FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol.9, No 1, 2002, pp. 15 - 25 UC 616.61-004

BALKAN ENDEMIC NEPHROPATHY IN ROMANIA

Gheorghe Gluhovschi[1], Florin Margineanu[2], Virginia Trandafirescu[1], Adalbert Schiller[1], Ligia Petrica[1], Silvia Velciov[1], Gheorghe Bozdog[1], Cristina Gluhovschi[1], Flaviu Bob[1]

[1]University of Medicine and Pharmacy "Victor Babes", Department of Nephrology, County Hospital, Timisoara, Romania,
[2]County Hospital, Drobeta Turnu Severin, Romania

Summary. *Balkan Endemic Nephropathy (BEN) is a disease with many similar epidemiological aspects in the countries in which it occurs. In spite of this, there are some particular aspects which have to be mentioned concerning this disease in Romania. BEN has a familial character; it is described in foci that consist of several families; sometimes families affected by the disease are found in the same village as families that are unaffected by BEN; also endemic villages may be encountered near unaffected villages.*
The clinico-biological picture of the disease is that of a tubulo-interstitial nephropathy. The etiology of BEN is unknown. Several factors are considered to be instrumental in the development of the disease: viral infections and mycotoxins.

Key words: *Balkan endemic nephropathy, epidemiology, etiology, kidney morphology, diagnosis, treatment*

Balkan endemic nephropathy (BEN) is a disease present in a limited geographical area in Romania, Bulgaria, Yugoslavia, Croatia and Bosnia.

It is classified as a tubulointerstitial disease, with progressive evolution towards end-stage renal failure.

In Romania it was first identified by Foarta and Negoescu in 1957, and first published in 1961.

Interest in the study of this disease has existed in Romania since the first reports appeared regarding BEN i.e.: epidemiological studies (1); the structure of the soil as instrumental in the etiology of the disease (2,3); cytogenetic studies (4,5); pathological studies (6); studies with regard to fertility impairment and evolution of pregnancy in BEN patients (7).

The most remarkable finding was the discovery by Georgescu et al. (6) of virus-like particles in the proximal renal tubules in BEN patients.

Studies on viral infections in BEN patients and in animals in the BEN area, the implication of mycotoxins, several immunology studies and studies regarding the structure of the soil have also been carried out, but they have been either infrequent or are at an incipient stage.

In recent years a hemodialysis centre has been set up in the vicinity of the endemic area, at Drobeta Tr. Severin, which has provided renal replacement treatment for BEN patients in end-stage renal failure.

Some patients benefited from renal transplant undertaken in Romania or abroad. However, the number of transplants is still small.

The findings regarding BEN have been communicated and published at various international congresses and in specialized journals.

A monography was published in 1994, in a bilingual edition, entitled Endemic Balkan Nephropathy by Gh. Gluhovschi, in collaboration with V. Stefanovic (Yugoslavia) and T. Dimitrov (Bulgaria) (8).

Epidemiology of the disease

BEN is found predominantly in Oltenia, and also in some areas of Banat (regions in the SW of Romania).

The best-known epidemiological studies are those of Lazarescu (1) and Zaharia (9), who described 5 hotbeds: in Oltenia, where they are located around the town of Drobeta Turnu-Severin, Strehaia, Vanjul Mare, Baia de Arama, and, in Banat, where there is one near Oravita.

The disease is present in the neighbouring villages, but not in towns, the BEN cases in the urban localities originating in the BEN villages (8).

According to Zaharia (9) and Lazarescu (1), the villages in which a large number of cases were found are: Erghevita, Bistrita, Poroina, Izvorul Anestilor, Severinesti, Simian, Cernet, Dodovita Veche, Fantana Domneasca, Ghelmingioaia (villages grouped in the neighbourhood of the town of Drobeta Turnu-Severin), Corcova, Jirov, Stangaceaua, Tamna, Breznita, Motru, Jeleni, Ruptura, Buicesti, Tantareni, Vagiulesti, Vanjul Mare, Batosi, Broscari.

In Banat the localities are: Secaseni, Jitin, Ticvanul Mare, Giurgiova. Lazarescu has mentioned cases in villages in the area adjacent to Baia de Arama.

An analysis performed by Margineanu in April, 2000, on 51 patients included in the hemodialysis centre T. Severin, has revealed the following localities as those with the largest number of patients undergoing renal replacement therapies: Hinova - 4 hemodialysed patients and one on peritoneal dialysis, Erghevita - 5 hemodialysed patients, Bistrita - 5 hemodialysed patients,

Husnicioara - 4 hemodialysed patients, Corcova - 4 hemodialysed patients, Simian - 5 hemodialysed patients, Valea Izvorului - 3 hemodialysed patients and 2 on peritoneal dialysis, Vanjulet - 2 hemodialysed patients and 1 on peritoneal dialysis; Rogova - 4 hemodialysed patients and 1 on peritoneal dialysis (Margineanu).

It should be mentioned that of the 74 patients on hemodialysis at the Hemodialysis Centre in Drobeta Turnu-Severin, 46 patients have BEN (66%), and of the 16 patients on peritoneal dialysis, 5 patients have BEN.

In the Department of Nephrology of the Drobeta Turnu-Severin County Hospital, (which consists of l2 beds), 21 NEB patients were hospitalized over a period of 3 months, between January 1st 2000 and March 31st 2000.

In the Department of Urology of the Drobeta Turnu-Severin County Hospital, of the 33 patients with tumors of the urinary tract, [20 males (60.6%) and 13 females (39.3%)], 29 live or come from BEN areas (Margineanu- unpublished data).

The epidemiological features of the disease, according to our own observations and to those of other authors are:

- Predominance in adults aged 30-50 years
- The disease has not been reported in children and adolescents
- Predominance in the female population
- Village populations are affected. The cases found in towns are persons originating in BEN villages who moved to town or into small towns that resulted from the development of BEN villages
- The main occupation of the BEN patients is farming
- BEN has a familial character, several members of a family being affected. These may belong to one generation or to 2 consecutive generations.
- Families with several BEN patients can be found beside families untouched by the disease:
- There are unaffected localities beside affected localities
- The disease shows a cyclical evolution. There are periods with a significant number of cases and periods in which the number of cases diminishes.
- Persons originating from the endemic zone and who have lived a long period in the endemic zone develop the disease even if they move to a new locality after a variable period of time
- Persons who leave the endemic zone in their childhood or in adolescence will not develop the disease.
- Persons coming from localities unaffected by BEN, may acquire the disease if they live several years in a locality in the endemic area
- There is a high incidence of tumors of the renal pelvis or of the ureter in BEN patients.

