Cadmium in urothelial carcinoma of the bladder

Tomasz Golabek[1], Barbara Darewicz[1], Jacek Kudelski[1], Katarzyna Socha[2],
Renata Markiewicz-Zukowska[2], Piotr Chlosta[1], Krzysztof Okoń[3], Maria Borawska[2]

[1]Department of Urology, Jagiellonian University, *Medical College* Kracow, Poland
[2]Department of Bromatology of the Medical University of Bialystok, Bialystok, Poland
[3]Department of Clinical and Experimental Pathomorphology, Jagiellonian University Medical College, Krakow, Poland

The aim of this study was to examine the relationship between cadmium (Cd) and bladder cancer (urothelial carcinoma of the bladder). Cadmium concentrations in two 36-sample series of bladder cancer tissue and blood, from patients with the neoplasm, were matched with those of the control group. The amount of heavy metal in every tissue sample was determined using atomic absorption spectrometry. This was correlated with tumour stage. While the median cadmium concentration levels reached statistically lower values in the bladder cancer tissue, as compared with the non-cancer one (11.695 ng/g and 56.32 ng/g respectively, $p < 0.001$), the median Cd levels in the blood of the patients with this carcinoma showed no statistical difference when compared to those of the control group (8.237 µg/l and 7.556 µg/l respectively, $p = 0.121$). The median levels of cadmium in the bladder tissue, depending on the stage of the tumour, compared with the tissue without the neoplasm, observed the same relationship for both non-muscle invasive and muscle-invasive tumours ($p < 0.002$ and $p < 0.02$ respectively). This study has shown that patients with urothelial carcinoma of the bladder had lower tissue cadmium levels than people without tumour while no difference in the Cd blood levels between the two groups of patients under investigation was found.

Key words: bladder cancer, cadmium, blood tissue.

Introduction

Bladder cancer is the fourth most common cancer in men and the ninth most common malignancy in women. It accounts for 3.0% of all cancer deaths in the male population and 1.5% of all cancer deaths in females [1].

Epidemiological and experimental data indicate a significant connection between chemical compounds and serious disorders as well as the development and progression of several cancers including urinary tract tumours [2-9]. Many chemicals, including heavy metals and trace elements, have been shown to be carcinogens for bladder cancer [10-12]. Experimental studies have demonstrated that cadmium can induce both benign and malignant tumour formation at various sites including the urinary tract [13-16]. Similarly, two epidemiological analyses of people exposed to Cd have reported possible associations between cadmium and bladder urothelial carcinoma [17, 18]. Additionally, higher Cd levels in the urine of patients with this tumour have been reported, further suggesting its contribution to carcinogenesis [19]. Thus far, however, there has been no demonstrable direct evidence for the association of cadmium with human urothelial carcinoma of the bladder.

In our study, we measured the concentration of cadmium in the tissue of the bladder tumour and compared this to the content in non-cancerous tissue. In addition, the concentration of Cd in the blood of patients with bladder tumours was compared to control samples from the patients with non-neoplasm or heavy metals-related diseases of the urinary system.

Finally, we evaluated the resultant levels depending on the stage of the tumour.

Material and methods

This study was approved by the Medical Ethics Committee for Human Studies of the Medical University in Bialystok, Poland, and all the procedures were carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study was conducted on 36 patients with histologically proven urothelial carcinoma of the bladder, without lymph node involvement or distant metastases. The patients included 30 men aged between 41 and 88 (on average 68.5 years old) and 6 women aged between 52 and 78 (on average 67 years old). Non-muscle invasive cancers (Tis – Ta – T1) occurred in 22 cases and muscle-invasive tumours (T2 – T3 – T4) in 14 patients. The grade of histological malignancy G1, G2 and G3 was found, respectively, in 10, 13, and 10 patients, whereas it was not possible to establish with certainty the actual grade of malignancy in 3 patients. Five patients had primary non-muscle invasive bladder cancer, whereas recurrent urothelial carcinoma of the bladder was diagnosed in 31 individuals (17 people with non-muscle invasive bladder cancer with disease duration of between 6 months and 4 years, and 14 cases with muscle invasive bladder cancer with primary non-muscle invasive bladder tumour diagnosed between 6 months and 2 years earlier). All individuals had normal renal and liver function tests and abdominal ultrasound did not reveal any abnormalities. Blood tests and urinalysis were within the normal range limits. None of our patients had renal, pancreatic or lung malignancy. Moreover, none of the men under investigation had testicular or prostate cancer.

