Research Article

Uroplakin IIIα Is a Marker in Bladder Cancer but Seems Not to Reflect Chemical Carcinogenesis

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Background. Uroplakins are glycoproteins investigated as potential markers of urothelial carcinoma. However, their role in chemical carcinogenesis is uncertain. In this study the diagnostic value of plasma and urine uroplakin IIIα (UPIIIα) levels in bladder cancer (BC) was investigated, particularly in the aspect of environmental exposure to chemical carcinogens, measured by DNA damage and detoxification ability in the BC smoking group. The correlation between uroplakin, 8-OHdG, and GSTπ was investigated.

Material and Methods. This study included 61 BC patients and 33 healthy controls. UPIIIα, 8-OHdG, and GSTπ levels were estimated by the immunoenzymatic method (ELISA).

Results. UPIIIα levels were elevated in BC patients in plasma (p ≤ 0.001) and in urine (p ≤ 0.001), as were 8-OHdG and GSTπ levels in urine. Moreover, the 8-OHdG level was higher in invasive or high grade tumors. A positive correlation between UPIIIα/GSTπ and 8-OHdG/GSTπ was observed, but no UPIIIα/8-OHdG correlation was noted.

Conclusion. The study showed the diagnostic value of urine and plasma UPIIIα in BC (good sensitivity, specificity, and predictive value). The lack of UPIIIα correlation with 8-OHdG and smoking suggests that UPIIIα does not reflect the environmental exposure. The increased levels of 8-OHdG and GSTπ in the invasive tumor stage indicate their value in BC monitoring.

1. Background

The most common urinary bladder neoplasm is carcinoma of the urothelium. Urothelium is a highly specialized type of transitional epithelium. It consists of a few, mainly three to five, cell layers. Characteristic for this type of epithelium is high elasticity and low permeability. It is a barrier between urine and human tissue [1]. The main components of the external layer of urothelium are glycoproteins, uroplakins (UP). A few types of uroplakins are known (UPIα, UPIβ, UPIIIα, and UPIIIβ) [2]. They form advanced spatial structures described as asymmetric unit membrane (AUM), due to the fact of its appearance in transmission electron microscopy (TEM), which results from the presence of uroplakin in the outer leaflet of the membrane [3]. Approximately 90% of the urothelium surface is covered by AUM [4]. One of the roles of UP is improving urothelium tightness, which decreases the permeability to ions and substances dissolved in urine [5]. Some UP such as UPIα take part in initiation of infections by binding with the protein FimH of fimbria type I in Escherichia coli. This mechanism is crucial in bacterial adhesion to the host cellular membrane [6]. The destruction of urothelium causes release of proteins into blood and urine [7].

Carcinoma of the bladder urothelium represents approximately 90% of all bladder cancers. Worldwide, bladder cancer (BC) is the seventh most common cancer among men and the seventeenth in women. About 70-80% of malignancies are superficial and are treated by surgery. BC recurrence is observed in 50-70% of patients, and development to invasive disease can be observed in approximately 5-20% of patients [8]. In diagnosis of BC radiological methods (ultrasonography, computed tomography), cytological examination of urine and invasive procedures (cystoscopy, transurethral resection of bladder tumor (TURB-T)) are used. Some laboratory tests could also be helpful in BC diagnosis, e.g., BTA (bladder tumor antigen), which detects H-protein of the complement complex in urine of patients with BC. The ImmunoCytest uses three types of monoclonal antibodies...
against mucin antigens and carcinoembryonic antigen (CEA) [9]. Other tests measure the level of nuclear matrix protein 22 (NMP22) or chromosomal aberrations (UroVysion test). The urological associations (American Urological Association, European Association of Urology) are skeptical about protein markers of bladder cancer, but the US Food and Drug Administration has approved some of them. Some reports have shown the possible usefulness of these markers [10, 11]. Even if cystoscopy cannot be replaced by another examination, there are some proteins, such as NMP22, which can substitute urine cytology [10]. Also the studies on new markers of BC among uroplakins are in progress [12–14].