Etiology of BEN

Although still unknown, several hypotheses have been proposed, however none of them has been confirmed as yet.

Suggested factors in the etiology of the disease are:

- infectious factors, mainly viruses
- toxic factors: mycotoxins, phenols, microelements, silicium
- ionizing radiation
- genetic factors
- immune factors.

Infectious factors

There is a strong argument for viral etiology. The existence of hemorrhagic endemic nephropathy caused by the Hanta virus predominantly found in South-East Asia and in the Scandinavian countries, in relatively defined epidemiological areas has determined, by analogy, the assumption that BEN is a chronic renal disease of viral etiology.

The investigations undertaken in order to isolate viruses from various biological samples taken from BEN patients, have failed to isolate any virus which could be the cause of the disease; thus, no viral etiological agent could be attributed.

However, the isolation of virus-like particles within the renal tubules has aroused interest in the study of viral etiology in this disease.

These particles were reported in Romania by Georgescu et al. (6) and Bruckner (10).

However, the virus-like structures are not specific to BEN, being also reported in electron microscopy on renal biopsies, in other renal diseases, too.

It is of great concern whether other microbial agents are involved in BEN. At present there is no evidence suggesting that infections with streptococcus or leptospira might be instrumental in causing this disease.

Furthermore, other bacteria (brucella, salmonella, shigella) and enterobacteriaceae, such as E. coli, Klebsiella, could not be related directly to BEN etiology, due to the fact that these microorganisms are found in other geographical areas too.

Also, the investigations carried out by Lazarescu (1) aimed at revealing infection with Rickettsia (Burnetti, Connori, Maseri) as the cause of BEN have been inconclusive.

The possibility of BEN being a form of chronic pyelonephritis is very unlikely, as BEN inhabits a limited geographical area, while pyelonephritis is found worldwide.

Genetic factors

One characteristic of BEN is the presence of the disease in several members of one family. This has raised the possibility of the involvement of a hereditary factor. Another hypothesis was that of the involvement of a toxic or viral factor.

The incidence of the familial factor is variable.

In Romania, Gh.Gluhovschi reported the presence of BEN in 2 or more members of the families of 25.7% of the patients investigated (11). A similar finding came

from Bruckner et al (10) among a group of 136 patients, while Bacila and Vintilescu (12) remarked familial incidence in 26% of the cases.

In Bulgaria, the familial factor is higher: 42% of the cases, reported by Puchlev (13), and 51.7% of the cases, reported by Toncheva (14).

Cytogenetic investigations have revealed chromosomial anomalies in BEN patients. They have been attributed by Bruckner et al. (4) and Tonea et al. (3) to a possible involvement of environmental factors, such as ionizing radiation, which might be instrumental in the etiology of the disease.

Gluhovschi (11) has shown that the chromosomal anomalies observed are similar to those which occur after exposure to ionizing radiation, chemical substances and viruses, etc.

Toxic factors

Several toxic factors have been attributed to BEN etiology. In the last decades, of these, mycotoxins have been paid increased attention.

Mycotoxins have been considered instrumental in the development of BEN.

Ochratoxin A – a mycotoxin, produced by Aspergillus Ochraceus, as well as by Penicillium verrucosum, and possessesing nephrotoxic and carcinogenic properties, has been incriminated in BEN. For these reasons it has been also ascribed to the development of tumors of the urinary tract found in this disease.

Ionizing radiation and radioactive elements. An increased uranium concentration has been observed in water from springs in the endemic area (the villages of Erghevita, Poroina and Simian) as compared to water from pipes in Tr.-Severin and Bucharest. In these areas, Tonea remarked, the presence of other radioactive elements, such as thorium, as well as of other microelements accompanying them, such as, zirconium, scandium and ytrium (3). Tonea et al. (3) have studied chromosomal modifications, resembling those produced by X-ray which they consider to be secondary to soil radioactivity.

Furthermore, Gluhovschi has found, in several BEN patients investigated, the presence of chromosomal abnormalities bearing similarities to those following irradiation (11).

Other authors, such as Andreescu et al., in Romania, have observed normal content of radioactive substances in the BEN area (15).

We assume that the involvement of ionizing radiation, as well as that of radioactive metals in the development of BEN is still difficult to define.

Silicon and silicates. An erosion ground layer lying on a silicate base has been observed in the BEN area. This layer is traversed by underground water, which may drain the silicates into the drinking water.

Markovic and Lebedev proposed the hypothesis that BEN is a disease in which the corrosive silicates play an important pathogenic role. They obtained interstitial lesions resembling those described in BEN, in guinea pigs and white mice, by feeding them with granite boronate and water from the BEN area (16).

The possible involvement of silicates in BEN etiopathogeny draws attention towards the structural components of the soil in the area of the disease.

Phenolic compounds. Analgesic nephropathy is an interstitial nephropathy which bears several clinical and pathological similarities to BEN. In addition, BEN associates to these characteristics the increased incidence of tumors of the urinary tract.

However, an increased ingestion of phenacetinic analgesics by the population in the endemic area has not been detected.

In the Banat endemic area of Secaseni, natural phenolic compounds in surface water have been observed (17), as well as paracresolic compounds. Studies conducted by Mustata et al. (2,18) pointed out that pollution of wells with phenolic compounds was found both in the Secaseni area and in Erghevita.

Phenolic compounds are believed to intervene in the occurrence of a chronic intoxication involving the kidney through a toxic, an allergic and even a blastogenic mechanism, which can produce modifications of the urinary tract (2,18).

Increased concentrations of phenols in the drinking water are also found in other areas where BEN has not been detected, which makes it difficult to explain their intervention in the pathogeny of the disease.

It has been assumed that BEN may be related to some polycyclical hydrocarbons or to some other toxic substances originating in the Pliocenic lignite in the soil in the BEN area. The latter could penetrate the drinking water.

These hydrocarbons are believed to develop, by long exposure, a nephrotoxic action that leads to BEN (19-21).