The tumours were removed by transurethral resection (TURBT) in 29 patients and by cystectomy in 7 patients. Just after the bladder or the tumour itself had been removed, samples of 1 g of cancer tissue were taken and stored after being snap frozen in liquid nitrogen. Before bladder or tumour removal, three-millilitre samples of venous blood were collected from the basilic vein using Vacutainer vacuum sets.

The control material consisted of 15 one-gram samples of bladder tissue taken from sex- and age-matched individuals, who had died from trauma (n = 15). Samples were taken during autopsy from 12 male cadavers aged 54-77 (on average 64 years old) and 3 females aged 57-76 (on average 65.5 years old).

Next, 15 three-millilitre samples of venous blood from the corresponding sex-and-age-matched patients, who were in fasting states with non-neoplasm or heavy metal-related diseases of the urinary system (1 woman with benign pelvic-ureteric junction obstruction, 3 with stress incontinence, 3 men with scrotal injury, 3 with renal injury, 2 with benign ure-

thral stricture and 3 with hydrocele), were collected in Vacutainer vacuum sets and used in the control group. There were 11 samples taken from male patients aged 43-86 (on average 67 years old), and 4 from females aged 52-78 (on average 67.5 years old). There was no difference in socioeconomic status between the cases and controls. Neither group had occupational exposure to cadmium.

Before analysis, the samples were mineralized in concentrated nitric acid (V) in a UniClever closed microwave system manufactured by Plazmatronika. The mineralization products were quantitatively transferred into polypropylene scintillation vessels. Cadmium was quantitatively determined in the blood samples that had undergone deproteinisation with 1 mol/l nitric acid (V) and 1% Triton X-100 added as a surface-active agent. The levels of investigated heavy metal concentration were analysed by the atomic absorption spectrometry technique with Zeeman background correction on a Hitachi Z-5000 spectrometer. The Cd content was calculated using readings on a standardization curve, formed by recording differences in absorbance and element concentration. Cadmium standard solution (1000 mg/l) traceable to SRM from NIST in 0.5 mol/l HNO_3 (Merck, Germany) was used to prepare working solutions for the calibration curve. The accuracy of the cadmium determination method was verified using the following certified standard materials: Seronorm 404108 for the whole blood and BCR-184 for bovine muscle. Accuracy (i.e. % of error) and coefficient of variation were calculated for the certified standards under investigation. The accuracy and precision coefficient of variation was 1.9% and 3.65% for Seronorm 404108 and 0.76% and 3.72% for BCR-184, respectively. The detection limit was 0.053 $\mu g/l$. The samples were evaluated in the Department of Bromatology of the Medical University of Bialystok, which is involved in a program of intra-laboratory comparative analyses of elements organized by the Institute of Nuclear Chemistry and Technology, and the National Institute of Public Health.

Since data in the studied group were not distributed in a Gaussian manner, the Mann-Whitney U test was used. A p value of < 0.05 was considered statistically significant. The Statistical Package for the Social Sciences (SPSS) was used for all statistical analyses.

Results

Median values of cadmium, determined in the bladder cancer tissue and blood of the patients with urothelial carcinoma of the bladder and the control group, are shown in Table I. Figures 1, 2 present Cd bladder tissue and blood concentration distribution of patients with bladder cancer and controls.

