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Tamm-Horsfall Protein Determination in Balkan Endemic Nephropathy

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Summary. Data on the excretion of Tamm-Horsfall protein (THP) in subjects living in an area of Balkan endemic nephropathy (BEN) are reported. The study subjects were divided into groups as follows: diseased, suspect, "at risk" and others, according to previously adopted criteria. The THP excretion in "at risk" subjects was found to be significantly higher as compared to control subjects. The difference between these two groups could not be registered by any other clinical or laboratory diagnostic methods. No difference in the excretion of THP was observed between the groups of others and control subjects. According to the results obtained, the excretion of THP may be considered a possibly useful additional diagnostic test for the detection of subjects with the latent, early subclinical phase of BEN. On the other hand, the data obtained shed some more light on the still obscure pathogenesis and natural history of BEN.

Key words: Tamm-Horsfall protein – Endemic (Balkan) nephropathy

Introduction

In the late fifties, the physicians in Bulgaria, Romania and Yugoslavia were faced with an "epidemic" chronic renal disease occurring in particular rural areas. Extensive research work attempted to clarify the nature of the disease [6, 21, 25]. The clinical manifestations of the disease were unlike other common renal diseases such as glomerulonephritis or pyelonephritis or hypertensive kidney disease. Initial studies indicated that BEN had similar clinical and patho-morphological characteristics to chronic interstitial nephritis [14, 15, 27], and the name *Balkan Endemic Nephropathy* (BEN) was accepted [27]. Since that time, many research studies on the ethiology of BEN have carried out but, unfortunately, the ethiology of BEN has remained obscure.

In the advanced phases of the disease, BEN presents clinically as a chronic tubulo-interstitial syndrome and can be diagnosed with a sufficient reliability on the basis of clinical and morphological criteria [19]. In the advanced phases (azotemia, uremia) it is not difficult to ascertain the existence of a renal disease, but difficulties arise in the differential diagnosis versus other renal diseases, which also present at 'end stage'. Therefore, much effort has been invested in the search for diagnostic procedures that would allow an early diagnosis to be made during the initial phases of the disease while clinical signs are still scarce or absent. An exact diagnosis is particularly difficult to approach when proteinuria can not be detected [12, 16, 22, 23].

Further advances in the diagnosis of BEN were achieved by the discovery that the tubular pattern of proteinuria was characteristic [10, 12, 16, 23]. Since then, more sophisticated methods of urinary protein multifractionation and determination of individual low molecular weight proteins have been employed in the diagnosis of BEN. The measurement of urinary beta-2-microglobulin excretion has been demonstrated to be a valuable indicator of tubular proteinuria, and recognized as a positive diagnostic sign of the presence of BEN as well as a useful method of the detection of patients suffering from BEN [8, 11, 17]. In an earlier study [2], we summarized the characteristics of proteinuria obtained by means of the available methods of protein separation in urine. Since 1980, when we introduced the method of Tamm-Horsfall protein (THP) determination in urine, urinary THP excretion has been studied in subjects from the endemic area of Slavonski Brod (Yugoslavia). This approach seemed relevant, because the excretion of THP takes place in a limited segment of the nephron, where the thick ascending limb of Henle's loop turns into the distal convoluted tubule [9]. It appeared reasonable to expect the THP excretion to be elevated in such a tubulo-interstitial disorder, because an increased excretion of THP was also reported on in a condition pathologically very similar to BEN, caused by cadmium poisoning [9].

Since 1980, we have published several reports on the results obtained, along with the description of the methodology [1, 4, 5, 18, 19]. The present paper records the results

	n	Values		Median	Significance versus Control
		min	max		
Diseased	34	8.4	153.0	26.5	$P < 0.01$
Suspect	102	1.8	171.4	21.5	$P < 0.01$
"At Risk"	99	3.6	150.0	23.5	$P < 0.01$
Others	230	2.6	137.1	14.3	$P = 0.64$
Control	73	1.8	52.9	17.8	

Fig. 1. Urinary THP excretion in the examined group of subjects, expressed as mg/g creatinine

