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BALKAN ENDEMIC NEPHROPATHY IN SERBIA: CURRENT STATUS AND FUTURE RESEARCH

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Summary. *During the 90s, wars and economic hardship hampered most attempts for serious research in Serbia, as well as in other BEN affected countries. This relative lull in intensive research provided an opportunity for identifying key problems and assessing priorities for future research. The following issues were singled out as relevant from this point of view: 1. What is the trend of BEN? Did it disappear? 2. Is exposure to the agent(s) still going on? 3. Which descriptive epidemiological features of BEN may be considered established and how they should be interpreted? 4. What are the most likely aetiological hypotheses to be pursued? 5. Assuring a proper study design. 6. Avoiding "circular" research. 7. Conducting transregional studies. 8. Ethical considerations. This first scientific meeting on BEN after more than a decade is an important opportunity to agree on priorities, establish interregional collaboration and proceed with research at an internationally acceptable and competitive level.*

Key words: *Balkan endemic nephropathy, etiology, Serbia, epidemiology, research*

Attention of the international scientific community to the problem of Balkan endemic nephropathy (BEN) was drawn in 1957-8 by two papers (1,2) based on observation in the Central Serbian municipality of Lazarevac. It was later demonstrated that Serbia was the most affected BEN region with a total of 366 settlements categorized into endemic (91) and possibly endemic (275) (3,4). Three events – BEN, upper urothelial tumours (UUT) and β_2-microglobulinuria were shown to be clustered in well-defined areas (5). Intensity of the endemic process was reflected in disappearance of complete families. In one instance, four of five siblings developed both BEN and UUT, while the fifth one had painless haematuria but refused further examination (6).

Intensive BEN research in Serbia, as well as in the rest of the Balkans, was brought to a standstill in the early 90s, due to political turmoil, wars, and economic hardship. The last major opportunity for Serbian scientists to present results of their research were two conferences held in Belgrade in 1988 and 1989, with proceedings published as a supplement to the Kidney International (7). Meetings that followed throughout the 90s were not devoted solely to BEN (e.g., BANTAO conferences) or were only individually attended by researchers from Serbia (e.g., IARC Meeting, Lyon, 1991).

In spite of all drawbacks, several reports on BEN in Serbia appeared in scientific journals over the last 10 years. With a few exceptions of field studies (8,9), they were based on previous field work (10), overall experience (11,12,13,14), laboratory results of in- or out-patients (15,16,17,18,19,20), case reports (21) and analysis of medical history or statistical data (22,23,24,25). Also, an updated and comprehensive review of BEN and UUT, in a form of a critical appraisal of the existing body of knowledge, was recently published as a bilingual monograph (26).

This relative lull in intensive research, as compared to the previous periods, should have helped in crystallizing ideas for future research. Since the resources are sparse, priorities have to be defined. Research that is more reliable is also needed. Furthermore, sociopolitical changes that took place in the meantime require more respect for humans, either patients or participants of field studies. In this context, the following issues appear relevant for research in Serbia, as well as in other BEN regions.

What is the trend of BEN? Did it disappear?

Natural history of BEN tended to take ever more protracted course over time (27,28). Simultaneously, the age distribution of the disease showed an apparent shift to the right, i.e., towards the older ages (27,28). A decrease in the incidence of terminal kidney failure and BEN death rates was observed in the South Morava region between 1978 and 1997 (25), but the trend of BEN in this area had quite irregular and unpredicted pattern in the past (28). In two endemic municipalities of the Outer Belgrade area (Barajevo and Lazarevac) the number of incident BEN cases did not change over the previous three decades but their median age increased for 3.5 years between 70s and 80s, and for another 7.5

years between 80s and 90s; as for UUT, an increase of 5 years between 80s and 90s was observed. In 90s, BEN cases in Petka developed terminal kidney failure 4 years later than during the previous decade (Radovanovic – unpublished data). Continuation of these trends would lead to eventual fading away of BEN and reduction of the UUT incidence to the rates observed in non-endemic areas.

Is exposure to the agent(s) still going on?

An ideal means of assessing the presence of BEN agent(s) in the environment would be a specific indicator of acute proximal tubular damage. Until such a test is suggested, standard indicators of tubular injury, e.g., β_2-microglobulin, should be relied upon. Hyper-β_2-microglobulinuria may not be decisive for establishing the diagnosis at an individual level, but its high prevalence is very useful epidemiological diagnostic tool. Two population groups are particularly relevant: children and recent immigrants to an endemic region.

In the early 90s, we tested forty 10-year old children from Petka for β_2-microglobulinuria by RIA method (Radovanovic et al – unpublished data). None was positive, but the number was too small for relevant inferences. Unless a few hundred children in each endemic and control settlements are tested, preferably on more than a single occasion, and the prevalence rates are similar and close to zero, one cannot rule out maintenance of contact with the agent(s) in a respective area.

Which descriptive epidemiological features of BEN may be considered established and how they should be interpreted?

Researchers on BEN usually perceive the facts in a way that supports their hypotheses. Thus, authors arguing that BEN is a form of glomerulonephritis used to diagnose the disease in the autochthonous urban population (29). The same holds true for proponents of genetic hypotheses (30), but not for other researchers. Many such examples are available.

Do Gypsies develop BEN? Genetically-oriented researchers firmly decline such a possibility (31,32). We recall only two Gypsies diagnosed by nephrologists as probable BEN cases. Both of them were atypical insofar as they kept working in their fields obtained by the land reform. Are the Gypsies spared due to their genetic set-up or because they are not exposed to agricultural activity as one of key criteria for establishing the diagnosis (33,34)?