It is worth mentioning that in Romania, lignite deposits have been found in the neighborhood of the BEN areas. The intervention of the above mentioned factors present in the soil should be accurately investigated in order to support the hypothesis that hydrocarbon compounds originating in the Pliocenic lignite are involved in the development of BEN.

However, similar soil structures are found in other areas in various countries, without cases of nephropathies similar to BEN.

Lead. For several years, lead has been considered an etiologic agent in BEN by some Bulgarian and Yugoslavian authors (22). Subsequent studies carried out in these countries failed to confirm this hypothesis.

Research conducted in our country regarding the intervention of lead in the development of BEN has revealed high concentrations of lead in the air and in the urine of BEN patients.

At present, it is assumed that lead is not instrumental in the occurrence of BEN (23).

Microelements. Microelements in the soil have been attributed to BEN etiopathogeny by analogy with

the relationship between the low quantity of iodine in the soil and the occurrence of the endemic goiter, as well as with that one between the manganese content of the soil and the development of cancer in animals, etc.

The following observations concerning the soil microelements in the BEN area are worth mentioning:

In Romania, Tonea (3) observed a high concentration of manganese, aluminium, chromium and iron in the drinking water in the endemic area.

However, up to now, no causal relationship between one of these elements or their combinations and BEN could bee established.

Selenium deficiency in the soil, food, and in the blood of the healthy population has been observed in several areas in Yugoslavia. This selenium deficiency might favour the susceptibility towards BEN.

Immune mechanisms

The hypothesis of underlying immune mechanisms related to the causal agent of the disease has been proposed in BEN pathogenesis. Craciun and Rosculescu introduced the hypothesis of the existence of a self-aggression mechanism in BEN, which could be initiated by an infectious agent.

The immunological studies carried out in BEN have not succeeded in revealing a well-defined immune mechanism. They have shown only occasional immune modifications in BEN patients.

Low values of the serum complement have been found in BEN patients: Gluhovschi (11) revealed its low levels in 13 out of the 20 BEN patients investigated, while Mustata et al. (2,18) reported this finding in 8 out of the 16 BEN patients. Freyria et al. (24) observed a decrease of the hemolytic activity of the complement (CH 50%), as well as of the C_3 levels in 25% of the BEN patients on hemodialysis. 17% of the BEN patients in the initial stage and 12% of symptom-free persons originating in BEN families showed diminution of the CH 50% activity. Modifications of the complement system were not recorded in healthy persons in the BEN area described.

Serum IgG and IgM immunoglobulins show normal values. Drugarin et al. described a subset of cytotoxic lymphocytes (CD 3, CD 16, CD 56) in BEN patients. This phenotype was observed in both T lymphocytes and NK lymphocytes. According to Drugarin et al. (25) the quantitative analysis of the various lymphocyte subpopulations shows no differences in these subpopulations in BEN patients when compared with normal subjects, originating in the BEN area, as well as with normal subjects from non-endemic areas.

Tatu et al. (20) showed decreased levels of B lymphocytes and increased levels of eosinophils in BEN patients.

The study of several cytokines which participate in the inflammatory process (interleukin 1,interleukin 6, tumor necrosis factor), carried out by Drugarin et al. (25), Tatu et al. (20), revealed normal values of these cytokines in most of the cases investigated.

Voiculescu (26) reported the participation of cellular immunity in BEN. T lymphocytes react against tuberculin and a renal tissue antigen in BEN patients .

Several immunohistochemical studies have provided arguments for the immune hypothesis in BEN. Their results are controversial, as several authors agree on the existence of glomerular and complement deposits in the glomeruli of the BEN patients, while other authors deny such deposits or describe them in small amounts.

Data that show immune deposits in the renal tubules have been provided by Trandafirescu (53).

Diagnosis of BEN

At an early stage, diagnosis of BEN is difficult. It raises problems particularly in persons who have migrated from the endemic zone.

At the subsequent stages of the disease diagnosis is easier to establish.

Diagnosis of BEN is established according to the following **criteria**:

- The geographical criterion: the patient was born in a BEN area, subsequently residing there for a number of years; or having been born elsewhere he moved into this area and became ill after a period of several years.
- The familial criterion: the patient comes from families in which there were cases of BEN or other elements arousing suspicion of this disease.
- The main occupation of the patients is farming
- The disease occurs predominantly in females
- The onset of the disease is insidious
- The overt disease stage is characterized by symptoms of well-tolerated, progressive chronic renal failure.
- The absence of signs or symptoms of another renal disease, either primary or secondary (28), edema is absent and hypertension is rare.
- **Laboratory findings**:
- anaemia, which is well tolerated
- mild proteinuria, of tubular type, which does not exceed l g/day, with increased elimination of beta 2 microglobulin.
- Renal function tests: initially, the concentration ability declines followed subsequently by decline of glomerular filtration rate.
- An increase in BUN and serum creatinine, as renal failure sets in, disorders of fluid and electrolyte, as well as disorders of the acid-base balance
- Urinalysis reveals low density urine, poor urinary sediment. Leucocyturia is present in case of associated urinary infection. Hematuria is observed when tumors of the urothelium are present.
- Ultrasound shows, in the advanced stages, shrunken kidneys, with increased echodensity.
- Plain X-ray and i.v. urography: in the advanced stages both kidneys are diminished in volume, without calicial modifications. They can reveal tumors of the urothelium.

- Renal biopsy: reveals tubulointerstitial nephritis, consisting of tubular atrophies and mild inflammatory interstitial infiltrate. As the disease advances, the tubular atrophies become diffuse, with progressive interstitial fibrosis. Slight glomerular lesions with mild mesangial and endothelial proliferation might also occur.

In the advanced stages renal biopsy reveals severe lesions of chronic renal failure, with tubulointerstitial and glomerular injury.

The positive diagnosis of BEN is very difficult and cannot be established in the absence of the geographical criterion. Other renal diseases which evolve to chronic renal failure should be ruled out before BEN is considered.

According to Hall (28), it is assumed that in BEN neither clinical features or laboratory findings are pathognomonic, nor do pathological data provide an element of certainty in the diagnosis of BEN. However they complete the clinical, biological and laboratory investigations undertaken.

The positive diagnosis of BEN can be established through clinical, biological, functional and laboratory data in the familial, geographical and epidemiological context, by ruling out other renal diseases to be found in the area.