Table I. Bladder tissue and blood cadmium concentrations in patients with bladder cancer versus controls

Group		N	Q1	Median	Q3	P
Cd blood, μg/l	bladder cancer cases	36	7.102	8.237	10.215	0.121
	controls	15	5.640	7.556	8.388	
Cd bladder tissue, ng/g	bladder cancer cases	36	4.858	11.695	36.350	0.001*
	controls	15	51.610	56.320	71.990	

*Cd – cadmium, n – number of samples, Q1 – lower quartile, Q3 – upper quartile; *statistically significant*

Fig. 1. Cadmium (Cd) bladder tissue concentration distribution of patients with bladder cancer and control group

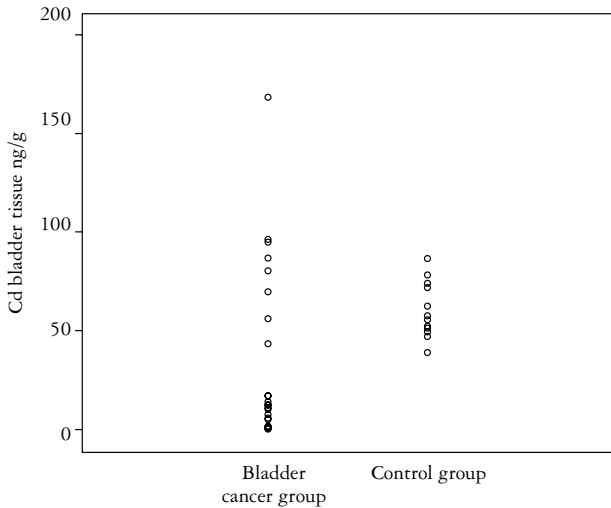**Fig. 2.** Cadmium (Cd) blood concentration distribution of patients with bladder cancer and control group

The median value of cadmium concentration in the bladder cancer tissue and in the non-cancer one was 11.695 ng/g and 56.32 ng/g, respectively. The difference in Cd concentration in the tissues was statistically significant ($p < 0.001$). There was no significant difference in the blood Cd levels between the groups (8.237 μg/l and 7.556 μg/l, respectively, $p = 0.121$). There was no statistical difference in the median value (quartile 1; quartile 3) of cadmium concentration in the bladder cancer tissue and in the non-cancer one found in men and women, when analysed by sex [12.3 ng/g (5.7; 46.7) vs. 14.1 ng/g (0.1; 20.6), $p = 0.103$, and 56.3 ng/g (51.6; 74.4) vs. 59.0 ng/g (51.0; 69.6), $p = 0.997$, respectively]. Similarly, no statistical difference in the blood Cd concentration between men and women with and without bladder cancer was observed [8.0 μg/l (7.1; 9.9) vs. 8.6 μg/l
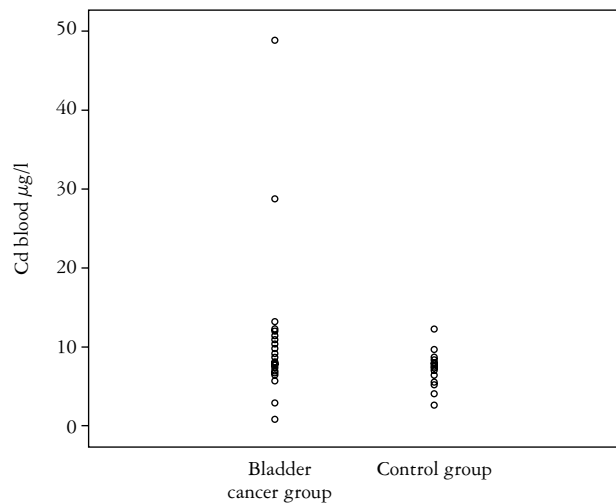
(6.5; 16.2), $p = 0.756$, and 7.4 μg/l (5.8; 8.3) vs. 7.9 μg/l (5.2; 8.7), $p = 0.734$, respectively).

