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AETIOLOGY OF BALKAN NEPHROPATHY: A REAPPRIASAL  
AFTER 30 YEARS

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**Key words:** Balkan nephropathy - Urothelial tumours - Aetiology

Balkan nephropathy (BN) has been a complete aetiological puzzle for many years. Intense research produced many controversial results, but also helped some clues to be traced. Thus, it became rather obvious that the disease is caused by environmental factors (though it may be, to some extent, genetically controlled). Among live agents, slow viruses are the only (and theoretically very attractive) possible cause of both BN and urothelial tumours, which occur frequently in BN endemic areas. Apart from occasional visualization of virus-like particles, other evidence supporting this hypothesis has been lacking. The role of biological products, particularly fungal toxins, needs to be further defined. An excess of any heavy metal or radioactive substance as a cause of BN is very unlikely indeed; however, a deficiency of a bio-essential element could not be ruled out. Many epidemiological data indicate that water might be a transmitter of the agent(s). A wide range of ions and minerals have been incriminated, but up to now most of the studies have provided negative or inconclusive results. Organic compounds in well water have never been sufficiently considered. Another ignored area are unstable and, particularly, photosensitive substances. Fertilizers, pesticides, analgesics, local herbs and teas have no causal relation to BN.

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INTRODUCTION

The first account of endemic Balkan nephropathy (BN) in an English-language journal was published over 30 years ago (8). Though the author was convinced that BN was related to chronic lead poisoning, the idea was never proved and was, thus, gradually abandoned.

The disease aroused a great deal of wide scientific interest with the organization of 20 major symposia by the affected countries, two meetings held by the World Health Organization (49, 50) and one CIBA-sponsored conference (48). A vast majority of the 3000 papers on the topic were published in proceedings and local journals, but several dozen appeared in more widely read publications. Many epidemiological, laboratory, histological and clinical features of BN have been explained, but its aetiology has yet to be determined. The following descriptive-epidemiological features have been generally agreed upon:

- the disease is known to exist in only three countries of south eastern Europe (Bulgaria, Romania and Yugoslavia);
- endemic foci are located in the vicinity of the Danube and its tributaries, often (except in Bulgaria) in flooded areas;
- clustering of cases within families is one of the most prominent features of the disease;
- there is a mosaic topographical distribution of cases, with spared households even in the most affected foci;
- an overt clinical picture is absent in children and adolescents; incidence begins to rise in the third decade of life and, except for the age group over 60, is proportional to age;
- the mortality risk from BN is not significantly different for males and females;
- transitional-cell tumours of the upper urothelium may be even 100 times more frequent in endemic areas than in neighbouring non-endemic areas.

Most authors also agree that:

- BN is associated with a rural way of life, while the native urban population has been spared;
- immigrants to an endemic area may develop the disease after sufficiently long (usually over 20 years) exposure;
- early emigration from an endemic area (in childhood) may prevent the occurrence of BN.

On the basis of the above facts, numerous hypotheses on the aetiology of BN have been proposed. Most of them are completely compatible with the epidemiological patterns of BN described and interpreted by their author(s). However, evidence that seems to be equally sound has often been provided from the same area in support of completely opposite ideas. Such controversy makes it difficult to either accept or reject most of the suggested clues for explaining the cause(s) of BN.

A critical review of the available facts, is necessary. For the sake of comprehensibility, the existing aetiological hypotheses have been grouped.

#### *Genetic factors*

Bulic (5) was the first of several authors who considered BN to be a solely or primarily hereditary disorder. He felt that the present topographical distribution of the disease reflects migration routes of a biologically handicapped ethnical group with kidneys prone to shrinkage. Multiplication of a genetic defect due to endogamic practice over the centuries was offered as an alternative explanation (16).

More recently, only one author (27) continues to ignore major environmental influences. According to him, all BN cases detected in Bulgaria have been genetically inter-related, i.e. they have been recruited from a pool of 360 families with 26,000 members. If his assessment is valid, non-members of a limited number of kinship groups might quite safely be exposed to the BN environment.

However, there is sound evidence that immigrants to an endemic area may develop the disease after having lived there long enough (30). It was demonstrated (7) that in an endemic area of Yugoslavia, the risk of developing BN was the same for the indigenous population and a large group of Ukrainians who inhabited the region some 80-90 years ago.

