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UPPER UROTHELIAL CARCINOMAS ASSOCIATED WITH BALKAN ENDEMIC NEPHROPATHY AND THEIR SIMILARITIES WITH UPPER UROTHELIAL CARCINOMAS IN ANALGESIC NEPHROPATHY

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Summary. *The incidence of upper urothelial carcinomas (UUC) is even more than hundred times higher in the foci of Balkan endemic nephropathy (BEN), by investigations of our team, and others. Every tenth patient suffers from both diseases. Etiology is unknown. It would seem that BEN is a tubulointerstitial nephritis associated with urothelial malignancy. We found that apoptosis plays an important role in the early phase of BEN, similarly as in other toxic nephropathies. Petronic shows great similarity between Balkan nephropathy and analgesic nephropathy, as well as between upper urothelial carcinomas associated with both diseases. Based on investigation of grade to stage ratio in 100 tumors from our series of UUC, the upper urothelial carcinomas from BEN areas are characterized by slow growth of tumor mass in comparison to the same carcinomas outside BEN regions in former Yugoslavia and the evaluated cut-off point % cErb-B2+ cells for aggressive tumors of grade 3 or stage 2 is slightly greater for the UUC in the general population. Smaller cell population in proliferation in the UUC from BEN regions than what is detected in the UUC outside these regions may explain BEN tumor resistance to putative toxic agent effect during tumor progression in grade 2, although the same toxin has probably realized tumor induction. It would seem that the cErb-B2 (a receptor for EGF) is differently involved in the control of a cell cycle proliferation in urothelial tumor associated with BEN.*

Key words: *Upper urothelial carcinomas, Balkan endemic nephropathy, analgesic nephropathy, cErb-B2*

In some rural areas along tributaries of big rivers in ex Yugoslavia the occurrence of Balkan nephropathy is endemic and common. In the same areas the incidence of upper urothelial carcinoma is also very high. Since 1953, a rapid increase of the pelvic and ureteral tumors has occurred at our Clinic of Urology in Belgrade (1). It became clear that these tumors happened more frequently of inhabitants in the villages with the Balkan endemic nephropathy (BEN). In addition, Balkan endemic nephropathy patients had more hematuria commonly, which

Table 1. Toxic nephropathies are characterized by apoptosis of tubular epithelial cells in the early phase of the disease evolution, including Balkan endemic nephropathy

TUBULOINTERSTITIAL NEPHRITIS ASSOCIATED WITH UROTHELIAL MALIGNANCES		APOPTOSIS OF RENAL TUBULAR CELLS
Analgesic nephropathy (AN) Analgesic abuse	**Upper urothelial carcinomas 10% /RCC** Jensen 1989 (6), McCredie 1993 (7)	**YES** Rocha and Michea 2001 (8)
Balkan endemic nephropathy (BEN) Toxin(s) + genetic predisposition 3q25/3q26 Toncheva 1991 (9)	**Upper urothelial carcinomas 10-30%** Petkovic (1,2) Petronic 1991, 2000 (3,4)	**YES** Savin and Petronic 2001 (10)
Chinese herbs (CHN) Aristolochia clematis-wheat (BEN areas)	**Upper urothelial carcinomas 40%** Slimming regimen + AA Cosyns 1994, 1999 (11,12)	
Ochratoxin Food and feed – (BEN areas)	**Urothelial carcinomas** Bach 1992 (13)	**YES** Schwerdt 1999 (14)
Penicillium polonicum Food spoilage mould (the Balkans)	**Karyomegaly** Mantle 1991 (15)	**YES** Mantle 1998 (16)
TOXIC NEPHROPATHY WITHOUT URINNARY TRACT MALIGNANCY		
Cyclosporine associated nephropathy / transplant patients		**YES** Thomas 1998 (17)

wasn't characteristic of that disease. The explanation was that every tenth Balkan endemic nephropathy patient in the sixties has been also affected with urothelial carcinoma, mostly of the upper part of the urinary tract (UUC) (2). The incidence of upper urothelial carcinomas is even more than hundred times higher in the foci of Balkan endemic nephropathy, according to investigations of our team, and others (3,4). Until now, the etiology of Balkan endemic nephropathy associated with urothelial tumors has not been elucidated.

