

Upper Urinary Tract Urothelial Cancer in Croatian and Bosnian Endemic Nephropathy Regions

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ABSTRACT

Objectives: Endemic nephropathy (EN) is a chronic tubulointerstitial renal disease associated with increased incidence of upper urinary tract urothelial cancer (UTUC) occurring predominantly in geographically limited areas in villages along big Danube river. Based on results obtained by the research it is confirmed that aristolochic acid is causative agent of endemic nephropathy (EN). **Aim:** The aim of this study was to determine characteristics of UTUC in two endemic areas and analyze trends in 5-year period. **Methods:** This study included all patients with UTUC with or without synchronous/metachronous urothelial cancer in urinary bladder, from Croatian and Bosnian EN and non-EN regions. We analyzed archival data bases from all patients with UTUC who were admitted and operated in Department for surgery and urology, General Hospital Josip Bencevic Slavonski Brod in the time period between 2005 and 2010. Analyzed groups of patients were divided and compared as EN group (that includes patients from Croatian and Bosnian EN region) and non-EN group (that includes patients from Croatian and Bosnian non-EN region). All surgical specimens were processed according to standardized pathological procedures. **Results:** Comparing patients basic characteristics from Croatian and Bosnian EN region there was no significant difference in gender (females were more frequently affected in both EN regions, $p=0.99$) or age ($p=0.43$) of patients. We found higher blood levels of urea and creatinine in group of patients from Croatian EN region when compared with group of patients from Bosnian EN region but the difference was not statistically significant ($p=0.79$ and $p=0.44$, respectively). In patients from Croatian EN group Hemoglobin levels were significantly lower than levels from Bosnian EN region patients, $p=0.0049$. In group of patients from Bosnian EN region ureteral tumors were more frequently observed than in Croatian group of patients, when compared with renal pelvis tumors but the difference was not statistically significant ($p=0.258$). **Conclusion:** Our data showed some differences in tumor localization between to investigated, relatively nearby situated, EN regions in Croatia and Bosnia. Statistically significant difference in hemoglobin levels between Croatian and Bosnian EN regions that should be further analyzed to give some reasonable explanation.

Keywords: endemic nephropathy, urinary tract, urothelial cancer, Croatia, Bosnia, regions.

1. INTRODUCTION

Endemic nephropathy (EN) is a chronic tubulointerstitial renal disease associated with increased incidence of upper urinary tract urothelial cancer occurring predominantly in geographically limited areas in villages along big Danube river tributaries in Croatia, Bosnia and Herzegovina, Bulgaria, Romania and Serbia (1, 2). In non-endemic regions upper urinary tract cancers (UTUC) are rare and accounts for 5-10 % of all urinary tract cancers, while in EN regions are more frequent and reported as 65% of all kidney cancers in adults (3, 4). Histologically, EN can be manifested as bilateral interstitial fibrosis of renal cortex with tubular atrophy with or without urothelial cancer of upper urinary tract (3, 5). Histology of re-

nal parenchyma can be very blind or with non-specific inflammatory/degenerative changes due to obstruction, diabetes or hypertension that usually occurs in older age and/or in patients with tumors in renal pelvis and/or ureter (6, 7). EN can be manifested solely as urothelial cancer of upper urinary tract proceeding renal impairment. EN occurs in particular households, but it does not have inherited features.

Based on results obtained by the research project led by the University of Zagreb and State University of New York it is confirmed that aristolochic acid is causative agent of EN, and this peculiar kidney disease is now considered to be an environmental form of worldwide present aristolochic acid nephropathy. Aristolactam-DNA adducts

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	CKD stage 1	CKD stage 2	CKD stage 3A	CKD stage 3B	CKD stage 4	CKD stage 5
ALL	3 (3.9%)	17 (22.4%)	15 (19.7%)	14 (18.4%)	12 (15.8%)	15 (19.7%)
EN	1	6	9	8	7	13
nonEN	2	11	6	6	5	2
EN-HR	0	2	3	1	2	6
EN-BiH	1	4	6	7	5	7