Differential diagnosis of BEN takes account of other diseases encountered in the BEN area, mainly chronic glomerulonephritis and chronic pyelonephritis.

Another disease which requires differential diagnosis is analgesic nephropathy.

BEN is distinguished more easily from diabetic nephropathy and lupus nephritis.

Evolution of BEN

Generally, BEN evolves towards renal failure following the stages presented above.

There are rare cases which show proteinuria or beta 2 microglobulinuria on screening tests; on check-ups performed in these patients a few years later, these alterations and other BEN signs were not observed any more.

The disease evolves over a long period of time showing no signs of renal failure. This phase reveals symptoms such as: reduced proteinuria of tubular type, beta 2 microglobulinuria, anaemia, impaired concentration ability. Ultrasound examination shows kidneys of normal size.

Subsequently, the disease evolves towards the onset of azotaemia, which is well tolerated for a period of 2-7 years. Finally, the patient evolves towards end-stage renal disease, when renal replacement therapies (hemodialysis or peritoneal dialysis) are required or renal transplantation performed.

Clinical features of BEN

BEN represents a renal disease with chronic evolution. It has: an initial stage, when the clinical and biological data is very faint and an overt stage, when the clinical and biological features of the disease are clearly present.

It is considered that BEN has a long latency period, which leads to the occurrence of the disease after the age of 30.

The disease is prevalent in females, the female/male ratio being 3:1.

Occupation of BEN patients: The great majority of BEN patients are involved in farming activities.

Personal history: History of a renal disease is absent. Exceptionally, urinary infections or rhynopharyngeal infections are found – they require a differential diagnosis with glomerular diseases and with pyelonephritis.

Onset of BEN

The disease starts insidiously. Symptoms of acute nephritis are absent.

Commonly, early symptoms are: asthenia, anorexia, dyspeptic disorders, weight loss, headaches, dizziness.

Signs which draw attention to a renal disease are rarely present: lumbar pains, polakyuria, macroscopic hematuria.

However, at this stage 2 elements draw attention to the disease: proteinuria and anaemia.

Anaemia is present from the beginning in most patients, without presenting individual hematologic features.

Mild proteinuria may be also present.

Other signs that have been observed are: declined concentration ability (29); reduction of the renal flow, indicated by Tm PAH .

The overt stage of the disease

Once diagnosed, BEN may present various stages. According to Strahinjic and Stefanovic (30), BEN evolves along 5 succesive stages:

- the stage in which the renal function is normal,
- the stage of compensated chronic renal failure, without azotaemia. It corresponds to the stage in which residual renal function is low.
- the stage of renal failure with asymptomatic azotaemia, manifested in stable values of BUN in the long run.
- the stage of renal failure with symptomatic azotaemia
- end-stage renal failure

The patient will show the clinical and biological symptoms of the corresponding stage.

The clinical features of BEN

- asthenia, digestive disorders: anorexia, nausea and vomiting; weight loss; nervous disordes: headache, dizziness, somnolence, muscular spasams.

- lombalgias are rarely present; polakyuria and dysuria are present especially in patients with associated urinary tract infections or with tumors of the urinary tract. Polakyuria, nocturia, and polydipsia are present at the stage of chronic renal failure.
- the skin is pale, with yellowish pigmentation. Xantochromia can be relatively frequent in BN patients.
- edema is usually absent. Discrete oedema can be observed at the advanced stages of the disease due to hydroelectrolytic disturbances.
- hypertension is a symptom with low incidence in BN (16,6% of the patients, according to Gluhovschi). It is not severe and its mechanisms are still unknown (11).

Biological data

ESR is moderately increased in the initial stages of the disease; it is higher in patients with significant anaemia.

Anaemia is a constant syndrome in BEN. It occurs in the first stage of the disease, it is most frequently normochromic or normocytic and is relatively well-tolerated.

It is caused by: reduced erythropoietin synthesis, moderate hemolysis, digestive or urinary losses.

Several authors have reported a relationship between anaemia and the degree of renal function impairment (5, 10).

Azotemia shows high values at the stage in which the disease is usually diagnosed. It is well-tolerated, allowing patients to go about life normally for a long period of time.

Decreased serum complement has been reported in certain patients.

Urinalysis

The urinary sediment is generally poor in elements. Hematuria can be found in patients who have urinary tract tumors or associated urinary lithiasis.

Leucocyturia can be also found in BEN. It may be associated with urinary tract infections.

Proteinuria is a constant symptom of the disease. It is mild and usually it does not exceed 0.5 g/24 hours.

Qualitatively, proteinuria is of tubular type. It is supposed to be due to the tubular lesions characteristic of this disease.

Various types of proteinuria have been described in BEN, however other authors have failed to detect them.

Gluhovschi (11) studied proteinuria in BEN patients using an anti-rabbit serum against urinary proteins. In certain patients two fractions of tubular type proteinuria were observed, with beta and gamma migration, respectively, which were not found in other patients. The source of these fractions could not be established .

Quantitatively, beta 2 microglobulin is the main protein component of tubular proteinuria.

Karlsson and Lenkei, in Romania, (31) showed an increased urinary elimination of beta 2 microglobulin in an apparently healthy population at high risk of developing BEN. These authors assume that radioimmunologic assessment of this protein may be sensitive enough to detect tubular proteinuria even in its initial stages, and suggest that it should be utilized as an early diagnosis test in BEN (31).

Hall and Vasilievic, in Yugoslavia (59), and Sattler et al. in Bulgaria (60), have obtained similar results.

Enzymuria in BEN

Gluhovschi found 11 patients in a group of 20 BEN patients, (55%) showing increased values of urinary leucine aminopeptidase activity (11).

Bruckner et al. (10) investigated lysozymuria in 655 apparently healthy inhabitants (most of them children) of the endemic zone and of Bucharest. They pointed out the presence of lysozyme associated with increased elimination of the light chains of immunoglobulins, beta 2 microglobulin and guanase, far more frequently observed in children originating from villages in the BEN zone (34).

Hydroxyprolinuria is increased in BEN patients. However, the values of hydroxyprolinuria are not different from those of other patients with renal failure (11).

Renal function tests

At the first stage of BEN, the tubular functions are impaired.