BC is often classified as an environment-related neoplasm. The main risk factor of BC is tobacco smoking [12, 15]. Exposure to other carcinogens such as aromatic amines, polycyclic aromatic hydrocarbons, or arsenic could also lead to BC growth [12]. It has been established that carcinoenic xenobiotics conjugated with endogenic substances in the body are dissolved and excreted in urine. In the urinary bladder these complexes undergo partial hydrolysis which produces carcinogenic carboxydrates [16]. This process develops in people exposed to aromatic amines and could lead to BC [17]. Another study also showed higher BC morbidity in the population exposed to arsenic compounds in drinking water [18]. Another example of the influence of life style on BC development is smoking. Most BC patients are present or former tobacco smokers. Smoking is established as the main BC risk factor [12]. The final effect of exposure to carcinoenic xenobiotics depends on detoxification abilities in the body. Due to the listed facts it seems useful to search for new BC diagnostic markers, which could reflect the environmental impact and human detoxification parameters.

The aim of our study was to evaluate the diagnostic value of UPIIIa in plasma and urine of patients with BC, particularly in the aspect of environmental exposure to chemical carcinogens, measured by 8-OHdG level in the BC smoking group and the correlation with detoxification ability evaluated by the glutathione transference \( \pi \) (GST\( \pi \)) isoenzyme level. The choice of noninvasive markers which reflect the environmental risk of carcinogens could improve the early diagnostics of BC. It is interesting to evaluate whether UPIIIa could be one of these markers.

Uroplakin IIIa is an isoform of UPIII isolated from AUM [19]. A recent study demonstrated a higher level of UPIIIa in urine of BC patients [11]. It was the first report which showed the possible usefulness of UPIIIa as a urine marker of BC. Other data have confirmed higher UPIII (not UPIIIa) concentration in serum of patients with BC [7]. According to our knowledge no research on UPIIIa in plasma has been reported. Furthermore, none of the published studies evaluated simultaneously UPIIIa in urine and blood, and none established the relation of UPIIIa to xenobiotic exposure and detoxification markers.

One marker which is reported to reflect the exposure to genotoxic xenobiotics is 8-hydroxy-2'-deoxyguanosine (8-OHdG) [20, 21]. The level of 8-OHdG in urine is reported as more specific for DNA damage than its level in blood. 8-OHdG is the most common product of oxidative DNA damage and is proportional to DNA damage. Many carcino-gens disrupt oxidative balance by excessive production of reactive oxygen species (ROS), which plays an important role in pathogenesis of neoplasms. Some studies have shown that urine level of 8-OHdG could be an important marker of genetic damage due to xenobiotic exposure [22].

Glutathione transferase (GST) is an important detoxification enzyme. It plays a basic role in xenobiotics transformation, especially in conversion of aromatic hydrocarbons into mercapturic acid. GST\( \pi \) (an isoform of GST) is particularly involved in carcinogenesis. Our former studies have shown a statistically significantly higher urine level of glutathione S-transference \( \pi \) (GST\( \pi \)) in BC patients [14, 23]. Moreover a twofold increase of GST\( \pi \) was observed in urine of BC patients in another study [24].

To achieve the aim of the study, we evaluated the levels of UPIIIa, 8-OHdG and GST\( \pi \) in BC patients in comparison to healthy controls (C) and also analyzed the differences in parameters between BC smokers and BC nonsmokers (environmental exposure to chemical carcinogens). The correlation between UPIIIa and listed markers was investigated by a mathematical method and also using a special test for diagnostic value. In order to perform the preliminary study of the prognostic value of listed markers, all results were related to BC stage (NMIBC and MIBC) and grade (low grade (LG) and high grade (HG)).