Table 1. CKD stages for all investigated group of patients

	EN (HR+BiH)	NonEN (HR+BiH)	EN-HR	EN-BiH
Creatinin (median, range, umol/l)	172 (56-1581)	113 (65-597)	213 (87-747)	156 (56-1581)
Urea (median, range, mmol/l)	9.8 (3-51.8)	8.15 (4.8-28)	11.8 (3-30.3)	9.25 (4-51.8)
Hemoglobin (mean value, ± standard deviation)	104.36 ± 24.03	119.37 ± 16.54	90.76 ± 24.07	110.77 ± 21.51
MDRD mean value, ± standard deviation)	35.42 ± 25.15	51.28 ± 25.52	31.64 ± 24.99	37.19 ± 25.44
Tumor localization (P-pyelon, U-ureter)	P-28 U-23 P+U-5	P-17 U-17 P+U-10	P-13 U-6 P+U-1	P-15 U-17 P+U-4
Tumor grade (LG/HG)	LG-14 HG-41	LG-18 HG-24	LG-4 HG-15	LG-10 HG-26
Tumor invasion (pTa/pT1 vrs pT2-pT4)	pTa/pT1-15 pT2-pT4-40	pTa/pT1-16 pT2-pT4-27	pTa/pT1-5 pT2-pT4-14	pTa/pT1-10 pT2-pT4-26
Synchronous and/or metachronous tumors	9/56 (16%)	12/34 (35.3%)	3/20 (15%)	6/36 (16.7%)

Table 2. Summarized clinical and pathological results in tested groups of patients

were detected in renal cortex as A:T > T:A mutations in the 5'-CpApG-3' context accumulating on the non-transcribed strand of the TP53 gene in EN/UTUC cases and presents as biomarkers of AA exposure in this geographical region (8, 9). Study of Jelakovic B et al. on renal cell carcinoma (RCC) observed the characteristic global mutational signature consistent with the mutagen effects of AA in 62.5% of tested RCC from EN region that was otherwise absent in the control RCC samples from non-endemic region (10).

Various studies from different EN regions and EN like diseases, such as AAN or Chinese herb nephropathy showed changes in numbers, distribution and histological characteristics of UTUC (4, 11, 12). Belicza et al observed changes in ratio between UTUC and RCC with relative decrease of urothelial carcinomas when compared with data from time period 20 years before investigation in the same EN region in Croatia (3). Changes in occurrence of UTUC as well as some characteristics of disease for EN region in Serbia were also observed by Cukuranovic et al. They found slow decrease in incidence of UTUC in Serbian EN region and in the same time increased tumor stage and grade when compared with results from earlier time periods (4).

The aim of this study was to determine characteristics of UTUC in two endemic areas and analyze trends in 5-year period.

2. PATIENTS AND METHODS

Patients with UTUC who underwent surgery in General Hospital Josip Bencevic, Slavonski Brod between 2005-2010 from both endemic and nearby non-endemic area were included in the study. General Hospital Josip Bencevic is situated near Croatian and Bosnian EN region and majority of patients from both EN regions were

treated in that hospital. This study included all patients with UTUC, with or without synchronous/metachronous urothelial cancer in urinary bladder, from Croatian and Bosnian EN and non-EN regions. The analyzed variables were: gender, age, hemoglobin, urea and creatinine blood levels, tumor localization, tumor grade and pTNM. We analyzed archival data bases from all patients with UTUC who were admitted and operated in Department for surgery and urology, General Hospital Josip Bencevic Slavonski Brod in the time period between 2005 and 2010. All operated patients were treatment naive. The study was approved by the Bioethics Committee of the General Hospital „Josip Bencevic“

Analyzed groups of patients were divided and compared as EN group (that includes patients from Croatian and Bosnian EN region) and non-EN group (that includes patients from Croatian and Bosnian non-EN region). We analyzed data from postoperative pathological reports, clinical history and preoperative questionnaires obtained from patients. We analyzed age, gender, tumor location, tumor multifocality, grade and stage from pathohistological reports, while place of residence (EN/nonEN) and clinical parameters such as blood levels of urea, creatinine and hemoglobin were obtained out of patients clinical history and questionnaires. All surgical specimens were processed according to standardized pathological procedures, while tumor grade and stage were assessed according to the WHO Classification of Tumours of the Urinary System and Male Genital Organs, 2016 (13). GFR was estimated using MDRD formula.

Statistical analysis was performed with MedCalc program, version 11.6.0.0. For statistical data processing for comparison of continuous variables, the T test of independent and dependent samples was used, i.e. the

Mann-Whitney test, depending on the symmetry of the data distribution. The significance level was $P < 0.05$.

3. RESULTS

In the time period between 2005 and 2010 in the Department of Urology and Surgery in General Hospital Josip Bencevic total of 102 patients were operated and treated due to primary UTUC. In the whole group median age was 72 years (41-85), and there was more female than male patients (M:F=42:60). Median and range of urea and creatinine were 9,2 (3-51,8) mmol/l and 135 (56-1581) μ mol/l, respectively. Mean value of eGFR for the whole group was $42,1 \pm 26,34$ while hemoglobin mean level was 110.84 ± 22.3 . CKD stage for all patients was shown in Table 1.