Balkan endemic nephropathy is a tubulointerstitial nephritis associated with urothelial malignancy

The lesions of tubules and vasculature in BEN develop from discrete foci of tubular atrophy, vas afferent hyalinosis and interstitial fibrosis, into vast parenchymal fibrosis and they result in the smallest kidneys of few centimeters in length (5). There is a body of evidence that Balkan endemic nephropathy is apparently a toxic nephropathy, like analgesic nephropathy, Chinese herbs nephropathy (induced by nephrotoxin Aristolohia clematis), or nephropathies in ochratoxicosis and diet of food contaminated by spoilage mould Penicillium aurantiogriseum (polonicum). Even if they all do not represent single entity, the remarkable similarities in the clinical course and several pathological findings will assign a comparable pathogenesis. It has been already documented that apoptosis takes place in the pathogenesis of BEN and other toxic nephropathies (8,10,14,16,17), (Table 1).

Our investigation of early phase of BEN explains how apoptosis (programmed cell death) of tubular cells and endothelial cells of intertubular capillaries displays the possibility for long-term repeated noxious action which would result in renal ischemia (Table 2). Ischemia may further facilitate apoptosis (18)**.** Interstitial inflammatory response in BEN is faint.

Table 2. Vasoconstrictive molecule action and capillarosclerosis / chronic ischemia seem to potentiate direct effect of putative nephrotoxin on tubular cells, in susceptible persons.

nephrotoxin action + genetic predisposition		
tubules	direct toxic effects	initiate tubular cell apoptosis
small vessels	vasoconstriction chronic ischemia	accelerates apoptosis
Result in		tubular atrophy interstitial fibrosis

All these nephropathies, including BEN, are characterized by very frequent occurrence of urothelial carcinomas. It is not a rule, and a number of toxic nephropathies are not related to the renal malignancies, like cyclosporine nephropathy. Vice versa, in the regions with BEN, several members of a household may be affected either by one or both diseases, urothelial carcinoma and BEN. They appeared concomitantly in range of 10 to 30% of cases (1,2,4). BEN is unlikely to be the additional lesion to (urothelial) malignancy, but it is a nephropathy induced by the same toxin(s) which initiate both carcinomas and/or nephropathy in susceptible persons. We may presume that the biological features of the UUC associated with BEN or with other toxic nephropathies are very similar. On the contrary, it is possible that some tumor characteristics are quite different from those that are usually seen in the same urothelial carcinomas in the general population or those generated outside endemic regions. Toncheva et al implicated the 3q chromosome aberration in affected people in BEN regions (9).

Similarities of analgesic nephropathy and endemic nephropathy

Similarities are given in the Table 3.

Slow growth of the upper urothelial carcinomas from the foci of Balkan endemic nepropathy

Upper urothelial carcinomas are especially common malignancies in BEN areas, and they are frequently developed in the patients with analgesic nephropathy. One of the major characteristics of the UUC from BEN regions and in analgesic abusers is slow growth of tumor mass. We conclude upper urothelial tumors from BEN regions are less malignant than those outside these regions in former Yugoslavia, comparing tumor grade and stage. The difference in grade to stage ratio for individual tumors at clinical presentations with respect to endemic nephropathy is in favor of delayed tumor growth in the regions with BEN ($n = 100$, $F = 5.66$, $p = 0.019$). Tumors in BEN regions may progress into high grade 3 of cell atypia and start to invade the muscle wall of urothelium. Tumors outside BEN regions may progress into malignancy grade 2 and invade muscle wall of urothelium (Figure 1). The former group of tumors have a better overall survival (8 and 3 years), (19). The presence of some inhibiting molecules was identified in the patient's serum from BEN regions (20). We find (according to investigation of Dr Savin M.) that cells grow more slowly in the primary culture of tumor tissue when tumors were obtained from patients from the settlements in foci of BEN (not yet published).