Moreover, tissue and blood cadmium levels were calculated for the two tumour stage groups (one with non-muscle invasive and the other with muscle invasive cancers). When processed by the non-parametric Mann-Whitney U test, the results showed that the median levels of Cd in the bladder tissue, depending on the stage of the tumour, were significantly lower for both non-muscle invasive and muscle-invasive tumours, as compared with the tissue without the neoplasm ($p < 0.002$ and $p < 0.02$ respectively). There was no marked difference in the cadmium blood levels between people with urothelial carcinoma of the bladder and the controls, when analysed by the two tumour stage groups (see Table II).

Table II. Bladder tissue and blood cadmium concentrations in patients with non-muscle-invasive vs. muscle-invasive bladder cancer

Group		N	Q1	Median	Q3	P (vs. control)
Cd blood, μg/l	Tis + Ta + T1	22	7.107	8.457	10.512	0.112
	T2 + T3 + T4	14	6.994	7.816	9.567	0.553
Cd bladder tissue, ng/g	Tis + Ta + T1	22	3.930	10.610	23.913	0.002*
	T2 + T3 + T4	14	5.073	12.495	49.478	0.02*

statistically significant, n – number of samples, Q1 – lower quartile, Q3 – upper quartile

Discussion

In this study we observed lower tissue cadmium levels in the patients with urothelial carcinoma of the bladder and no difference in the Cd blood levels between the two groups of patients under investigation.

Two published case-control studies have suggested an association between exposure to cadmium and the risk of urinary bladder cancer, as tumours were more frequently observed in people who had occupational exposure to Cd [17, 18]. These observations could theoretically be related to the fact that the urinary system is involved in the cadmium removal process out of the human organism [20]. Thus, whenever this element is present in urine, it may directly act on the urothelium, especially inside the bladder, which functions as a temporary reservoir for urine and as such is exposed to cadmium action for a longer time. The above theory has been supported by a study that showed increased Cd levels in the urine of people with bladder cancer as compared with healthy individuals [19]. Moreover, higher cadmium blood levels in men with urothelial bladder cancer have been observed in one study [21]. Furthermore, an experimental study demonstrated that cadmium is able to malignantly transform human urothelial cells [22]. Additionally, it has been shown that tumour heterotransplants produced by the urothelial cell line UROtsa malignantly transformed by Cd had epithelial features consistent with those of the typical urothelial carcinoma of the bladder [23]. Moreover, one study observed Kindlin-2 expression (a class of focal adhesion proteins implicated in integrin activation) in urothelium of archival human bladder cancer specimens and no such expression in normal urothelium [24].

The mechanisms involved in cadmium-induced carcinogenesis are, however, unclear. They likely include free radical-mediated genetic damage, inhibition of DNA repair, and stimulation of cell proliferation or apoptosis [15, 25, 26].

Our study does not support the above epidemiological data. We can only speculate why our findings do not validate previous studies. To date, evidence for a link between Cd and bladder cancer was found only in two case-control studies of people with bladder tumour who had occupational exposure to cadmium compounds [17, 18]. One of those studies reported only a weak association between the heavy metal and tumour, whereas the other found a significant increase in urothelial carcinoma of the bladder in men and women who had high exposure to Cd. Epidemiological studies, however, do not prove causality. Moreover, these types of studies, in particular case-control or retrospective ones, are often associated with serious selection bias [27].

One study has shown increased cadmium levels in the urine of subjects with bladder cancer, as compared with healthy individuals [19]. However, this study could be underpowered as the sample size was small (6 of 10 patients with bladder cancer had elevated urine Cd levels). Of added interest is the evidence suggesting that urinary cadmium levels increase with age, which is also an important risk factor for bladder cancer [28].

One study found significantly higher cadmium blood levels in people with bladder cancer than in controls [21]. This report, however, was limited to men. Moreover, patients with bladder cancer were significantly older than those without malignant disease. Both male gender and age have been identified as risk factors for urothelial carcinoma of the bladder [29].

It is also possible that neoplastic bladder tissue for some unclear reason does not accumulate cadmium. This would explain the statistically lower levels of Cd which we observed in the bladder tissue of people with urothelial carcinoma compared to those in men and women without the tumour. However, in such cases we would expect lower levels of the heavy metal in patients with muscle-invasive tumours than in subjects with non-muscle-invasive cancers.