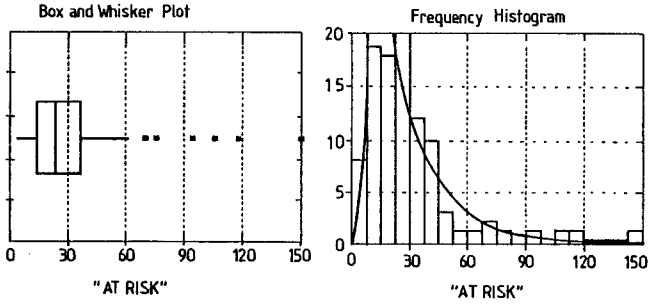

Fig. 2. Urinary THP excretion presented in Box and Whisker plot, and frequency histogram of subjects "at risk" in the endemic area

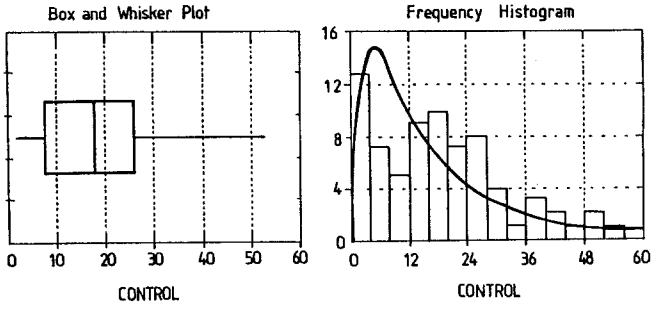

Fig. 3. Urinary THP excretion presented in Box and Whisker plot, and frequency histogram of control subjects from a non-endemic area. A difference in THP excretion between the groups of "at risk" (Fig. 2) and control subjects (Fig. 3) is obvious

obtained in the studies on THP in BEN, conducted in the endemic area of Slavonski Brod.

Material and Methods

THP excretion was determined in urine samples of 564 subjects of both sexes aged 18–78 years, living in the BEN area, and in 73 subjects from the control area where BEN had not been observed. Subjects from the endemic area were divided into the following groups according to the previously defined criteria [10, 27]: diseased, suspect and "at risk". The subjects were considered diseased when exhibiting proteinuria (sulfosalicylic acid test or urocomb strip test positive and/or beta-2-microglobulin positive in native urine), anemia (Hb below 120 and 113 g/l in males and females, respectively) and blood creatinine above 132.6 μmol/l, or proteinuria, anemia and positive family history of BEN, or all the four elements together. The subjects were considered suspect when they had both proteinuria and anemia or proteinuria and a positive family medical history of BEN. The subjects were considered to be "at risk" if they had no clinical or laboratory signs of kidney disease but belonged to the households with some members suffering from BEN. Overnight urines were collected in plastic blottles over 8–12 hours. The THP concentration in urine samples was measured by electrophoresis in 1% agarose gel containing serum against THP (3.0 μl/cm^2). The volume of antigen applied was 5 μl. THP was isolated from a pool of human urine by a modified method of Tamm and Horsfall [24]. Crude precipitate was further purified by Sephadex G-200 gel chromatography in 6 mol/l urea, pH 8.5. Antiserum against THP was prepared by immunization of rabbits with isolated antigen in complete Freund adjuvant. Before determination of THP, 5 ml of total urine sample was dialyzed overnight at 4 °C, the volume reduced to 2.5 ml and 2.0 ml of concentration added to 0.1 sodium dodecyl sulphate (63 g/l), followed by incubation at 37 °C for 1 hour. THP purified from normal urine was used as standard. Working standard solutions were obtained by dilution with 3 g/l solution of sodium dodecyl sulphate. Serum and urine creatinine concentrations were measured by the automated kinetic Jaffé method on the Abbott VP. The excretion of THP was expressed in mg/g creatinine. The statistical evaluation was done by Mann-Whitney-Wilcoxon test.

Results

The results of our recent studies, supplementing those obtained previously, are summarized in Fig. 1. As can be seen from the table, the study subjects from the diseased and suspect groups excreted significantly higher amounts of THP than those belonging to the control group, which is consistent with both our previous findings and our expectations. The group of subjects designated as "at risk" was also found to excrete more THP than the normal control subjects from a non-endemic village. This difference was statistically significant ($P = 0.0007$). To illustrate the difference between the "at risk" and control groups, the data are presented on the Box and Wisker Plot and frequency histograms (Fig. 2 and 3). No significant difference was recorded between the groups of others and controls, but was found to exist when these were compared to the groups of diseased, suspect and "at risk" subjects.