Lazarescu assumes that the decline of the concentration ability of the kidney is one of the first signs of renal failure in these patients and the most constant one (1).

The urinary acidification is impaired, a fact attributed by Polenakovic and Stefanovic (35) to lesions of the distal tubule.

Glomerular filtration rate is normal during the early stages of the disease and is declined in the advanced stages.

Ultrasound

Shows no modifications in the early stages of the disease.

In the advanced stages of BEN, both kidneys are shrunken, with increased echodensity of the parenchyma and the renal pelvis. Because of the increased echodensity they can be hardly differentiated from the surrounding tissues.

Echographic examination is useful in the diagnosis of the disease in the BEN areas, as well as in the diagnosis of the urothelium tumors.

Radiologic investigations

Do not indicate modifications at the first stages of the disease.

At the advanced stages, both kidneys are symmetrically diminished in volume, with smooth contours and without significant pyelocaliceal modifications. The dimensions of the kidneys are the smallest found in nephrology.

Urography is useful in the diagnosis of the urothelium tumors, more frequently found in BEN, as well as in that of certain obstructive factors which may be present in BEN patients.

Ascending pyelography is used when urothelium tumors are suspected and more rarely for the diagnosis of other obstructive factors.

Computed tomography is utilized in order to identify urinary tract tumors.

Renal biopsy is rarely utilized in BEN diagnosis because most patients show up in the advanced stages of the disease, in which biopsy is difficult to perform due to the reduced size of the kidneys, which have increased hardening.

Aspects of pregnancy in BEN

A study carried out in our country by Gh.Gluhovschi et al. has revealed that abortions, premature deliveries, fetal hypotrophy and fetal mortality are far more frequent in pregnant women originating in the endemic area as compared to those originating in areas unaffected by BEN. This observation could be ascribed to the presence of an environmental factor related to BEN etiology (7, 8).

Present data related to BEN etiology does not reveal a conclusive factor which could be attributed to the occurence of the disease. The factors presented above may provide evidence for their possible involvement in BEN etiopathogeny. They can be correlated with certain features of the disease, without any one factor or a group of factors providing an answer to all the clinical, biological, pathological and, especially, epidemiological aspects of BEN. The investigations conducted into the individual characteristics of pregnancy in women from the BEN area could argue for the involvement of one or several factors present in the area in the evolution of pregnancy.

We assume that the data collected up to now as a result of the studies regarding BEN etiology and which initiated new lines of research, require further investigations. Furthermore it is necessary to approach new directions of study by utilizing modern technologies currently available in medical research in order to describe BEN as a disease with a definite etiology, pathogeny and clinico-biological picture. The latest data should be conduced to the pathological lesions and the evolution of the disease, thus providing adequate medical approach to the disease.

Tumors of the urinary tract associated with BEN

It has been reported that BEN is a nephropathy which is far more frequently associated with tumors of the urinary tract than other nephropathies. This association raises the question of an etiological agent of BEN with mutagenic properties.

Tumors of the urothelium have a high incidence and only rarely tumors of the renal parenchyma are described.

The tumors of the urothelium are found predominantly in the renal pelvis and in the urothelium and less frequently in the urinary bladder.

Surgery is required in the treatment of urothelial tumors. Bladder tumors can be approached by endoscopy.

As far as the incidence of urinary tract tumors is concerned, of the 33 patients at the Urology Department of the Drobeta Turnu-Severin County Hospital, 29 live in or originate from the BEN area.

Pathological aspects in BEN

Studies have been performed both on material from renal biopsies and on necropathic material.

Renal biopsies are carried out easily at an early stage of the disease, when they provide useful data for diagnosis. At the advanced stages the fibrotic kidney causes difficulties in taking samples of bioptic material. In fact, the sclerotic lesions which are similar to other renal diseases, are less useful.

As a rule, necropathic examination utilizes kidneys from patients in the advanced stages of the disease.

The pathological investigations have been performed by means of light microscopy, electron microscopy and immunofluorescence. Histochemical studies have also been mentioned.

Necroscopic examination

The initial stage of the disease: limited data exists with regard to this stage.

Both kidneys have normal dimensions. The calyceal system is normal.

The advanced stages of the disease: most data refers to this phase.

Both kidneys show diminished volumes, usually symmetrically. They can reach very small dimensions, up to 30-40 grams. It has been stated that the kidneys of BEN patients can reach the smallest dimensions to be found in nephrology.

The difference between the two kidneys normally does not exceed 12 grams, a fact which differentiates BEN from chronic pyelonephritis, in which there is sometimes a clear difference between the two kidneys.

The existence of both small and shrunken kidneys at the final stages is a characteristic of BEN.

Their surface is pale grey and usually finely granulated or smooth. Hardening of the kidneys is increased.

The renal capsule is thickened and adherent to the renal parenchyma.

On the surface of the cross-section, which is uniformly grey in colour, there is a generalized atrophy of the renal parenchyma.

The cortex can reach a thickness of 1mm or even less.

Congenital malformations are exceptional in patients from the endemic zone.

Sometimes granulations of a few mm in size, with

sero mucous or hemorrhagic content and thickening and ulcerations of the bladder and renal pelvis can be observed.

Polyps and papillomas have been observed in BEN patients in the pelvis, ureter and urinary bladder. Increased incidence of tumors of the urinary tract has been reported in these patients.

Light microscopy

The lesions are mainly located in the tubulointerstitial region. Glomerular injury is also present.

Glomerular lesions. At the initial stages, mild focal lesions of the endothelial and mesangial proliferative type, with focal thickening of the capillary wall have been recorded.

The studies of Zosin et al. (29), Bruckner et al. (10) have revealed, at the initial stages of the disease, the prevalence of glomerular lesions when compared to the incidence of interstitial lesions.

At the advanced stages of the disease, on sections taken from different patients or even from the same patient, glomerular damage of various degrees has been observed. It has shown a lesional polymorphism represented by lesions of: proliferative glomerulitis, membranous glomerulitis, lobular glomerulitis, glomerular sclerosis and hyalinosis (36).

The more advanced the pathological process is, the more frequent the occurence of lesions of sclerotic glomerulitis are. Ferluga et al. (37) have reported: global sclerosis in 80% of the cases, segmental sclerosis in 10% of the cases, hyalinosis in 8% of the cases, hypercellularity in 4% of the cases.