2. Materials and Methods

The study group is comprised of 61 BC patients of the Urology and Oncological Urology Department (Wroclaw Medical University), hospitalized between September 2014 and July 2015. The group consisted of 51 men (84%) and 10 women (16%), medium age 66 (41–88). All patients were informed about the study, participation was voluntary, and all signed written informed consent. The control group (C) collected by Biobank in Wroclaw is comprised of 33 healthy persons, 28 men (85%) and 5 women (15%). The percentage of smokers in the C group was the same as in the BC group. No statistically significant differences in characteristics between groups BC and C were found (Table 1).

Histopathological examination of tissue taken by TURB-T or radical cystectomy was performed in the Department of Pathomorphology and Oncological Cytology (Wroclaw Medical University). Based on histopathological results, patients were divided into subgroups, according to tumor stage T (TNM (Tumor Nodules Metastases), 2002) and grade (low grade/high grade, WHO/International Society of Urological Pathology, ISUP System 2004). The subgroups were Ta (n=28), T1 (n=18), T2 (n=4), T3 (n=5), T1S (n=6); low grade (LG; n=29) (53%); high grade (HG) in 32 (47%). Also subgroups of NMIBC (nonmuscle invasive bladder cancer: Ta+T1; n=46) and MIBC (muscle invasive bladder cancer: T2+T3+T1S; n=15) were selected (Table 1).

The material for laboratory tests was human blood and urine. In the BC group the material was obtained one day before surgical and pharmacological treatment. In the morning urine samples were collected in polystyrene containers (Aptaca, Italy) and then centrifuged for 10 minutes (1438xg at
4°C), and the obtained supernatant was removed to Eppendorf tubes and stored at −80°C for further investigation.

Blood samples were collected into plastic tubes (BD Vacutainer, Na, citrate buffer, USA), with an anticoagulant. The tubes were centrifuged at 1438xg for at least 10 min at 4°C. The supernatant (plasma) was frozen at −80°C until being analyzed.

Our primary UPIIIa test showed its very low level in serum (in picograms). Due to this, tests were performed in plasma. The level of UPIIIa was measured in nanograms per plasma milliliter.

UPIIIa level was measured in urine and plasma by an immunoenzymatic (ELISA) Enzyme-Linked Immunosorbent Assay Kit, USCN Life Science Inc., PRC (designed by Cloud-Clone Corp. USA). GST-π was assessed in urine by Human Pi GST ELA-EKF Diagnostic and 8-OHdG was measured by Check Elisa JaJCA, Japan Institute for the Control of Aging) and GSTπ activity (Human Pi GST ELA-EKF Diagnostic, Ireland) were detected in urine using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions in the listed test.

### 3. Measurement of Markers

#### 3.1. UPIIIa

UPIIIa level Enzyme-Linked Immunosorbent Assay Kit, USCN Life Science Inc., was detected according to the manufacturer’s instruction.

The microplate was precoated with an antibody specific to UPIIIa. Standards or samples (100 μl) were added to the appropriate microplate wells with a biotin-conjugated antibody specific to UPIIIa. Next, avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated (2 h, 37°C). Next TMB (3,3′,5,5′-tetramethylbenzidine) substrate solution was added, which caused that only those wells that contained UPIIIa, biotin-conjugated antibody, and enzyme-conjugated avidin displayed a change in color. The enzyme-substrate reaction was terminated by the addition of 0.1 M sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of UPIIIa in the samples was then determined by comparing the absorption of the samples to the standard curve.

#### 3.2. 8-OHdG and GSTπ

8-OHdG levels (Check Elisa JaJCA, Japan Institute for the Control of Aging) and GSTπ activity (Human Pi GST ELA-EKF Diagnostic, Ireland) were detected using the enzyme-linked immunosorbent assay (ELISA).

### 4. Statistical Analysis

Statistical analysis was conducted with Statistica PL software (version 12.1). The normality of distribution was checked with the Kolomogorov-Smirnov test and the Lilliefors test. Student’s t-test for parametric data and the Mann-Whitney U test for nonparametric data were used for variables. The values of p<0.05 were considered statistically significant. The associations between continuous variables were analyzed by Spearman’s test for nonparametric data and Pearson’s test for parametric data. Also, sensitivity, specificity, accuracy (ACC) of method, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR−), and odds ratio (OR) were determined.