Discussion

Our previous studies on the determination of THP in urine of subjects from the endemic area revealed the subjects designated as diseased to excrete more THP than the control subjects. The subjects designated as suspect were also found to excrete higher amounts of THP than the normal control subjects from a non-endemic area. This finding was also consistent with our expectations, because the former were the subjects with clinical signs of a renal disease, most probably BEN, presenting a clinical picture of nephropathy without azotemia. It is known, that any renal disease may result in an increased excretion of THP. During our previous stud-

ies, however, our attention was particularly attracted by the difference in the excretion between the subjects belonging to the "at risk" group and those designated as controls [4]. The THP excretion was then observed to differ between the "at risk" and control groups of subjects, but the sample was too small to allow any valid statistical evaluation. This difference between the two groups of subjects, however, could not be recorded by any other, even very sensitive, methods of urine protein multifractionation currently used to demonstrate the tubular type of proteinuria. Then a preliminary conclusion was made that early subclinical phases of BEN, undetectable by any other method could possibly be detected by the determination of THP in urine [4, 5]. Now, supported by a greater number of subjects under study allowing the results to be statistically evaluated, this preliminary conclusion can be considered valid. In other words, the urinary THP determination could be a valuable additional diagnostic method in recording early subclinical phases of BEN that are not demonstrable by other clinical methods.

On the other hand, no difference in the excretion of THP was found between the groups of others and controls, as can be seen in Fig. 1. If we assume that BEN develops as a consequence of a long-term action of an noxious agent on the population exposed to it, we could imagine that only a minor portion of the population will develop a manifest kidney disease exhibiting the characteristics of BEN, whereas the other part of the population will develop latent subclinical renal lesions. Among the latter, only a minority may be expected to become overtly diseased whereas some of them will never develop a disease which could be diagnosed as BEN. In such a way, the pathogenesis of BEN can be conceived as being consistent with the iceberg phenomenon. Such a concept is quite widely present when the pathogenesis of a number of diseases, especially renal diseases, is concerned, as figuratively described by Mallick [13]. Only about 1/10 or even less is visible to or detectable by a doctor. The kidney biopsy findings obtained in these early phases of the disease would be very helpful in elucidating this problem, but, unfortunately, no such findings have been available.

Further, it should be emphasized that no difference in the excretion of THP was observed between the group designated as others, i.e. the subjects seemingly free of any apparent kidney disease, living in the endemic area, who did not belong to households with members suffering or having died from BEN, and the group of control subjects. This finding appears to point to the possibility that the subjects living in households with members suffering or having died from BEN actually were at higher risk, i.e. that they were exposed to a hypothetic noxious agent, which was either locally present in a higher concentration or it was a result of as yet unknown factors increasing the predisposition for the disease. All these considerations appear to lead to verification of the hypothesis searching for the causes of BEN among ecological factors, requiring, of course, further studies.

Conclusions

- Both our previous and present studies on urinary excretion of THP in subjects from an area endemic for BEN have shown that the determination of THP might serve as an additional diagnostic tool in the detection of early phases of BEN.

- Differences observed in the excretion of THP between the normal control subjects from a non-endemic area and those from an endemic area but free of any signs of a renal disease and living in households with BEN previously recorded, support the concept according to which the pathogenesis and natural course of BEN resembles the so-called iceberg concept: kidney lesions reflecting the action of a hypothetic chronic noxious agent proceed latently in most cases, whereas the manifest picture of BEN develops in a small number of such subjects only.

- Differences in the excretion of THP between the seemingly normal subjects from households with previously recorded BEN and other normal subjects from an endemic area give rise to a speculative theory according to which the intensity of action of a hypothetic noxious agent varies among particular locations of the endemic area. These findings speak in favor of the ecological hypotheses on the development of BEN.

- The results obtained provide a basis for further studies which might reveal whether these preliminary concepts have made a contribution to a better understanding of the pathogenesis of BEN.

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