Glomerular lesions, though present at the onset of the disease, probably advance very slowly. According to Dojcinov et al. (38), they do not have an important impact upon the renal function and clinical manifestation of the disease.

The process of glomerular sclerosis advances and leads to obsolescent glomeruli.

Bowman's capsule thickens with collagen-type fibres, gradually disappearing into the interstium.

Interstitial lesions. The main lesions encountered in BEN are considered as follows.

At the initial stages, the interstitial lesions consist of interstitial focal oedema and mononuclear infiltrates.

At the advanced stages, the sclerosis process sets in. It is usually acellular, but sometimes it is associated with a lymphoplasmocyte infiltrate.

Georgescu et al. (6) consider that sclerosis and hyalinosis are reactive processes which increase in intensity from the medulla to the cortex.

It is considered that the poor or even cellular infiltrate, the presence of frequent periglomerular sclerosis, as well as the location of the inflammatory process predominantly in the subcapsular region, allow the differentiation from chronic pyelonephritis (37).

The process of sclerosis can be acellular or poor in elements from the very first stages, the acellular interstial fibrosis being predominant.

Sclerosis spreads around the atrophic tubules and surrounds sclerotic or apparently normal glomeruli (37). It can replace the affected glomeruli and tubules.

The interstitum is considered to represent the main area of activity of the infectious or toxic agent. It is believed to initially determine a cellular reaction and an oedema, followed by a proliferation of the fixed elements of the connective tissue, with a consecutive acellular sclerosis.

Tubular lesions. They are consistently present in BEN.

At the initial stage, the tubular epithelium shows variable lesions: simple dystrophia, hydroprotidic degenerescence, pseudocystic dilatation, atrophy. The basement membrane shows thickening and splitting (PAS coloration). The process may lead towards destruction of the tubule or towards typical or atypical regeneration.

At the advanced stages, tubular atrophies predominate, which may be focal or segmental. Tubular atrophy may determine the narrowing of the lumen and even tubular collapse associated with the disappearance of the lumen.

The proximal tubules are mainly affected.

The tubular epithelium becomes flat. A nuclear polymorphism without mitotic activity has been observed (37).

The collecting tubules show a marked cellular proliferation with micropapillar projections.

Tubular atrophy is accompanied by zones of interstitial fibrosis.

The peritubular network of capillaries is reduced, the capillary basement membrane thickens and scleroses of the peritubular capillaries are observed.

Tubular lesions are supposed to precede the other lesions (13).

Dojcinov et al. consider that the atrophied tubules belong to nephrons in which the glomeruli are concomitantly affected (38).

Vascular lesions have a high incidence (up to 80% of the cases, according to Ferluga (37)).

They predominantly affect the arcuate and interlobular arteries, the latter showing a thickening of the walls and reduction of the lumen (35)).

According to Ferluga (37), the lesions are multifocal, rarely diffuse, sometimes severe; the intima is predominantly affected, but other vascular layers are also involved: hyalin deposits, later to replace the muscular cells in the medium layer .

The process also affects the elastic internal membrane, which grows thick and irregular (6,35,36).

The intrarenal venous system does not show evident pathological alteration. The juxtaglomerular apparatus can be affected in some BN patients; it is involved when an extensive periglomerular sclerosis is present. It consists of atrophic lesions.

The lymphatics show periglomerular, perivascular and intertubular dilatations (35).

Electron microscopy

Glomerular lesions. In the stage of compensated chronic renal failure: Bruckner has observed hypertrophy of the endothelial cells, which obstruct the capillary lumen. Giant vacuoles are present in the cytoplasm of these cells. The glomerular basement membrane becomes thickened. In the epithelial glomerular cells there are vacuoles containing electron-dense material, but these cells do not undergo a process of hypertrophy (39).

Other modifications remarked:

- The basement membrane of Bowman's capsule is thickened and cleaved
- Focal proliferation of the mesangium, increase in the mesangial area by development of the matrix.

Tubular lesions. Consist of atrophy and flattening of the tubular epithelium. The basement membrane shows thickening and there is cleavage of the lamina densa. A slight nuclear polymorphism, unaccompanied by mitotic activity, is present.

The lesions are predominantly in the proximal convoluted tubules. Besides the mitochondrial alterations and focal or diffuse disappearance of the mitochondrial crista, changes of shape and chromatin distribution of the nuclei are remarked.

Cytoplasmic vesicles by degeneration of the tubular epithelium and the presence of several necrosis centres are described.

The presence of virus-like particles within the proximal tubules has been observed (9,35).

Apostolov considers these particles to be coronaviruses, which would determine a slow infection in humans.

Interstitial lesions consist mainly of:

- Accumulation of proteic material in the interstitium
- Increase in the number of fibroblasts
- Extension of the cytoplasm of the endothelial cells along the capillary walls (63).

Vascular lesions A focal thickening of the pericapillary and intertubular space is observed, which contains an increased quantity of homogeneous material and a large number of collagen fibers of various dimensions.

Immunohistochemical studies

Some studies have revealed glomerular and tubular deposits of immunoglobulins and complement in patients with BEN (40), while others have revealed the absence of these deposits or the presence of non-specific deposits (13,41).

Apostolov found, by immunofluorescence, IgG deposits in the tubular basement membrane in each of the 7 cases of BEN investigated.

The presence of immune deposits in the tubular basement membrane could be an argument for the involvement of some immune mechanisms in BEN.

Pathological particularities

Microcrystaluria has been restricted to convoluted tubules, especially in the proximal tubules. These crystalloid formations, usually round, have an amorphous and lamellar structure. In polarized light they are birefringent and fluorescent.

Microcrystaluria in proximal convoluted tubules is not solely specific in BEN, however it has a higher incidence in this disease than in other renal diseases (Georgescu).

Medical Treatment of BEN

There is no etiological treatment.

Prophylactic treatment

- Moving out of the endemic area at an early age, preferably under the age of 20
- People from other villages should avoid settling in the endemic zone

Polenakovic and Stefanovic further recommend (16):

- Avoidance of environmental endemic factors
- Avoidance of genetic inheritance through marriage to healthy partners

Other authors recommend use of water from non-endemic areas.

General management

Physical effort should be restricted; periods of rest are recommended.

Calorie intake adjusted: according to specific physical activities.

