71 patients (34 males, aged 47-83 years) with BEN diagnosed using recently defined criteria: i) farmers living in endangered villages, ii) a familial history positive for BEN, iii) low-molecular-weight proteinuria, iv) proteinuria, v) impairment of kidney function, vi) anemia, vii) symmetrically shrunken kidneys[5,11]. Diagnosis of BEN was established in patients who, in addition to the first two criteria, had either low-molecular-weight proteinuria or proteinuria and at least one of the remaining criteria but after exclusion of other kidney diseases. Adult polycystic kidney disease, obstructive uropathy, nephrolithiasis were excluded using ultrasound and, if necessary, other imaging methods, in addition to medical and family history, and laboratory examination. If glomerulonephritis was suspected (only two patients, both under the age of 50 years) a kidney biopsy was done, diagnosis of glomerulonephritis was confirmed, and the patients were excluded from the study. We also omitted all patients from BEN families with diabetes mellitus, as well as those with the criteria for hypertensive nephrosclerosis[12]. In addition, no patients were involved in whom overlapping of BEN and other nephropathies could not be confirmed at the time of the study.

The Ethics Committee of the Foča Medical Faculty, University of East Sarajevo evaluated and approved this study (decision No 01/08, January 17, 2008), and both patients and healthy controls gave their informed consent.

We determined hemoglobin, serum levels of urea and creatinine using standard biochemical methods. Estimated GFR (eGFR) was calculated using the abbreviated MDRD study equation[13]. We measured urine protein by a colorimetric method with pyrogallol red and expressed it as mg protein/mmol creatinine (normal value <20 mg/mmol creatinine). Urine albumin was determined by a photometric color method with bromcresol green (Olympus AU 400 analyzer, Olympus, Tokyo, Japan) (normal value <3.4 mg/mmol creatinine) and urine alpha1-microglobulin (alpha1-MG) by immunonephelometric assay (BN II nephelometer, Dade Behring, Deerfield, IL, USA) (normal value <1.5 mg/mmol creatinine). We used fresh random urine specimens for all analyses.

One dedicated doctor (SM) examined the kidneys by ultrasound using an Esaote MyLab™Gamma portable system (Esaote S.p.A, Genova, Italy) with a sector probe of 3.5 MHz. The longest craniocaudal length and minimal parenchymal thickness (the shortest distance from the renal sinus fat to the renal capsule) were measured on each kidney. Data on kidney cysts, stones and other morphological abnormalities were also registered.

Descriptive statistics are reported as mean values and standard deviation (SD) for the continuous variables or as frequencies for categorical variables. We compared the variables among the three groups using one-way analysis of variance (ANOVA) accompanied by Tukey multiple comparison tests or the Chi-square test to estimate differences between categorical variables. Analysis of covariance (ANCOVA) was used to adjust differences in kidney dimensions in accordance with age and eGFR. We performed all analyses using the Statistical Package for the Social Sciences (SPSS), version 21 (SPSS, IBM Corp., Armonk, NY).

Results

Table 1 presents the demographic characteristics, blood pressure and laboratory findings for the three groups examined. No significant differences were found between healthy subjects from BEN and non-BEN families for any of the presented variables. BEN patients were significantly older and had significantly higher systolic and diastolic blood pressure than healthy persons from BEN and non-BEN families. As expected, BEN patients excreted larger amounts of all three measured proteins in their urine and eGFR was lower than values for the other two groups examined.

Both kidneys were of similar length in healthy subjects from BEN and non-BEN families and were significantly longer than in BEN patients. Parenchymal thickness in BEN patients was slightly but not significantly smaller than in the healthy groups (Table 2).

When the subjects of each group were separated according to eGFR, we found no significant difference in kidney length between the groups of healthy persons irrespective of the eGFR value. Kidneys of the healthy groups were significantly longer than those in BEN patients both for stage 1 ($p = 0.039$) and stage 2 ($p = 0.044$) of chronic kidney disease (Figure 1). In addition, cysts were more frequently present in BEN patients (15/71) than in healthy subjects from BEN (2/74) and non-BEN families (2/59), and the difference between the groups was highly

Table 1: Main characteristics and laboratory findings of healthy persons from Balkan endemic nephropathy (BEN) families, non-BEN families and BEN patients.