There was no statistically significant difference in CKD stages between EN and nonEN groups, but there is a trend showing higher CKD stages in patients from EN regions. There was no significant difference in CKD stages between Croatian and Bosnian EN foci.

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Out of all analyzed patients 56 were from EN region (Bosnian and Croatian) and 46 were residents from non-EN region. In EN group patients were statistically significantly older, with median age of 74.5 years (41-84 y) compared with patients from nonEN group with median age of 71.5 years (54-85 y), $p=0.015$. Female gender was significantly more frequent in EN group (M:F=18:40) when compared with nonEN group (M:F=24:20), $p=0.029$.

Our results regarding creatinine, urea and hemoglobin levels, MDRD and tumors localization, grade, pT and presence of synchronous/metachronous tumors were shown in Table 2.

Comparing patients basic characteristics from Croatian and Bosnian EN region there was no significant difference in gender (females were more frequently affected in both EN regions, $p=0.99$) or age ($p=0.43$) of patients. We found higher blood levels of urea and creatinine in group of patients from Croatian EN region when compared with group of patients from Bosnian EN region but the difference was not statistically significant ($p=0.79$ and $p=0.44$, respectively). However, we found statistically significant difference in Hemoglobin levels between to analyzed group of patients from different EN regions. In patients from Croatian EN group Hemoglobin levels were significantly lower than levels from Bosnian EN region patients, $p=0.0049$.

There was also no statistically significant difference in eGFR (MDRD), tumor grade, tumor invasiveness, presence of multiple tumors or family history between Croatian and Bosnian EN groups of patients ($p=0.50$, $p=0.83$, $p=0.84$, $p=0.93$, $p=0.15$, respectively).

In group of patients from Bosnian EN region ureteral tumors were more frequently observed than in Croatian group of patients, when compared with renal pelvis tu-

mors but the difference was not statistically significant ($p=0.258$).

3. DISCUSSION

Aim of this study was to investigate characteristics of patients from to relatively nearby situated EN regions and to compare our findings with other studies. Analyzed age and gender distribution between patients from EN and nonEN regions our results showed older age of patients with UTUC in group of patients from EN regions with female predominance in the same group, that was expected and these results are compatible with other similar studies (3, 4). Regarding tumor localization within upper urinary tract system, we found some differences in comparison with other studies. Most studies indicated renal pelvis/pyelon as a more frequent site of urothelial cancer in EN regions as well as in nonEN regions when compared with ureteral tumor site (12, 14, 15,).

In our study, when we analyzed patients from Croatian and Bosnian EN regions as one group, we found slightly more frequently tumors originating in renal pelvis than in ureter. However, when compared tumors sites between Croatian and Bosnian EN regions as to different groups we found urothelial cancer in renal pelvis more frequent in Croatian focus while in Bosnian EN region tumors were equally distributed in renal pelvis and ureter. We also found that in patients with urothelial tumors arising from ureter number of synchronous and/or metachronous tumors was significantly higher when compared with urothelial cancers arising from renal pelvis/pyelon. This finding suggests need for more frequent clinical surveillance of patients with tumors arising from ureter.

Results of our study are compatible with other studies of urothelial tumors connected with EN regarding depth of tumor invasion and tumor grade. In our study, urothelial tumors diagnosed in upper urinary tract were more frequently high grade with more frequently present tumor invasion classified as pT2-pT4. This finding is also present in studies from nonEN regions (4, 14, 15).

Comparing our results we found differences in CKD stages between our study and Taiwanese study conducted by Chen and co. In Chens study, almost 40% of patients were in CKD stage 0-2, while in our study only 16% of patients from EN region were in the same stage. In our study 29.5% of patients from EN regions were classified as CKD stage 5, while the same stage in study by Chen was found in 15,2 % of patients (12). Found differences could be result of different genetic predisposition, amount of ingested AA and/or time period of ingestion as well as other accompanying comorbidities.

Comparing CKD stages between ours investigated groups of patients we found that patients from nonEN regions were more frequently in CKD stage 0-2 (13/20) while CKD stage 5 was more frequently found in group of patients from EN region (13/15). Our findings could indicate more severe impairment of kidney function that is probably due to EN. Although, we did not found significant differences in CKD stage between patients from Croatian and Bosnian EN regions we did found more

elevated blood levels of urea and creatinine in group of patients from Croatian EN region and more decreased levels of Hemoglobin in the same group when compared with patients from Bosnian EN group. We did not find good explanation that could justify found differences in blood levels of urea, creatinine and hemoglobin between those to populations (especially because patients were operated in similar age, gender with similar type and tumor grade). The difference could be in different genetic predisposition that is still not very well investigated part in pathogenesis of EN.