Although light microscopy assessment may not reveal any pathological peculiarity of those UUC associated with BEN or analgesic nephropathy, our molecular investigation confirms slow evolution of the UUC from the BEN regions. On a series of one hundred upper urothelial carcinomas from the patients who underwent surgery at the Clinic of Urology in Belgrade from 1977-2000, we applied an immunohistochemic analysis of the receptor of epidermal growth factor expression, cErb-B2. The evaluated cut-off point % cErb-B2+ cells for aggressive tumors of high grade / stage 2 is slightly greater for the UUC in the general population, than that for the UUC in BEN areas (1% of positive cells, and >0, respectively), Figure 2. The cErb-B2 positive score

(overexpression) usually means mutation of the *cerb-B2* gene. In addition, those UUC with overexpression of cErb-B2, coming from regions with BEN, exhibit significantly lower increase in proliferation to apoptosis ratio at clinical presentation. It would seem that cErb-2 is differently involved in the control of a cell cycle proliferation in tumors associated with BEN, which are with rare metastases. The cErb-B2 signaling in the UUC from the BEN regions is a consequence of different threshold levels of cErb-B2 (homo and hetero) ligation to EGF receptors, resulting possibly in different intracellular signal transduction via surface cErb-B2 R.

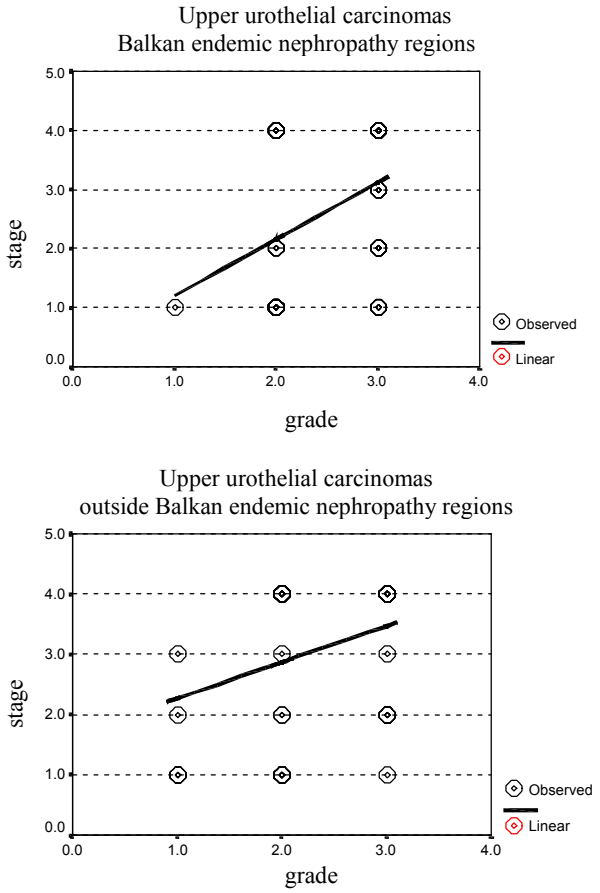

Fig. 1. The grade to stage ratio of the upper urothelial carcinomas from different regions.

It has been recognized the signal specificity and diversity of cErbB2 ligation on cell surface resulting in proliferation in one carcinoma cell line, but inhibition of proliferation in another cell line from breast carcinoma with the same amount of cErb-B2 molecules (21). Our current investigation on a group of 100 UUC has clearly made known an inclination of the UUC from BEN regions to much more tumor cell apoptosis and lesser cells in proliferation in tumors with cErb-B2 overexpression, in comparison to the cErb-B2 negative UUC.