In our study we found no difference in the cadmium blood levels between the two groups of patients under investigation. This is an important observation, as blood Cd reflects current exposure rather than whole-body burdens, whereas urinary cadmium reflects total burden [20]. On the other hand, Cd accumulated in the body influences the blood cadmium concentration as it does not decrease to the pre-exposure levels after exposure ceases. Thus, Cd in blood may serve as a good estimate of the accumulated body burden of heavy metal [30]. Therefore, the absence of difference in cadmium blood levels between subjects with bladder cancer and controls provides indirect evidence that both groups of people had similar exposure to Cd. North-Eastern Poland is regarded as un-unpolluted region and has been found to have low concentrations of Cd in soil [31]. This is mainly due to a lack of anthropogenic sources of heavy metals such as industrial waste, mining or smelting activity. Automobile exhausts, municipal effluents and smoking are the major sources of cadmium in this area. As none of our subjects including controls had occupational exposure to Cd, no acute increase in blood cadmium levels among patients with bladder cancer and controls was observed. It has to be noted, however, that a very high tissue cadmium level (168.37 ng/g) was found in one patient with bladder cancer. This 67-year-old man also has a high blood Cd concentration (48.88 μg/l). Although he had no occupational exposure to cadmium, he was living for more than 25 years in the lower Silesian region of Poland prior to moving to the North-Eastern part of the country.

The lower Silesian region has been known for a high concentration of cadmium in soil [32].

In summary, this study showed that patients with urothelial carcinoma of the bladder had lower tissue cadmium levels than people without tumour while no difference in the Cd blood levels between the two groups of patients under investigation was found. Further studies are required to assess the biological significance of these parameters. Moreover, large epidemiological prospective studies, collecting information on occupational, residual and environmental exposure to cadmium compounds and their possible association with bladder cancer, are needed.

Authors declare no conflict of interest.