4. CONCLUSION

Our data showed some differences in tumor localization between to investigated, relatively nearby situated, EN regions in Croatia and Bosnia. When compared tumors sites between Croatian and Bosnian EN regions as to different groups we found urothelial cancer in renal pelvis more frequent in Croatian focus while in Bosnian EN region tumors were equally distributed in renal pelvis and ureter. We also found that in patients with urothelial tumors arising from ureter number of synchronous and/or metachronous tumors was significantly higher when compared with urothelial cancers arising from renal pelvis/pyelon. We also found differences in urea and creatinine levels that were not statistically significant and statistically significant difference in hemoglobin levels between Croatian and Bosnian EN regions that should be further analyzed to give some reasonable explanation.

- Conflict of interest: none declared.

REFERENCES

- Grollman AP, Scarborough J, Jelakovic B. Aristolochic acid nephropathy: An environmental and iatrogenic disease. *Advances in Molecular Toxicology*, ed Fishbein JC. Elsevier, Amsterdam, 3rd Ed, 2009: 211-22.
- Đukanović L, Radovanović Z. Balkan endemic nephropathy. U: De Broe ME, Porter GA, Bennett WM, Verpooten GA, ur. *Clinical Nephrotoxins*, Dordrecht, The Netherlands: Kluwer Academic Publishers, 2003; 587-601.
- Belicza M, Demirović A, Tomić K, et al. Comparison of occurrence of upper urinary tract carcinomas in the region with endemic villages and non-endemic nephropathy region in Croatia. *Coll Antropol*. 2008; 32(4): 1204-7.
- Cukuranovic R, Ignjatovic I, Visnjic M, Velickovic LJ, Petrovic B, Potic M, Stefanovic V. Characteristics of upper urothelial carcinoma in an area of Balkan endemic nephropathy in south Serbia. A fifty-year retrospective study. *Tumori*. 2010; 96(5): 674-9.
- Vukelić M, Sostarić B, Belicza M. Pathomorphology of Balkan endemic nephropathy. *Food Chem Toxicol*. 1992; 30(3): 193-200.
- Jelaković B, Nikolić J, Radovanović Z. i sur. Consensus statement on screening, diagnosis, classification and treatment of endemic (Balkan) nephropathy *Nephrol Dial Transplant*. 2014; 29: 2020-7 .
- Ferluga D, Hvala A, Vizjak A. i sur. Renal function, protein excretion, and pathology of Balkan endemic nephropathy. III. Light and electron microscopic studies. *Kidney Int*. 1991; 40, Suppl 34: 57-67.
- Moriya M, Slade N, Brdar B, et al. TP53 mutational signature for aristolochic acid: An environmental carcinogen. *Int J Cancer*. 2011; 129: 1532-6.
- Jelakovic B, Karanopvić S, Vuković-Lela I, Miller F, Edwards K. et al. Aristolactam-DNA adducts are a biomarker of environmental exposure to aristolochic acid. *Kidney Int*. 2012; 81: 559-67.
- Jelaković B, Castells X, Tomić K, Ardin M, Karanović S, Zavadil J. Renal cell carcinomas of chronic kidney disease patients harbor the mutational signature of carcinogenic aristolochic acid. *Int J Cancer*. 2015; 136(12): 2967-72.
- Nortier JL, Martinez MC, Schmeiser HH, Arlt VM, Bieler CA, Petein M, Depierreux MF, De Pauw L, Abramowicz D, Verstraeten P, Vanherweghem JL. Urothelial carcinoma associated with the use of a Chinese herb (*Aristolochia fangchi*). *N Engl J Med*. 2000; 342(23): 1686-92.
- Chen CH, Dickman KG, Moriya M, et al. Aristolochic acid-associated urothelial cancer in Taiwan. *Proc Natl Acad Sci USA*. 2012; 109(21): 8241-6.
- Comperat E, Al-Ahmadie H. Epithelial tumours of the upper urinary tract. In: Moch H, Humphrey PA, Ulbright TM and Reuter VW, ed. *WHO Classification of Tumours of the Urinary system and Male genital Organs*, IARC Lyon, 2016: 131.
- Shariat SF, Favaretto RL, Gupta A, et al. Gender differences in radical nephroureterectomy for upper tract urothelial carcinoma. *World J Urol*. 2011; 29: 481-6.
- Langner C, Hutterer G, Chromecki T, Winkelmayer I, Rehak P, Zigeuner R. pT classification, grade, and vascular invasion as prognostic indicators in urothelial carcinoma of the upper urinary tract. *Mod Pathol*. 2006 Feb; 19(2): 272-9.