The receiver operating characteristic (ROC) curves were estimated. The area under the curve (AUC) and best cut-off point were calculated employing ROC analysis which evaluated the relation between sensitivity and specificity of markers.

The study was approved by the Ethics Committee of Wroclaw Medical University (KB-292/2-16). The participation was voluntary, respectful of human rights.

### 5. Results

#### 5.1. Uroplakin IIIa

The mean urine level of UPIIIa was 2.44 ng/mg of creatinine (cr.) in the group of patients with BC and 1.02 ng/mg cr. in the control group. The difference was statistically significant (p<0.001) (Table 2). The results of UPIIIa measurement without calculating the creatinine

### Table 1: Population characteristics.

| Population characteristic | Patients (%) | Controls (%) |
|---------------------------|--------------|--------------|
| No.                       | 61           | 33           |
| Male                      | 31 (84)      | 28 (85)      |
| Female                    | 10 (16)      | 5 (15)       |
| Age, years                | 66 (41-88)   | 65 (54-81)   |
| Smokers                   | 47 (77)      | 24 (73)      |
| Nonsmokers                | 14 (23)      | 9 (27)       |
| Clinical staging          |              |              |
| Ta                        | 28 (46)      | -            |
| T1                        | 18 (30)      | -            |
| T2                        | 4 (6)        | -            |
| T3                        | 5 (8)        | -            |
| TIS                       | 6 (10)       | -            |
| Clinical grading          |              |              |
| LG                        | 29 (53)      | -            |
| HG                        | 32 (47)      | -            |
| Clinical subgroups        |              |              |
| NMIBC                     | 46 (75)      | -            |
| MIBC                      | 15 (25)      | -            |

Ta, T1, T2, and TIS: subgroups of BC according to tumor stage T (TNM); LG: low grade; HG: high grade; NMIBC: nonmuscle invasive bladder cancer; MIBC: muscle invasive bladder cancer. TNM: Tumor Nodules Metastases. 2002.
| Group       | UPIIIa in urine [ng/mg cr.] | UPIIIa in plasma [ng/ml] | p-value in urine | p-value in plasma |
|-------------|-----------------------------|--------------------------|------------------|------------------|
|             | Mean | SD  | Me  | Mean | SD  | Me  |                  |                  |
| C           | 1.02 | 0.54| 0.98 | 0.58 | 0.31| 0.65 | **p<0.001**      | **p<0.001**      |
| BC          | 2.44 | 1.41| 2.11 | 1.47 | 0.76| 1.32 | BC vs C          | BC vs C          |
| NMIBC       | 2.38 | 1.36| 2.10 | 1.37 | 0.56| 1.25 | **p=0.5978**     | **p=0.4312**     |
| MIBC        | 2.64 | 1.59| 2.23 | 1.77 | 1.15| 1.32 | NMIBC vs MIBC    | NMIBC vs MIBC    |
| LG          | 2.44 | 1.37| 2.11 | 1.42 | 0.62| 1.32 | LG vs C          | LG vs C          |
| HG          | 2.44 | 1.48| 2.21 | 1.52 | 0.90| 1.22 | LG vs HG         | LG vs HG         |
| Non-smoking BC | 2.02 | 1.11| 2.02 | 0.96 | 0.61| 0.96 | **p> 0.05**      | **p> 0.05**      |
| Smoking BC  | 2.11 | 1.48| 2.11 | 1.32 | 0.81| 1.32 | non-smoking BC vs smoking BC | non-smoking BC vs smoking BC |
| Non-smoking C | 1.28 | 0.41| 1.17 | 0.68 | 0.33| 0.69 | **p>0.05**       | **p>0.05**       |
| Smoking C   | 0.94 | 0.56| 0.91 | 0.553| 0.30| 0.65 | non-smoking C vs smoking C | non-smoking C vs smoking C |