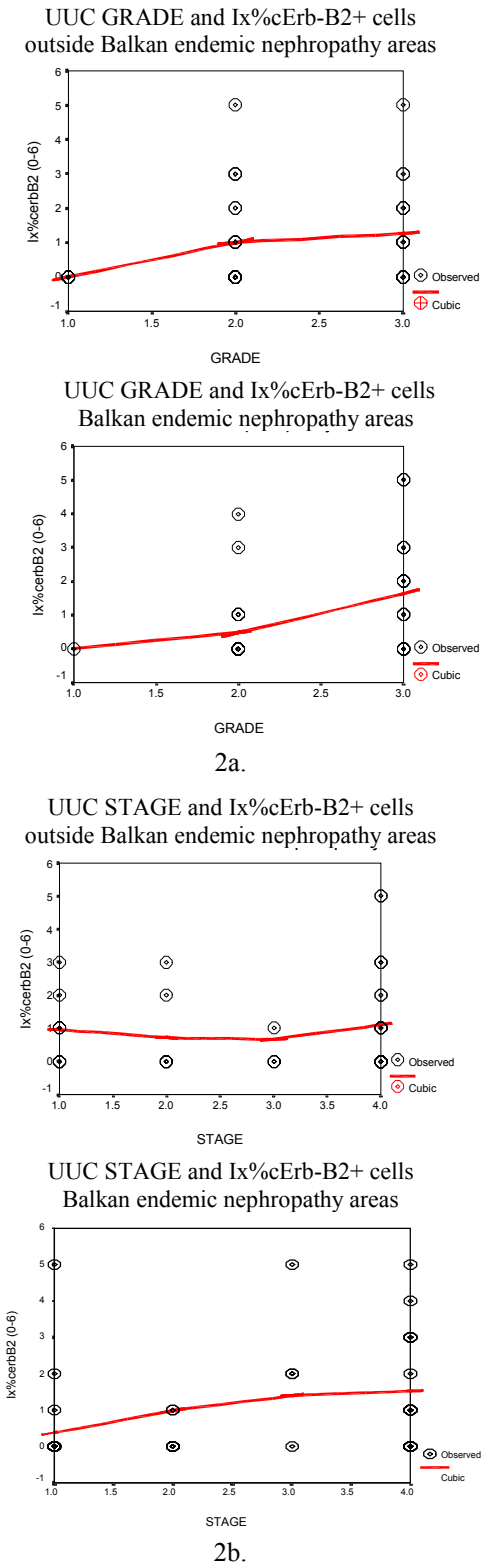

Fig. 2. The relation between Index of cErb-B2 and grade (a), or stage (b) of the upper urothelial carcinomas (UUC) from the foci of the Balkan endemic nephropathy and outside these regions in former Yugoslavia. Index of cErb-B2+ cells is in range 0-; 0 = 0; 1 = 0-1%; 2 = 1-10%; 3 = 10-33%; 4 = 33-66%; 5 = 66-100%, 6=100%.