	BEN patients	Healthy subjects from BEN families	Healthy subjects from non-BEN families	p
Gender, M/F	34/37	39/35	19/40	0.052
Age, years	64.4 ± 12.0	49.1 ± 12.2	49.2 ± 12.3	<0.0001
Systolic BP, mmHg	166 ± 27	139 ± 25	133 ± 31	<0.0001
Diastolic BP, mmHg	96 ± 13	86 ± 13	73 ± 25	<0.0001
Hemoglobin, g/L	131 ± 17	137 ± 14	136 ± 13	0.199
eGFR, ml/min/1.73m^2	66.5 ± 27.9	109.1 ± 23.9	114.8 ± 29.7	<0.0001
U-protein, mg/mmol creatinine	42.1 ± 45.0	12.5 ± 8.3	15.3 ± 7.9	0.001
U-albumin, mg/mmol creatinine	26.9 ± 28.5	0.8 ± 1.0	0.5 ± 1.1	<0.0001
U-alpha1-microglobulin, mg/mmol creatinine	7.6 ± 6.2	0.3 ± 0.7	0.3 ± 0.5	<0.0001

BEN: Balkan endemic nephropathy, M: males, F: females, BP: blood pressure, eGFR: glomerular filtration rate estimated by MDRD study equation, U: urine.

Table 2: Kidney length and parenchymal thickness in healthy persons from Balkan endemic nephropathy (BEN) families, non-BEN families and BEN patients.

	BEN patients	Healthy subjects from BEN families	Healthy subjects from non-BEN families	ANOVA	ANCOVA
Kidney length, cm					
Right	9.8 ± 1.1	10.7 ± 1.0	10.6 ± 0.9	<0.0001	<0.0001
Left	10.3 ± 1.2	11.4 ± 1.0	11.2 ± 1.1	<0.0001	<0.0001
Mean	9.9 ± 1.3	11.0 ± 0.8	10.9 ± 0.8	<0.0001	<0.0001
Parenchymal thickness, mm					
Right	12.7 ± 2.0	12.8 ± 2.8	12.9 ± 3.1	0.892	0.796
Left	14.1 ± 2.7	14.5 ± 2.5	14.3 ± 2.9	0.694	0.589
Mean	13.4 ± 1.9	13.6 ± 2.5	13.6 ± 2.7	0.878	0.286

BEN: Balkan endemic nephropathy, ANCOVA included age and eGFR as covariates.

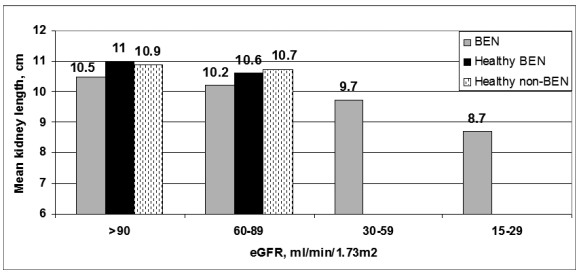

Figure 1: Kidney length in healthy persons from Balkan endemic nephropathy (BEN) families, non-BEN families and BEN patients divided according to estimated glomerular filtration rate (eGFR).

significant (p =0.0014). A small number of persons in all three groups had stones. Two patients with BEN were nephrectomized due to urothelial carcinoma.

The values for eGFR, urinary excretion of albumin, alpha1-MG, kidney length and parenchymal thickness was compared between subgroups of BEN patients and healthy subjects from BEN families formed depending on the family history of BEN. Mean values of these variables in patients whose father or mother or both suffered from BEN did not differ significantly either in the BEN patient group or the group of healthy members from BEN families. Nevertheless, BEN patients who had a mother with BEN had a lower eGFR (64 ± 37 ml/min/1.73m^2) than those with a father (73 ± 23 ml/min/1.73m^2) or both parents (72 ± 14ml/min/1.73m^2) with BEN, as well as higher urinary excretion of albumin (33.5 ± 24.7 mg/mmol creatinine vs. 30.1 ± 40.5 mg/mmol creatinine vs. 30.1 ± 40.5 mg/mmol creatinine) and alpha1-MG (12.2 ± 2.8 mg/mmol creatinine vs. 8.4 ± 8.4 mg/mmol creatinine vs. 2.1 ± 3.4 mg/mmol creatinine) and shorter kidney length (9.1± 1.8 cm vs. 10.0 ± 1.3 cm vs. 10.3 ± 1.1). However, the differences between all these variables did not reach statistical significance.

Discussion

In the present study, we measured kidney dimensions in two groups of healthy persons from BEN villages that differed in family history: one with a positive and the other with a negative family history of BEN. All persons in both groups had normal blood pressure, and all laboratory findings were within normal ranges. Our main objective was to resolve the dilemma of whether kidney size of BEN family members without any laboratory sign of BEN differed from those in healthy members of non-BEN families. Kidney length and parenchymal thickness, measured by ultrasound, were similar in both healthy

groups. While kidney length of each healthy group was significantly longer than that for BEN patients, we found no significant difference in eGFR, kidney dimensions and urinary excretion of albumin and alpha1-MG in BEN patients and healthy persons from BEN families depending on whether the mother or father or both suffered from BEN.