Fluid intake: adjusted according to the phase of the disease, strictly related to loss of fluids. In the phase of compensatory polyuria, there should be appropriate fluid intake, thus avoiding dehydration.

Sodium intake: according to requirements

- Normal intake - in the initial stage of the disease
- Increased sodium to correct urinary losses in cases of associated salt losing nephritis
- Salt intake must be restricted in patients with hypertension and heart failure
- Adequate sodium balance in the advanced stage of the disease
- Protein intake: adjusted according to the stage of the disease is recommended:
- Normal intake in phases of BEN without renal failure
- Low-protein diet is recommended in patients with azotaemia. It is correlated with the degree of renal failure

Symptomatic treatment

Anaemia is a precocious symptom of the disease. Treatment is recommended from the moment of its diagnosis.

The treatment includes: iron, folic acid, recombinant human erythropoetin.

Treatment with iron aims to solve the iron deffi-ciency component of anemia.

Other factors which might lead to iron loss are also corrected:

- endocrine factors (prolonged menstruation)
- post abortum conditions
- peptic ulcer accompanied by digestive hemorrhages
- surgical interventions accompanied by blood loss.

Treatment with folic acid is also implemented in cases of renal failure.

Treatment with recombinant human erythropoetin is indicated both in the pre-dialysis phase and in hemodi-alysed patients. The experience of the hemodialysis centre in Drobeta Turnu Severin shows the effectiveness of this treatment in hemodialysed BEN patients.

Treatment of hypertension. It is recommended in the rare cases of hypertension associated to BEN. Cal-cium channel blockers (amlodipine) and betablockers, vasodilators with direct action upon the vascular smooth muscles are utilized.

The use of conversion enzyme inhibitors is avoided because they diminish renal function.

The pathogenic treatment of renal failure, of phosphocalcic metabolism disorders, of renal acidosis, etc. is also recommended.

Positive results obtained by using synthesis anabo-lisers (decadurabolin, decanofort) have been reported.

Renal replacement therapies

Hemodialysis

The BEN patients can benefit from hemodialysis. The patients from the endemic areas of Oltenia are treated at the hemodialysis centre of Drobeta Turnu-Severin.

On April 10th 2000 the hemodialysis centre in this town had 74 patients on hemodialysis, of which 46 were with BEN (66%); 4 of the BEN patients had been on hemodialysis since 1994.

The adequacy of hemodialysis in BEN patients is satisfactory.

The patients from the endemic area around the town of Oravita, particulary located in Ciudanovita, are on hemodialysis at the hemodialysis centre of Resita. How-ever, the BEN cases are rare in this area.

Peritoneal dialysis

Peritoneal dialysis has been introduced on a regular basis during the last 2 years at the Dialysis Centre of Drobeta Turnu-Severin.

Of the 16 patients hemodialysed on April 10, 2000, 5 were with BEN.

Renal transplantation

It is a method which has been successfully utilized in BEN.

Results of renal transplantation were first published in Romania in 1979, when Pasare et al. reported, at the 4th Symposium held at Nis, a case of a patient with BEN who was observed for 6 years after renal transplanta-tion. The patient showed a good general condition, go-ing about life as normal (42).

The Hemodialysis Centre at Turnu Severin regis-tered 8 patients who had undergone transplantations in the last 5 years, 3 of whom were BEN patients.

Of the BEN patients, one underwent renal trans-plantation in 1997 and the graft is still functioning. The second patient survived 3 years and death occurred through bronchopneumonia; the third survived for 2 years, showing progressive chronic rejection.

Of the 3 patients undergoing transplantation, 2 were females and 1 male. All three of them received the transplanted kidney from their own mothers.

Studies carried out on the transplant BEN patients have indicated positive results in the grafts originating from both living and cadaver donors.

Touraine et al (43), using cadaver grafts, obtained positive results, the evolution of BEN patients being similar to that of graft patients with chronic renal failure of other etiologies. Recurrence of BEN in the graft has not been observed.

References

1. Lazarescu R. Contributii la studiul nefropatiei endemice in Romania. Teza de doctorat, IMF Bucuresti, 1966.
2. Mustata N, Basarab I, Serafin T. Complementul seric la bol-navii cu nefropatie endemica. Viata Medicala 1968; 15: 169.
3. Tonea TR, Nicoara S, Butoianu E, et al. Modificarile cromo-zomiale la doua cazuri de nefropatie cronica endemica. Med. Interna.1967; XVIII, 10: 1179.
4. Bruckner I, Motoiu Raileanu I. Chromosomal changes in en-demic nephropathy. Rev Roum Med Int. 1971; 8: 75.
5. Gluhovschi G, Sabo I. Biochemical changes in the Balkan ne-phropathy in Endemic (Balkan) Nephropathy. Endemic (Bal-kan) Nephropathy. University of Nis, 1981: 93.
6. Georgescu L, Litvac B, Manescu N, et al. Particules virales dans le rein de la nephropathie endemic balkanique. Sem Hop Paris 1970; 46, 53: 3526.
7. Gluhovschi GH, Trandafirescu V, Munteanu I, Margineanu Gh. Aspects of pregnancy in endemic Balkan Nephropathy. In: Munteanu I, Rippmann ET, Hrubaru N (eds), Maternal – fetal risk in gestosis. Preeclampsia and pregnangy induced hyperten-sion. Organisation Gestosis – Press 1966: 15.
8. Gluhovschi G, Stefanovic V, Dimitrov T, et al. Nefropatia en-demica balcanica (Endemic Balkan Nephropathy). Ed. Helicon, Timisoara 1994.
9. Zaharia C. Nefropatia endemica familiara (N.E.F.). Teza de doctorat, IMF Bucuresti, 1968.
10. Bruckner I, Zosin C, Lazarescu R, et al. A clinical study of ne-phropathy of an endemic character in the People's Republic of Rumania. Intern Symp Endemic Nephropathy. Acad Sci Press, Sofia, 1965: 25.
11. Gluhovschi Gh. Contributie la studiul nefropatiei endemice bal-canice. Teza de doctorat. Institutul de Medicina Timisoara 1973.
12. Bacila E, Vintilescu D. A new focus of endemic nephropathy in the Romanian People's Republic. Intern Symp Endemic Ne-phropathy, Bulgarian Acad Sci Press, Sofia 1965: 122.