Also, are the Moslems spared from BEN and, if so, is it because they do not eat pork as suggested by Apostolski et al. (35)? We saw a few Moslems diagnosed with BEN in Janja, an allegedly hypoendemic semi-urban settlement in North-East Bosnia. There was no apparent familial clustering and BEN diagnoses were not convincing. Most endemic parts of Bosnia except Janja were inhabited by non-Moslems. Bulgarian Moslems ("White Gypsies") used to develop BEN (31). Is then exposure or religion an issue?

What are the most likely aetiological hypotheses to be pursued?

According to an account of possible agents (Z. Radovanovic – unpublished paper), there are three main candidate causes of BEN.

First, mycotoxins. Over many years, ochratoxin A was the prime suspect. In 1991, an entire scientific meeting was devoted to this toxin as a culprit (36). Some ochratoxin A – DNA adducts were even considered as "characteristic" for UUT in BEN areas (37), but the evidence on ochratoxin A involvement in BEN aetiology is still mainly geographical (38). Due to the toxicity profile of ochratoxin A, another mycotoxin, produced by *Penicillium polonicum*, has been proposed as the cause of BEN through the mechanism of apoptosis (39). The hypothesis on mycotoxins fits most of the epidemiological features of BEN and BEN-associated UUT.

Second, viruses. Many common viruses have been implicated. Though frequently published in reputable journals, such studies were usually based on a poor methodological design and could not endure the challenge of a scrutinized reappraisal. Coronaviruses were recently isolated from the tissue of both BEN (40) and UUT patients from BEN areas (41). As indicated elsewhere (42), any viral hypothesis, in order to be seriously considered, should comprise biologically plausible explanation of the observed descriptive epidemiological features of BEN (stability of endemic foci, mosaic pattern, the need for long term exposure, etc.).

Third, organic substances dissolved in water. The prime candidate in this group are polycyclic aromatic hydrocarbons and aromatic amines leached from low caloric coals (43,44). There are apparent differences in water content of BEN endemic and non-endemic areas, but demonstration of a cause-effect relationship is still far away.

Some additional clues might also be followed. One of them points to a common weed, birthwort (*Aristolochia clematitis*). The idea was put forward many years ago (45) but buried in oblivion until a similarity between BEN and Chinese herbal nephropathy, caused by aristolochic acid, was demonstrated (46). While the role of birthwort in the etiology of Chinese herbal nephropathy has been clearly established, exposure to this plant in BEN areas is not apparent.

Deficiency rather than abundance of an agent might also be considered (47). Attention was drawn to selenium (48) but no convincing evidence has ever been provided.

Another possible clue is genetic origin of the disease. There is overwhelming evidence that BEN is environmentally induced. One such argument is that three

ethnic groups living in the same endemic area had the same risk of developing BEN (49). Still, susceptibility may well be affected by some inherited characteristics. The most authoritative group dealing nowadays with genetic mechanisms in BEN occurrence assumes multifactorial (polygenic) origin of the disease (32), implying an important role of non-genetic factors. It brings us back to the search for environmental causes. As predicted (50), genetic epidemiology may help in identifying susceptible individuals, their diagnosis, prognosis and treatment.

There are at least two dozen additional hypotheses that were never categorically ruled out. None of them at this stage of BEN research deserves particular attention.

Assuring a proper study design

There are numerous examples of anecdotal blunders in BEN research due to various methodological shortcomings (absent or poorly selected comparison group, lack of laboratory quality control, diagnostic errors, etc.). Expectations are frequently built-in into the results, e.g., alleged BEN cases that were born and lived outside endemic areas were diagnosed only by proponents of genetic hypotheses. There is no ideal, but only appropriate study design.

As an example, for studying organic substances in well water samples (51,52), three groups of households were defined: a) affected (at least 3 household members diagnosed with BEN); b) non-affected in the same settlement, and c) non-affected in a non-endemic settlement. In groups b) and c) none of the household members had any kidney disease or tumours of urinary organs. Non-affected (control) households were individually matched to the affected ones. Matched criteria were altitude and depth of the well. Households were selected only if well water was regularly used, at least for animal consumption. Samples were coded and sent to Denver, CO. If the objectives were different (e.g., measuring acute tubular damage), the research plan would be changed (only members drinking well water would be selected for studying).

Avoiding "circular" research

Many researchers keep publishing results based on the same or slightly modified model. Repeatability does not prove anything. There is a need for progression from observation (generating a hypothesis) to analytical studies (hypothesis testing) and, if applicable, to experimental epidemiological design (confirmation). It is the only way to sort out which hypotheses are worth pursuing and to prevent many other haunting around for decades without being discarded.

Conducting transregional studies

A myriad of aetiological hypotheses have been restricted to a single endemic focus. They may excellently fit descriptive epidemiological features of BEN in a particular setting, only to be shown irrelevant when tested elsewhere. One of many such examples is the exposure of BEN affected people to natural foci of infection in oak forests (53).

Checking for LCAT deficiency (54) outside South Morava region is long overdue. Demonstration of predisposing genes for BEN in the region between 3q25 and 3q26 (55) from samples obtained outside Bulgaria would much corroborate this hypothesis.

Ethical considerations

The need for informed consent has been generally ignored in most if not all endemic regions. A drastic eye witnessed example: In a non-affected household in the village of Beljina, parents were obsessed by the future of one child, ignoring the prospects of the other one, a lively 12-year old girl. The explanation was that there was no hope for her because she underwent a serious surgery. They did not know that girl was just "a healthy control from a non-affected household", subjected to open kidney biopsy in order to satisfy someone's research ambitions.

Malpractice is nowadays better defined and condemned. There are also strict rules of proper scientific conduct. As for field studies, detailed ethical procedures have been set up (56,57,58,59). In Europe today, adherence to basic ethical considerations is a must.

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