During the last decade interest in BEN has re-awakened and, in addition to research on BEN etiology, significant attention has been paid to biomarkers of the disease. Most studies concerned the diagnostic value of biochemical markers, primarily various urinary proteins[14-16]. Although reduced kidney size is considered as characteristic of BEN, only a few research groups have investigated kidney size. The main objective of such studies was the establishment of the stage at which kidney shrinkage starts because diametrically opposing views existed regarding this issue[6,17]. Recent studies involving a large enough group of patients have shown that reduced kidney size appears in the early stages of BEN[7-9] as confirmed here. However, it remained unclear whether or not kidney size of healthy members of BEN families differed from that in healthy persons from families not burdened with BEN. Dimitrov and colleagues[9,18] compared kidney dimensions in offspring of BEN and non-BEN parents from Vratza, Bulgaria. After adjusting for confounders, they found significantly shorter kidney length and smaller cortex width in BEN offspring when compared with non-BEN offspring. Arsenović et al[19] also compared kidney size and function in family members of BEN and non BEN hemodialyzed patients and detected shorter kidney length in BEN than in non-BEN family members, although the difference was insignificant. Moreover, Aleckovic-Halilovic et al[10] observed no statistically significant difference in kidney length between subjects with positive and negative family histories of BEN. However, all these studies included a few BEN family members with pathological urinary findings (low-molecular-weight proteinuria, microalbuminuria and/or proteinuria) indicating that they might have been at the early stage of BEN. Moreover, after detailed examination Arsenović et al[19] reported that five BEN family members without previously known BEN had enough criteria for BEN or suspected-BEN. In addition, after five follow-up years, Hanjangsit et al[18] diagnosed BEN in 14 of their cohort and suspected BEN in 23 BEN offspring. At the beginning of follow-up, these persons had slightly shorter kidney length and significantly smaller cortex width than BEN offspring who did not develop disease. It is true that there is no specific biomarker for diagnosis of BEN and that low-molecular proteinuria and microalbuminuria are intermittent in the early stages of BEN[5]. Therefore, it is not easy to exclude the existence of the disease at early stages. Nevertheless, the question arises whether the differences registered in kidney size between members of BEN and non-BEN families described in the above mentioned studies were due to the inclusion of some BEN patients in an early stage of the disease among the group of BEN family members. That directed us to undertake the present study where the main objective was to find out kidney size in healthy BEN family members without any sign of kidney disease. The two groups of healthy persons, one from BEN and the other from non-BEN families did not differ either in age, gender, blood pressure, eGFR or urinary protein excretion and all values were within the normal range. We detected no microalbuminuria or proteinuria in any of these participants, either in the first phase of screening (urine dipstick test) or in the second phase (biochemical urine analysis). There was no difference in kidney length and parenchymal thickness between healthy subjects from BEN and non-BEN families. It is considered that BEN does not occur in people under 20 years of age[20]. No one was younger than 30 years in either group of healthy subjects. A shift of BEN towards older ages is well-known[20,21] and recently Hanjangsit and colleagues[18] showed in a five-year prospective study of BEN offspring that increased incidence of BEN started at 45 years. Assuming that some of the subjects from our BEN healthy group might have undetected disease, we divided both healthy groups according to age into the following subgroups: under 45, between 45 and 60 and over 60 years. Kidney length decreased with age but insignificantly in both BEN (11.2 ± 0.7 cm; 11.1 ± 0.7 cm; 10.6 ± 0.8 cm) and non-BEN (10.9 ± 0.8 cm; 11.0 ± 0.8 cm; 10.8 ± 1.0 cm) subgroups. All these results and analyses indicated that kidney length of BEN family members without any sign of kidney disease did not differ from those of healthy non-BEN family members.

For the first time, Dimitrov et al[9] reported an association of parental history of BEN with kidney size and function. They showed that BEN offspring with a mother suffering from BEN had significantly shorter kidney length and increased urinary excretion of albumin, total protein and beta2-microglobulin than those having a father with BEN. In the present study we found no differences in kidney length, parenchymal thickness, eGFR or urinary excretion of albumin and alpha1-MG in healthy BEN family members and BEN patients having a mother or father or both suffering from BEN. Although BEN patients with a maternal history of BEN had somewhat lower eGFR, higher urinary excretion of albumin and alpha1-MG and shorter kidney length than those with a father or both parents with BEN, the differences were not significant.

The present study was cross-sectional which is its main limitation. There is no doubt that further monitoring of the healthy subjects from both groups would show how kidney function and size change over time. Hanjangsit and coworkers[22] found that kidney size was strongly associated with BEN incidence when parental history was not taken into account. A few persons in the group of healthy members of BEN families presented here, at the time of examination were younger than 45 years, had kidney size between 9.5 cm and 10 cm but normal laboratory findings. As kidney size is an important predictor of BEN, these individuals require careful monitoring.

Conclusion

Kidney length of BEN family members without any laboratory sign of kidney disease did not differ from values for healthy members from non-BEN families, but was significantly higher than in BEN patients. No significant association was found between parental history of BEN and kidney size or function in both BEN patients and healthy members from BEN families.

Conflict of interest

Authors declare no conflict of interest.

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