UPIIIa: uroplakin IIa; C: control group; BC: patient group; NMIBC: nonmuscle invasive BC; MIBC: muscle invasive BC; LG: low grade; HG: high grade; SD: standard deviation; p: statistically significant difference; Me: median
level were also higher than in the control group C. The mean plasma level of UPIIIa in BC patients (1.47 ng/ml) was higher than in the control group C (0.58 ng/ml) (p \leq 0.001). There was no statistically significant difference of UPIIIa level in either plasma or urine between nonmuscle invasive bladder cancer (NMIBC) and invasive bladder cancer (MIBC), but in both groups the UPIIIa level was higher than in the control C (p \leq 0.001). Similar results were obtained in LG and HG groups in BC. No significant difference in UPIIIa level in urine or plasma between low grade and high grade BC tumor was noted (Table 2). A significant difference between LG/control group and also HG/control was observed (Table 2). The comparison of HG/control and LG/control showed statistically significant differences (p \leq 0.001), which is interesting and indicates additional value of UPIIIa in LG diagnosis. No significant difference between UPIIIa level in urine or plasma between BC smokers and nonsmoking patients was observed, in either urine or plasma (Table 2). Only between BC smokers and healthy controls was the difference significant in both urine and plasma (p = 0.05). The examination of control group C showed a lack of difference between UPIIIa level in C smokers and C nonsmokers (Table 2). It confirms that smoking does not influence UPIIIa level in healthy people.

The receiver operating characteristic (ROC) analysis was performed to estimate the diagnostic and prognostic value of UPIIIa. It was found that the measurement of UPIIIa level has a 69% sensitivity and 89% specificity in urine and a little higher in plasma (79%, 91%). The area under the curve (AUC) was calculated as 0.78 in urine (Figure 1) and 0.85 in plasma (Figure 2). It indicates good diagnostic value of UPIIIa in plasma (over 0.8).

The PPV was a little higher in plasma (0.94) but also high in urine (0.913) (Table 3). The results show that the level of UPIIIa measured by immunoenzymatic methods has good diagnostic value in bladder cancer. Also the prognostic value (PPV) seems to be high, but there are no significant differences in UPIIIa level according to grade and stage of BC.

5.2. 8-OHdG. The mean 8-OHdG level was 19.06 ng/mg cr. in the BC group, and it was significantly higher than in control group C (11.84 ng/mg cr.; p = 0.003) (Table 4). Also a significant difference was observed between NMIBC (16.96 ng/ml cr.) and MIBC (25.48 ng/mg cr. p = 0.013). Likewise 8-OHdG level was higher in the HG group (24.15 ng/mg cr.) than in the LG group (14.44 ng/mg cr.) p = 0.001 (Table 4). Mean level of 8-OHdG in the group of smoking patients was 19.22 ng/mg cr. and 18.22 ng/mg cr. in nonsmokers. This difference between smoking and nonsmoking BC was not statistically significant.

Data shown in Table 4 indicate that the oxidative damage of DNA was more intensive in patients with advanced BC (MIBC).

5.3. GST \(\pi\). The mean urine level of GST\(\pi\) in the BC group was 15.81 ng/mg cr. and was significantly higher than in control group C, 4.62 ng/mg cr. (p = 0.001). No significant differences in GST\(\pi\) level in urine were observed in comparison of NMIBC and MIBC between LG and HG groups. Mean level of GST\(\pi\) in the group of smoking patients (n=33, 80%) was 15.22 ng/mg cr. and it was 18.23 ng/mg cr. in nonsmokers (n=8, 20%) (Table 5).