References

1. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. CA Cancer J Clin 2005; 55: 10-30.
2. U.S. Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. Toxicological profile for lead. Atlanta, GA 1999; 196-205.
3. Poniedziałek B, Rzymski P, Wiktorowicz K. In vitro effect of cadmium on the function of human lymphocytes and neutrophils. Centr Eur J Immunol 2012; 37: 110-113.
4. Hartwig A. Role of DNA repair inhibition in lead-and cadmium-induced genotoxicity: a review. Environ Health Perspect 1994; 102 Suppl 3: 45-50.
5. Jabłonowski Z, Reszka E, Gromadzińska J, et al. Hypermethylation of p16 and DAPK promoter gene regions in patients with non-invasive urinary bladder cancer. Arch Med Sci 2011; 7: 512-516.
6. Kiszka K, Rudnicka-Sosin L, Tomaszewska R, et al. Morphological characteristics of potentially malignant pulmonary nodules in high-risk male smokers detected in lung cancer screening trial in Cracow, Poland. Pol J Pathol 2013; 64: 114-120.
7. Marszałek A, Szylberg L, Wiśniewska E, Janiczek M. Impact of COX-2, IL-1β, TNF-α, IL-4 and IL-10 on the process of carcinogenesis in the large bowel. Pol J Pathol 2012; 63: 221-227.
8. Dąbroś W, Adamczyk A, Ciurkot K, Kordowiak AM. Vanadium compounds affect growth and morphology of human rhabdomyosarcoma cell line. Pol J Pathol 2011; 62: 262-268.
9. Romanowicz-Makowska H, Forma E, Bryś M, et al. Concentration of cadmium, nickel and aluminium in female breast cancer. Pol J Pathol 2011; 62: 257-261.
10. Morrison AS, Cole P. Epidemiology of bladder cancer. Urol Clin North Am 1976; 3: 13-29.
11. Golabek T, Darewicz B, Borawska M, et al. Lead concentration in the bladder tissue and blood of patients with bladder cancer. Scand J Urol Nephrol 2009; 25: 1-4.
12. Golabek T, Darewicz B, Borawska M, et al. Copper, zinc, and Cu/Zn ratio in transitional cell carcinoma of the bladder. Urol Int 2012; 89: 342-347.
13. Achanzar WE, Diwan BA, Liu J, et al. Cadmium-induced malignant transformation of human prostate epithelial cells. Cancer Res 2001; 61: 455-458.
14. Takenaka S, Oldiges H, König H, et al. Carcinogenicity of cadmium chloride aerosols in W rats. J Natl Cancer Inst 1983; 70: 367-373.
15. Verougstraete V, Lison D, Hotz P. Cadmium, lung and prostate cancer: a systematic review of recent epidemiological data. J Toxicol Environ Health B Crit Rev 2003; 6: 227-255.
16. Klatka J, Remer M, Dobrowolski R, et al. The content of cadmium, cobalt and nickel in laryngeal carcinoma. Arch Med Sci 2011; 7: 517-522.
17. Siemiatycki J, Dewar R, Nadon L, Gérin M. Occupational risk factors for bladder cancer: results from a case-control study in Montreal, Quebec, Canada. Am J Epidemiol 1994; 140: 1061-1080.
18. Kellen E, Zeegers MP, Hond ED, Buntinx F. Blood cadmium may be associated with bladder carcinogenesis: the Belgian case-control study on bladder cancer. Cancer Detect Prev 2007; 31: 77-82.
19. Darewicz G, Malczyk E, Darewicz J. Investigations of urinary cadmium content in patients with urinary bladder carcinoma. Int Urol Nephrol 1998; 30: 137-139.
20. U.S. Agency for Toxic Substances and Disease Registry. A Toxicological Profile for Cadmium. U.S. Department of Health and Human Services, Atlanta, GA 2012; 4-7.
21. Feki-Tounsi M, Olmedo P, Gil F, et al. Cadmium in blood of Tunisian men and risk of bladder cancer: interactions with arsenic exposure and smoking. Environ Sci Pollut Res Int 2013; 20: 7204-7213.
22. Somji S, Zhou XD, Garrett SH, et al. Urothelial cells malignantly transformed by exposure to cadmium (Cd(+2)) and arsenite (As(+3)) have increased resistance to Cd(+2) and As(+3)-induced cell death. Toxicol Sci 2006; 94: 293-301.
23. Sens DA, Park S, Gurel V, et al. Inorganic cadmium- and arsenite-induced malignant transformation of human bladder urothelial cells. Toxicol Sci 2004; 79: 56-63.
24. Talaat S, Somji S, Toni C, et al. Kindlin-2 expression in arsenite- and cadmium-transformed bladder cancer cell lines and in archival specimens of human bladder cancer. Urology 2011; 77: 1507.e1-7.
25. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003; 192: 95-117.
26. Bertin G, Averbeck D. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie 2006; 88: 1549-1559.
27. Mezei G, Kheifets L. Selection bias and its implications for case-control studies: a case study of magnetic field exposure and childhood leukaemia. Int J Epidemiol 2006; 35: 397-406.
28. Kowal NE, Johnson DE, Kraemer DF, Pahren HR. Normal levels of cadmium in diet, urine, blood, and tissues of inhabitants of the United States. J Toxicol Environ Health 1979; 5: 995-1014.
29. Babjuk M, Burger M, Zigeuner R, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol 2013; 64: 639-653.
30. Alfvén T, Järup L, Elinder CG. Cadmium and lead in blood in relation to low bone mineral density and tubular proteinuria. Environ Health Perspect 2002; 110: 699-702.
31. Biernacka E, Małuszyński M. The content of cadmium, lead and selenium in soils from selected sites in Poland. Polish J Environ Stud 2006; 15: 7-9.
32. Bojakowska I. Cadmium in the mineral sources of Poland and its potential emission in the environment. Ochrona Srodowiska Zasobow Naturalnych 2009; 40: 22-30.

Address for correspondence

Tomasz Golabek
Department of Urology
Collegium Medicum of the Jagiellonian University
Kracow, Poland
e-mail: elementare@op.pl