Several other facts, agreed upon by most (though not all) investigators, such as the protective effect of early emigration, phenomenon of simultaneous deaths, etc., do not support the idea of BN as a classical hereditary disease, though it may be, to some extent, genetically controlled. It has been suggested (46) that either a single gene with incomplete penetrance or, more probably, polygene inheritance may be involved. In both cases, there is a need to identify environmental factors responsible for the manifestation of the gene(s).

Recent cytogenetic studies of lymphocyte cultures from peripheral blood of a limited number of

probands in Bulgaria led the authors to claim the existence of a specific chromosome marker in BN (45). Similar comprehensive studies in Romania (64) failed to demonstrate these chromosomal aberrations. Alterations observed were, therefore, related to exposure to the BN endemic environment. A report of an increased frequency of HLA B<sub>18</sub> in BN patients, as compared to healthy members of the same village (29), could not be confirmed in another endemic region (44).

#### *Live agents and their products*

No single bacteria is currently under consideration as a possible cause of BN, though some (streptococci, leptospirae, *E. coli*, etc.) used to be mentioned in this context repeatedly.

Many authors believe in the viral aetiology of BN, though convincing evidence has been lacking. Three ideas used to be more widely accepted: the West Nile virus transmitted by migratory birds (20), coronavirus propagated from pigs (2), and arboviruses from natural foci of infection (3).

Subsequent serological studies have failed to support any relation between BN and the West Nile virus (42) or several other viruses (12, 23). Interpretation of cytoplasmic inclusions as coronavirus used to be supported by a claim that the disease was associated with pig husbandry and that Moslems, who do not raise pigs, were not affected (2). No epidemiological evidence supports these suggestions, and coronavirus has never been seen again by any other virologist. The third hypothesis - that of arbovirus infection - was based purely on epidemiological data: in an endemic village, all of the BN victims had happened to hide, during World War II, in dense forests (3). However, in most other BN endemic areas, exposure to such natural foci of infection is limited to males (hunting, forestry), while both sexes are affected by BN.

It has recently been suggested (37) that, if the disease is of viral aetiology, exposure takes place at home or in the courtyard, with rodents excreting slow viruses acting as a reservoir. Several epidemiological features of BN may be explained in this way. The medical geography of BN, its mozaic pattern and the stability of its foci would thus be a reflection of the topographical distribution of the virus reservoir. The absence of interhuman transmission can be explained by the fact that humans do not excrete the agent and the familial pattern of the disease and the similar mortality in both sexes, by common exposure in (or around) the house. Visitors to affected households would not be at risk for contracting BN because of the susceptibility of the viruses, which are likely to be transmitted only early in the morning, shortly after being excreted by the rodents. The increased incidence of BN seen in the mid-fifties could be related to an ecological imbalance caused by DDT poisoning of cats, who act as a natural barrier between humans and rodents. However, only inference drawn

from epidemiological observations, rather than any proof, supports this possibility.

The weed *Aristolochia clematitis*, already known for its nephrotoxic effect in animals (25), was at one time suspected to be a cause of BN. Ivic (18) hypothesized that its flattened seeds had been milled and consumed with flour. The active principle of this plant - aristolochic acid - was later implicated as "one of the most effective carcinogenic substances yet known", causing multiple tumours of urothelium and other tissues (26). However, no one has ever demonstrated any correlation between this agent and BN or an excessive frequency of urothelial tumours in BN endemic areas.

The idea of fungal toxins as a cause of BN has attracted several groups of researchers. There are two reasons that most studies have been concentrated on Ochratoxin A: 1) it is a casual factor of nephropathy in swine (21) and 2) it can be determined by quantitative techniques (unlike many other mycotoxins).

Ochratoxin A contamination of cereals is common in BN endemic areas (32, 35). The significance of this finding is limited by the fact that such contamination in neighbouring non-endemic regions (35) is also higher than that reported elsewhere (22). Even data reported in support of the hypothesis of Ochratoxin A as a cause of BN (35) revealed that the differences between foodstuffs from BN endemic and control villages were not significant and, furthermore, that the highest concentrations of Ochratoxin A were recorded in beans from control rather than endemic areas. Similarly non-impressive and/or inconsistent differences have been obtained by comparison of blood samples (17, 36). Porcine nephropathy is not present in BN endemic areas, and BN is not known in parts of the world where Ochratoxin A affects pig husbandry.