Table 3. Characteristics of analgesic and Balkan endemic nephropathy

Analgesic nephropathy	Balkan endemic nephropathy
- exposure to the agent should persist for 15-20 years before the occurrence of the disease	- exposure to the agent should persist for 20 years before the occurrence of the disease
- the disease is manifested between the ages of 40 and 60	- the disease used to become manifested between the ages of 20 and 40 years, but now it is manifested between the ages 40 and 60
- pathohistologically it is characterized by changes in the renal tubulo-interstitium and papillary necrosis	- pathohistologically it is characterized by changes in the renal tubulo-interstitium
- it is not sex related, but depends on duration and level of exposure to the noxious agent	- it is not sex related, but depends on duration and level of exposure to the noxious agent
- the disease is chronic and progresses to terminal renal failure	- the disease is chronic and progresses to terminal renal failure
- high incidence of upper urothelial carcinoma (renal, pelvis, ureter). They are much more common than in patients affected by other renal diseases or individuals with no renal diseases	- high incidence of upper urothelial carcinoma (renal, pelvis, ureter). They are much more common than in patients affected by other renal diseases or individuals with no renal diseases
- urothelial carcinoma in the urinary bladder is somewhat more common or occurs in the same percentage as in patients with other renal diseases or no renal diseases	- urothelial carcinoma in the urinary bladder is somewhat more common or occurs in the same percentage as in patients with other renal diseases or no renal diseases
- the ratio of the carcinoma of the upper urothelium and the same tumors in the urinary bladder is 11 vs. 1, while this ratio in patients without analgesic nephropathy or endemic nephropathy is 1 vs. 15	- the ratio of the carcinoma of the upper urothelium and the same tumors in the urinary bladder is 15 vs. 1, while this ratio in patients without analgesic nephropathy or endemic nephropathy is 1 vs. 17
- carcinoma of the upper urothelium occurring in analgesic nephropathy is frequently accompanied with renal failure, more commonly than in people without renal diseases or even patients with other renal diseases, except for analgesic nephropathy and endemic nephropathy	- carcinoma of the upper urothelium occurring in endemic nephropathy is frequently accompanied with renal failure, more commonly than in people without renal diseases or even patients with other renal diseases, except for analgesic nephropathy and endemic nephropathy
- carcinoma of the upper urothelium occurs in about 8 % of patients with analgesic nephropathy	- carcinoma of the upper urothelium occurs in about 10 % of patients with endemic nephropathy
- bilateral occurrence of upper urothelial carcinoma in patients with analgesic nephropathy is recorded in about 5% (simultaneous or successive)	- bilateral occurrence of upper urothelial carcinoma in patients with endemic nephropathy is recorded in about 10-14% (simultaneous or successive)
- multiple occurrence of upper urothelial carcinoma in patients with analgesic nephropathy is frequent	- multiple occurrence of upper urothelial carcinoma in patients with analgesic nephropathy is recorded in 1/3 of the cases
- in patients with analgesic nephropathy hyperplasia, dysplasia and other urothelial atypias are found along with the whole urothelium. They are located on several different places and manifested to a different degree	- in patients with endemic nephropathy hyperplasia, dysplasia and other urothelial atypias are found along with the whole urothelium. They are located on several different places and manifested to a different degree (even carcinoma in situ)
- discontinuation of exposure to the noxious agent after 20 years does not provide protection from the occurrence of upper urothelial carcinoma	- discontinuation of exposure to the noxious agent after 15 - 20 years does not provide protection from the occurrence of upper urothelial carcinoma
- the agent is mildly cancerogenic for the renal parenchyma and very carcinogenic for the upper urothelium	- the agent is mildly carcinogenic for the renal parenchyma and very carcinogenic for the upper urothelium
- in the terminal stage of renal failure (even in anuria) 44% of patients with analgesic nephropathy subjected to dialysis (hemo- or peritoneal) develop urothelial carcinoma as compared to 14% of those with transplanted kidney (urothelial carcinoma of their own kidney). These carcinomas on the urothelium do not occur in patients in the terminal stage of renal failure induced by other renal diseases, except for endemic nephropathy	- in the terminal stage of renal failure (even in anuria) 44% of patients with endemic nephropathy subjected to dialysis (hemo- or peritoneal) develop urothelial carcinoma as compared to 14% of those with transplanted kidney (urothelial carcinoma of their own kidney). These carcinomas on the urothelium do not occur in patients in the terminal stage of renal failure induced by other renal diseases, except for analgesic nephropathy

From (ref 4) Petronic V. *Tumors of the upper urothelium and endemic nephropathy*; in: Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic Lj, Petronic V (eds): *Endemic nephropathy*. Office for Textbooks and Teaching Aids, Belgrade, 2000: 350-439.