13. Puhlev A, Popov NG, Astrug A et al. La nephropatie endemique en Bulgaria. Arch de l'Union Med Balk 1965; III, 5: 559.
14. Toncheva D, Dimitrov T. Genetic predisposition to Balkan endemic nephropathy. Nephron 1996, 72: 564.
15. Andriescu E, Ancusa M, Telegut M, et al. Preliminary note on the microelement concentration in the Secaseni area (Banat) water supplies. Intern Symp Endemic Nephropathy, Bulgarian Acad Sci Press, Sofia,1965.
16. Markovic B, Lebedev S. Role etiopathogenique des silicates erosifs dans la nephrite endemique. Press Med 1965, 73: 401.
17. Ancusa M, Bacila E, Vintilescu D. Study of the general mortality rare in the Secaseni area (Banat). Intern Symp Endemic Nephropathy, Bulgarian Acad Sci Press, Sofia, 1965.
18. Mustata N, Matei S, Serafin T. Relatii intre poluarea cu compusi fenolici naturali a apelor si fantanilor din satul Secaseni (judetul Caras-Severin) si nefropatia endemica. Viata Medicala 1971, XVIII, 11: 489.
19. Feder GL, Radovanovic Z, Finkelman RB. Relationship between weathered coal deposits and the etiology of Balkan endemic nephropathy. Kidney Int 1991; 40(suppl 34): 9.
20. Tatu CA, Orem WH, Finkelman RB, Feder GL. The etiology of Balkan Endemic Nephropathy: Still more questions than answers. Environmental Health Perspectives 1988; 106: 689.
21. Tatu C.A, Feder G.L, Paunescu V, et al. Balkan Endemic Nephropathy: the possible role of the geological environment. Fiziologia – Physiology, 1999; 9: 6.
22. Gaon J, Griggs RC, Vasiljevic M, Alibegovic S. Testing of hypothesis of lead, a possible etiological agent of chronic nephropathy in Yugoslavia. Intern Symp Endemic Nephropathy, Bulgarian Acad Sci Press, Sofia 1965: 124.
23. Vedeen R.P. Environmental renal disease. Lead, cadmium and Balkan endemic nephropathy. Kidney Int 1991: 40(suppl) :4.
24. Freyria AM, Touraine JL, Stefanovic V, et al. Complement components in endemic nephropathy. Endemic (Balkan) Nephropathy. University of Nis, 1979: 81.
25. Drugarin D, Bona C.A, Noveanu L, et al. CD 3+, CD 16+, CD 56+ cells: a novel subset of T lymphocytes from patients with Balkanic nephropathy. Roum Arch Microbiol Immunol 1995; 54: 255
26. Voiculescu C, Rogoz S, Stanciu L, Rosca T. Virological and immunological study of 20 patients with Balkan endemic nephropathy. Rev Roum Med Virol 1983; 34: 203.
27. Trandafirescu V. Aspecte de imunofluorescenta in nefropatiile glomerulare. Teza de doctorat.Institutul de Medicina Timisoara, 1974.
28. Hall PW. Balkan endemic nephropathy. More questions than answers. Nephron 1992, 62: 1.
29. Zosin C, Georgescu L, Manescu N, et al. Aspects anatomoclinique de la nephropatie endemique balcanique. Sem Hop Paris, 1966; 42: 194.
30. Strahinjic S, Stefanovic V. Clinical feature and diagnosis of endemic nephropathy. In: Current Topics in Endemic (Balkan) Nephropathy, University Press, Nis, 1987: 1.
31. Karlsson FA, Lenkei R. Urinary excretion of albumin and beta-2 microglobulin in a population from an area where Balkan nephropathy is endemic. Scand J Clin Lab Invest 1977; 37: 169.
32. Hall PW, Vasilievic M. Beta-2-microglobulin excretion as an index of renal tubular disorders with special reference to endemic Balkan nephropathy. J Lab Clin Med 1973; 81: 897.
33. Sattler TA, Dimitrov TS, Hall PW. Relation between endemic (Balkan) nephropathy and urinary tract tumors. Lancet 1977, i: 278.
34. Bruckner I, Serban M, Nichifor E. Signification of lyzozymuria in endemic nephropathy. Second Symp Endemic Nephropathy, Sofia, 1972: 14A.
35. Polenakovic MH, Stefanovic V. Balkan nephropathy. In: Davison AM, Cameron JS, Grunfeld JP, Kerr DNS, Ritz E, Winearls CG (eds), Oxford Textbook of Clinical Nephrology II Ed, Oxford University Press, Oxford, 1998: 1201.
36. Georgescu L, Vasiliu L. Some particular morphological lesions in endemic nephritis of Balkans. Roum Med Rev 1970; 14: 3.
37. Ferluga D, Hvala A, Vizjak A, et al. Renal function, protein excretion and pathology of Balkan endemic nephropathy. IV Light and electron-microscopic study. Kidney Int 1991; 40 (suppl 34) :57.
38. Dojcinov D, Strahinjic S, Stefanovic V. Pathology of the kidney in the early phases of endemic (Balkan) nephropathy. In: Endemic (Balkan) Nephropathy, Univ Press, Nis, 1979: 91-104.
39. Bruckner I, Petrovici A, Lazarescu R, Voiculescu R. Aspectul electrono-optic al rinichiului in nefropatia endemica balcanica. St Cerc Med Interna 1967; 8: 153.
40. Macanovic M. Immune machanisms in the pathogenesis of endemic nephropathy. In: Danilovic V (ed), Proceedings of II Symposium sur la Nephropathie Endemique, Acad Serb Sci Arts, Belgrade, 1979: 129.
41. Polenakovic M, Hrisoho D. Balkan endemic nephropathy. Immunofluorescent microscopic examination. In: Proceedings of the II Symposium sur la Nephropatie Endemique, Academie Serbe des Sciences et Arts, Belgrade, 1979: 137.
42. Pasare G, Melencu M, Parvulescu G. Our first case of renal graft in a patient with Balkan nephropathy. In: Endemic (Balkan) nephropathy, University of Nis, 1979: 270.
43. Touraine JL, Malik MC, Stefanovic V, et al. Kidney transplantation in endemic nephropathy. In: Endemic (Balkan) nephropathy, University of Nis, 1979: 177.