Though data on Ochratoxin A have not been encouraging, a hypothesis on mycotoxin or a combination of fungal toxins is consistent with epidemiological observations (1), and there are moulds isolates from BN endemic areas which have been shown to produce (other) nephrotoxic components (51).

#### *Inanimate environment*

Many heavy metals and trace elements have been implicated as causes of BN. Not only chronologically, lead had the lead (8). However, apart from morphological and pathophysiological differences, (15), lead poisoning could not be demonstrated in BN patients (11). The same holds true for cadmium nephropathy (10). A joint Yugoslav-Japanese meeting on the possible link between itai-itai disease and BN (6), helped rule out the hypothesis that cadmium toxicity might be a cause of BN.

The roles of manganese, mercury, chromium and several other substances have also been considered.

As a rule, the enthusiasm of the authors exceeded the conclusiveness of their results or seemingly impressive findings could not be confirmed on a broader scale. The most recent study of this type was based on a single sampling from 7 wells (31). Though the maximum concentrations allowed for each substance were not exceeded, the conclusion was reached that, in an agglomeration of affected families around wells with higher cadmium, chromium, manganese, cobalt and mercury content (no data on "agglomeration" have been provided), simultaneous exposure to several nephrotoxic substances had enhanced their effect.

The idea was originally advanced in relation to uranium intoxication. Radioactive substances may induce histological changes resembling BN. However, in spite of occasional alarming reports (28), concentrations of uranium and other radioactive substances in the BN endemic environment were found to be within permissible limits (19, 31). For its reason, Stefanov (43) hypothesized a synergistic effect of uranium and numerous trace elements, none of them exceeding their maximal concentrations admitted according to international standards.

It should be borne in mind that BN endemic foci are not confined to a single geological era or geochemical unit (24). Actually, the disease occurs in two major geochemical provinces of the Balkan peninsula (one with Pb-Zn-Sb and another with Cu), and there is no trace element present in an excessive amount in either the rocks, soils, stream sediments or groundwater of both of them. However, the theoretical possibility that a deficiency, rather than an excess, of a bio-essential element is associated with BN (24) could not be ruled out.

All BN endemic foci seem to have common hydrogeological characteristics (40). This fact has led many authors to speculate on the role of water as a transmitter of the agent(s). The most ambitious study on this possibility, supported by U.S.A. PL-480 funds, claimed a correlation between BN occurrence and a number of characteristics of wells and the quality of water, such as depth of the well, height of the water column, electroconductivity, silicate, nitrate and boron content, radon emanation etc. (33, 34). Re-evaluation of the same data revealed that none of the conclusions could be supported by the results obtained (39).

Analysis of wells in different parts of Yugoslavia, using sophisticated analytical techniques, failed to demonstrate that the concentration of any of a wide range of ions and minerals was higher in affected, as compared to control, villages (9). However, organic compounds in well water of BN endemic areas have never been sufficiently studied. This holds true particularly for phytotoxins. Another area that has been ignored is that of photosensitive substances, as suggested by G. Feder (personal communication). Unstable substances, such as nitrites, may have extremely high levels in well water of endemic foci (41).

*Other hypotheses*

The fact that BN may develop and progress many years after a patient has left an endemic area has led several authors to speculate that BN is an autoimmune disease. As revealed by Hall (14), the evidence supporting this concept has been lacking.

Hall (13) postulated that BN might be a light-chain nephropathy, i.e. that an overload of beta-2-microglobulin, most likely produced by inapparent papillary transitional cell tumours, damages kidney tubules and causes the disease. Even if this is true, the question remains as to which environmental factor increases the production of beta-2-microglobulin. A population-based study indicated that hyperbeta-2-microglobulinaemia follows, rather than precedes, overt kidney damage (38).

Fertilizers and pesticides have been occasionally mentioned at scientific meetings on the aetiology of BN, but the disease already existed before their extensive use. Apart from papillary necrosis, BN has many histological and clinical similarities to analgesic nephropathy; epidemiological data, however, do not support the link with analgesic or any other type of drug abuse. Local herbs and/or teas could not be connected with BN in any way.

There is hardly any possibility - from endogamic practice to cosmic influences - that has not been mentioned in this context. Even geopathogenic zones have been recently related to BN occurrence (47). As is the case with many other hypotheses, the authors did not answer two basic questions: why BN does not occur worldwide and why only a specific cancer site has been involved.

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