5.4. Relationship between Markers. In order to evaluate whether UPIIIa reflects the exposure to chemical carcinogens, Spearman’s rank correlation was calculated for UPIIIa, GST\(\pi\) and 8-OHdG, especially in BC smokers. Positive correlations were observed in urine between UPIIIa and GST\(\pi\) (correlation ratio R:0.34) and between 8-OHdG and GST\(\pi\) (R:0.35). No significant correlation was noted for UPIIIa and 8-OHdG either in urine or plasma in the whole BC group (Table 6) and in BC smokers (Table 7).
Table 3: Diagnostic parameters of UPIIIa in bladder cancer.

| UPIIIa | Sensitivity | Specificity | PPV | NPV | ACC | LR+ | LR- |
|--------|-------------|-------------|-----|-----|-----|-----|-----|
| cr. in urine | 0.689 | 0.879 | 0.913 | 0.604 | 0.755 | 5.68 | 0.354 |
| in plasma | 0.787 | 0.909 | 0.941 | 0.698 | 0.83 | 8.656 | 0.234 |

PPV: positive predictive value; NPV: negative predictive value; ACC: accuracy; LR+: positive likelihood ratio; LR-: negative likelihood ratio.

Table 4: 8-OHdG levels in urine.

| Group          | Mean 8-OHdG in urine [ng/mg cr.] | SD | Me | p-value |
|----------------|---------------------------------|----|----|---------|
| BC             | 19.06                           | 14.29 | 15.0 | p=0.0031 BC vs C |
| C              | 11.84                           | 3.81 | 11.75 | p=0.0129 NMIBC vs MIBC |
| NMIBC          | 16.96                           | 11.96 | 13.68 | |
| MIBC           | 25.48                           | 18.89 | 18.25 | |
| LG             | 14.44                           | 7.36 | 12.62 | p=0.0011 LG vs HG |
| HG             | 24.15                           | 18.07 | 18.25 | |
| Non-smoking patients | 18.22 | 9.20 | 20.58 | p>0.05 non-smoking vs smoking |
| Smoking patients | 19.22 | 15.25 | 14.77 | |

UPIIIa: uroplakin IIIa; C: control group; BC: patient group; NMIBC: nonmuscle invasive BC; MIBC: muscle invasive BC; LG: low grade; HG: high grade; SD: standard deviation; p: statistically significant difference; Me: median

Table 5: GST\(\pi\) levels in urine.

| Group          | Mean GST\(\pi\) in urine [ng/mg cr.] | SD | Me | p-value |
|----------------|-------------------------------------|----|----|---------|
| BC             | 15.81                               | 21.08 | 8.68 | p=0.0005 BC vs C |
| C              | 4.62                                | 3.43 | 3.48 | |
| NMIBC          | 17.17                               | 23.23 | 8.57 | p=0.9606 NMIBC vs MIBC |
| MIBC           | 10.19                               | 5.34 | 9.12 | |
| LG             | 12.80                               | 16.99 | 7.91 | p=0.1281 LG vs HG |
| HG             | 20.05                               | 25.75 | 13.71 | |
| Non-smoking patients | 18.23 | 37.58 | 7.79 | p>0.05 non-smoking vs smoking |
| Smoking patients | 15.22 | 16.55 | 8.85 | |

UPIIIa: uroplakin IIIa; C: control group; BC: patient group; NMIBC: nonmuscle invasive BC; MIBC: muscle invasive BC; LG: low grade; HG: high grade; SD: standard deviation; p: statistically significant difference; Me: median

Table 6: Correlation ratio (R) between markers in BC group.

| Markers          | UPIIIa [urine] | UPIIIa [plasma] | 8-OHdG [urine] | GST\(\pi\) [urine] |
|------------------|----------------|-----------------|----------------|-----------------|
| UPIIIa [urine]   | -              | 0.123           | 0.194          | 0.344           |
| UPIIIa [plasma]  | 0.127          | -               | -0.042         | -0.073          |
| 8-OHdG [urine]   | 0.194          | -0.042          | -              | 0.349           |
| GST\(\pi\) [urine]| 0.344         | -0.073          | 0.349          | -              |
Table 7: Correlation ratio (R) between markers in BC smokers.