Smaller cell population in proliferation in the UUC from BEN regions than what is detected in the UUC outside these regions may explain BEN tumor resistance to putative toxic agent effect during tumor progression in grade 2, although the same toxin has probably realized tumor induction (22). This could alter tumor growth. The same option may act in analgesic nephropathy associated with urothelial tumors, by antipyretic analgesics taken in large doses over a prolonged period >20 years. Rocha and Michea (8) explain why only very high doses of the drugs, taken over a very long time, lead to chronic renal failure with renal papillary necrosis and late urothelial carcinomas. The therapeutic level of acetaminophen and / or salycilate that are directly toxic to rapidly proliferating cells of the inner medulla (mIMCD3) in culture might occur in renal medulla in vivo, when excessive amount of drugs are chronically ingested. Very small population of IMCD3 cells is proliferating and they are particularly vulnerable to toxic agents. In addition, genotoxic effects of acetaminophen that may contribute to the development of urothelial tumors that accompany analgesic nephropathy had been earlier described. These are: 1) direct inhibition of RNA reductase, which reduces cell growth by stopping DNA replication, 2) the relative number of cells in S phase increases, with damaged DNA leading to formation of sister chromatide exchange and chromosomal aberrations, 3) acetaminophen inhibits nucleotide excision repair.

Conclusion

With respect to what we displayed in this review, the similarities in the symptoms and clinical presentation between the upper urothelial carcinomas associated with the Balkan endemic nephropathy and analgesic nephropathy (toxic nephropathies) have become clear. Based on that, we may presume, with rather high likelihood that toxic nephropathy may lead to Balkan endemic nephropathy associated with urothelial tumors, although the nature of the putative toxin has not been identified, nor do we know whether it is one or several different toxic agents. An endemic occurrence of the Balkan nephropathy and upper urothelial carcinomas in different foci indicates that a toxic substance exists only in limited areas. The original speculation on the possible etiology factor of Balkan endemic nephropathy, by the authors who have discovered that disease, was that it might be a toxin, for instance a heavy metal (lead) (23). Later on this direction was abandoned, since the results of investigation of lead in endemic areas have not been conclusive. The results of our investigations are in favor of toxic etiology of Balkan endemic nephropathy associated with (upper) urothelial carcinomas (10,22,24).

