Microalbuminuria – The new marker for Balkan Endemic Nephropathy?

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Abstract

Several criteria are necessary to meet in order to have the diagnosis of Balkan endemic nephropathy established. One of them is tubular proteinuria type that may be found, but not in the early stage of the disease. Beta 2 microglobulin may be found in the early stage, but its determination is rather cumbersome and not suitable for a daily routine. Therefore, urinary albumin/creatinine ratio was determined in 8 patients (all females, aged 58,37±4,37 years) from the town of Šamac region (Bosnia and Herzegovina) as a measure of albumin excretion in order to establish useful marker in the early stage of the disease. Increased urinary albumin/creatinine ratio, was found in 50% of BEN patients. According to these preliminary results, microalbuminuria could be used as the reliable marker for the early detection of BEN.

Key words: Balkan endemic nephropathy, microalbuminuria, urinary albumin/creatinine ratio

Introduction

Balkan endemic nephropathy (BEN) is an unknown disease that ultimately leads to terminal uremia. It affects rural population of Croatia, Bosnia and Herzegovina, Serbia, Romania and Bulgaria. It is characterized primarily with tubulo-interstitial changes (1) and predominant tubular proteinuria type has been reported by now (2). Beta 2 microglobulin (B2M) has been used as a marker for early detection of the disease, but its use is rather complicated and not applicable for a daily routine. Quantitative measurement of urinary albumin as a marker of an early tubular changes has not been done by now. Therefore, we tested non-proteinuric urine samples of patients suffering from early stages of BEN for microalbuminuria in order to suggest possible new reliable BEN marker.

Patients and methods

Urinary albumin to creatinine ratio was determined in random urine samples in eight patients suffering from the early stage of BEN (all females, aged 58,37±4,37 years, range 51-63) from the town of Šamac region, Bosnia and Herzegovina

Patients were selected on the basis of established criteria. Half of them had biopsy-proven lesions compatible with BEN in a study done 17 years ago (1). Hypertension, diabetes mellitus, heart failure and urinary tract infection have been ruled out because those conditions give rise to microalbuminuria themselves.

Random urine samples were first tested on proteinuria with 20% sulfosalycilic acid (SSA) test. The SSA test is performed by mixing one part urine supernatant (e.g., 2.5 mL) with three parts 3 percent sulfosalicylic acid, and grading the resultant turbidity according to the following schema (the numbers in parentheses represent the approximate protein concentration): 0 = no turbidity (0 mg/dL), trace = slight turbidity (1 to 10 mg/dL), 1+ = turbidity through which print can be read (15 to 30 mg/dL), 2+ = white cloud without precipitate through which heavy black lines on a white background can be seen (40 to 100 mg/dL), 3+ = white cloud with fine precipitate through which heavy black lines cannot be seen (150 to 350 mg/dL), 4+ = flocculent precipitate (>500 mg/dL). Only those who proved non-proteinuric were tested on microalbuminuria using urinary albumin/creatinine ratio.

Albumin was determined by immunonephelometric method using BN System (Dade Behring) on the basis of scattered light that has passed through immune complexes made up of albumin and specific antibodies. Intensity of scattered light was proportional to the quantity of the albumin in a sample. Results were evaluated by comparing them with the standard of known concentration.

Creatinine in serum and urine was determined using auto-analyzer Dimension RxK (Dade Behring) with kinetic method by Jaffe, modified by Larsen (3).

Albumin to creatinine ratio was then calculated. The cutpoint for females is > or = 2,7 mg/mmol (25 mcg/mg) (4)

Diethylen Threeamino Penta Acetate (DTPA) clearance rate was determined by computerized method after the patients had been overloaded with fluid (1 L two hours prior to the test) and after they had voided. Tc99m DTPA was then applied in a dose of 185 MBq. Radioactivity was measured, memorized in a computer and clearance rate calculated.

The statistical analysis was done using chi square test. $P<0.05$ was taken to be significant. The results obtained were compared with the results obtained from healthy population, since between 5% and 10% of nondiabetic individuals have a urinary albumin excretion rate (UAER) within the

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microalbuminuric range in US (5,6) and between 5% and 7% in Europe (7,8).

Results

Urinary albumin/creatinine ratios in individual samples are shown in Table 1. Increased urinary albumin/creatinine ratio was found in 50% of BEN patients, what was statistically significant result ($p<0,05$).

Table 1. Urinary albumin/creatinine ratios in individual samples

Patient	Age (years)	DTPA C1	SeCr	SSA proteinuria	Urine Albumin /Creatinine ratio Biopsy	Previous
1	58	70,8		ST	11,3	YES
2	62	52,8		NT	0,88	NO
3	63	48,8		ST	14,9	YES
4	53		60	ST	10,4	NO
5	60	42,7		ST	1,4	YES
6	58	69,9		ST	0,58	NO
7	62	65,2		ST	1,29	YES
8	51		61	ST	3,1	NO

Legend: DTPA Cl: DTPA Clearance Rate (ml/min); SeCr: Serum Creatinine (mcmol/L), ST: Slight Turbidity, NT: No Turbidity

Discussion

Increased UAER could be the consequence of an augmented intraglomerular capillary pressure, it could reflect the existence of intrinsic glomerular damage that causes changes in glomerular barrier filtration, or it could be the consequence of a tubular alteration that impedes the normal reabsorption of filtered albumin (9). The latter refers to BEN, which is considered tubulo-interstitial nephropathy.

B2M is a good marker of tubulo-interstitial lesion (10) and has been used as one of the criteria for early detection of BEN. An attempt has been made with β2 microglobulinuria for this purpose, but this test is rather complicated and requires special circumstances, such as alkaline medium.

This is a pilot study, what represents its major limitation, but the extended study is underway. The limitation of this study is also gender uniformity. The reason for this is not greater affection of females with this disease. Males and females are affected equally (11). The reason is the size of the sample that is rather small as well as higher readiness of women to participate in BEN survey or to come for a check-up on their own (12). Limitations of this study was the sampling method, too. It was based on the phone contacts, which means that those who did not possess the phone or did not happen to be home by the time of our call did not have a chance to answer it. Finally, only volunteers took part, so that the sampling method was not random.

Six patients underwent DTPA clearance rate measurement. Technical reasons prevented the other two to undergo it, but serum creatinine was enough to document the early stage of the disease.

Conclusions

According to our preliminary results, microalbuminuria could be considered the reliable marker for the early detection of BEN, but further studies, with greater samples, are needed in order to have this conclusion supported or counteracted.

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