References

1. Petkovic S, Mutavdzic M, Petronic V, Markovic V. Les tumeurs du bassinet et de l' uretère - recherches cliniques et étiologiques. J Urol Néphrol 1971; 77(6): 429-439.
2. Petkovic S, Mutavzic M. Osvrt na istraživanja tumora pijeluma i uretera u žarištima endemske nefropatije. Zbornik radova II Simpozijum o endemskoj nefropatiji Srpske Akademije Nauka i Umetnosti, Beograd-Lazarevac 1977. Glas SANU 1979: 27.
3. Petronic V, Bukurov N, Djokic M, et al. Balkan endemic nephropathy and papillary transitional cell tumors of the renal pelvis and ureters. Kidney Int 1991; 40(S34): 77-79.
4. Petronic V. Tumors of the upper urothelium and endemic nephropathy; in: Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic Lj, Petronic V (eds): Endemic nephropathy. Office for Textbooks and Teaching Aids, Belgrade, 2000: 350-439.
5. Sindjic M. Morphological changes in kidneys affected by endemic nephropathy. In: Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic Lj, Petronic V (eds): Endemic nephropathy. Office for Textbooks and Teaching Aids, Belgrade, 2000: 153-276.
6. Jensen OM, Knudsen JB, Tomasson H, Sorensen BL. The Copenhagen case-control study of renal pelvis and ureter cancer: role of analgesics. Int J Cancer (USA) 1989; 44(6): 965-969.
7. Mc Credie M, Stewart JH, Day NE. Different roles for phenacetin and paracetamol in cancer of the kidney and renal pelvis. Int J Cancer 1993; 53(2): 245.
8. Rocha GM, Michea LF, Peters EM, et al. Direct toxicity of nonsteroidal anti-inflammatory drugs for renal medullary cells. Proc. Natl. Acad. Sci. USA: 200; 98(9): 5317-5322.
9. Toncheva DI, Gergov TD, Tzoneva MT, Bouchakliev ZP. Spontaneous and induced chromosome aberrations in Balkan endemic nephropathy. Kidney Int 1991;40(S34): 97-99.
10. Savin M, Bumbasirevic V, Djukanovic LJ, Petronic V. The significance of apoptosis for early diagnosis of Balkan nephropathy. Nephrol Dial Transplant 2001; 16(Suppl 6): 30-32.
11. Cosyns JP, Jadoul M, Sqifflet JP, et al. Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? Kidney Int 1994; 45(6): 1680-1683.
12. Cosyns JP, Jadoul M, Squifflet JP, et al. Urothelial Lesions in Chinese-Herb Nephropathy. Am J Kidney Dis 1999; 33(6): 1171-1173.
13. Bach PH, Cregg NJ, Delacruz L. Relevance of rat model papillary necrosis and upper urothelial carcinoma in understanding the role of ocharatoxin A in Balkan Endemic Nephropathy and its associated carcinoma. Food Chem Toxicol (England) 1992; 4: 205.
14. Schwerdt G, Freudinger R, Mildenberger S, et al. The nephrotoxin ochratoxin A induces apoptosis in cultured human proximal tubule cells. Cell Biol Toxicol 1999; 15(6): 405-15.
15. Mantl PG, McHugh KM, Adatia R, et al. Persistent karyomegaly caused by Penicillium nephrotoxins in the rat. Proc R Soc Lond B 1991; 246: 251-259.
16. Mantle PG, Miljkovic A, Udupa V, Dobrota M: Does apoptosis cause renal atrophy in Balkan endemic nephropathy? Lancet 1998;3, 352 (9134):1118-1119.
17. Thomas S, Takeshi A, Raymond P, et al. Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. Kidney Int 1998; 53(4): 897-908.
18. Khan S, Cleveland RP, Koch CJ, Schillings JR. Hypoxia induces renal tubular epithelial cell apoptosis in chronic renal disease. Lab Invest 1999 Sep; 79(9): 1089-1099
19. Jankovic S, Marinkovic J, Radovanovic Z. Survival of the upper-urothelial-cancer patients from the Balkan nephropathy endemic and nonendemic areas. Eur Urol 1998; 15: 59-61.
20. Ramic Z, Nikolic V, Radovanovic Z, et al. Serum immunosuppressive factors in endemic nephropathy: I. Effects on T cell proliferation. In: Strahinjić S, Stefanović V (eds.) Etiology of Endemic (Balkan) nephropathy. University Press, Nis, 1987: 71-78.
21. Brockhoff G, Heiss P, Schlegel J, et al. Epidermal growth factor receptor, c-erbB2 and c-erbB3 receptor interaction, and related cell cycle kinetics of SK-BR-3 and BT474 breast carcinoma cells. Cytometry 2001; 44(4): 338-348.
22. Petronic V, Savin M. Apoptosis and p53 status of the upper urothelial carcinomas from Balkan endemic regions. Nephrol

Dial Transplant 2001; 16(Suppl): 33-35.
23. Danilovic V. Chronic nephritis due to ingestion of lead – contaminated flour. Br Med J 1958; (1): 27-28.
24. Savin M, Petronic V, Sindjic M, et al. Apoptosis in upper urothelial carcinoma from Balkan endemic regions. Balkan J Urol 2000; 1(1): 27-35.
25. Savin M, Petronic V. The significance of molecular-biological characteristics of upper urothelial carcinomas associated with the Balkan endemic nephropathy. Facta Universitatis 2002; 9: 95-97.