|              | UPIIIa [urine] | UPIIIa [plasma] | 8-OHdG [urine] | GSTπ [urine] |
|--------------|----------------|----------------|----------------|-------------|
| UPIIIa       | -              | 0.175          | -0.138         | 0.239       |
| UPIIIa       | 0.175          | -              | 0.093          | 0.262       |
| 8-OHdG       | -0.138         | 0.093          | -              | 0.191       |
| GSTπ         | 0.239          | 0.262          | 0.191          | -           |

The absence of a relationship between UPIIIa and 8-OHdG indicated that UPIIIa is not a marker which reflects the environmental exposure. However, a correlation between UPIIIa and GSTπ showed its relevance in the detoxification process.

6. Discussion

Uroplakins are low molecular weight glycoproteins (15 to 47 kDa). Differences between uroplakins are based on the sequence of amino acids and number of transmembrane domains [4]. These proteins build the urothelial AUM [25]. UPIa and UPIb have 4 transmembrane domains but UPII and UPIIIa have only one domain whose C-end is turned into cytoplasm. The basic role of uroplakins is to form a barrier which protects tissue from substances dissolved in urine [4]. In mammals UPIII occurs in the isoforms UPIIIa, UPIIIb, and recently described UPIIIc. In contrast to UPIIIb (35 kDa), which is present in the urothelium, pericardium, and peritoneum, UPIIIa occurs only in the urothelium [4]. This knowledge was crucial to our choice of UPIIIa as a potential marker of BC.

Our preliminary research showed that UPIIIa concentration is higher in plasma than in serum. Due to this knowledge plasma was chosen as the material for research. Our study showed increased concentration of UPIIIa in plasma (p≤0.001) and in urine (p≤0.001) of BC patients in comparison to healthy control group C. Elevated UPIIIa level in urine of BC patients was previously described only by Lai et al. The results of this study showed high sensitivity (83%) and specificity (83%) of this marker (UPIIIa) in urine [11]. We noted lower sensitivity (69%) and higher specificity (88%) for UPIIIa in urine. The difference between our results and those reported before could be caused by different numbers of invasive and noninvasive causes in the examined groups. According to our knowledge there are no reports on UPIIIa level in plasma. The high sensitivity and specificity of UPIIIa in our study (79% and 91%, respectively) indicate its value in BC diagnosis.

Some reports have shown an increased amount of UPIII (without isoform consideration) in serum of BC patients. In this study the UPIII level was especially higher in MIBC (p≤0.001) than in NMIBC (p=0.040). Furthermore, this study emphasized that a higher serum UPIII level may be a prognostic marker with 66% specificity for MIBC and 33% for NMIBC [7]. The results of our study did not show a significant difference in isofrom of uroplakin UPIIIa concentration in urine and plasma between NMIBC and MIBC patients. The obtained results also showed no significant difference between urine or plasma UPIIIa level according to tumor grade: LG or HG. Similar results were described previously in urine for G1, G2, and G3 classification [11]. Other research made on UPIII (not UPIIIa) showed a difference between tumor grade and UPIII concentration but in serum, not in urine (G1+G2 group versus G3 group; p=0.005) [7]. The lack of differences in urine or plasma UPIIIa level between invasive and noninvasive BC suggests that this isofrom is not as good a marker in BC monitoring as serum UPIIIa level; however, further research is needed.

Bladder cancer is often classified as environmentally related. There is also abundant evidence that exposure to chemical carcinogens could cause BC. At first it was observed among azo-dyes workers exposed to aromatic amines and confirmed by others [26]. To date many other carcinogens have been found, such as arsenic or aromatic hydrocarbons [12]. The analysis of environmental risk factors for BC development indicates smoking as the most important cause of BC [12]. Also in our BC group the majority (77%) were former or actual smokers. Our comparison of UPIIIa level in BC smokers with BC nonsmokers did not show any difference either in urine or plasma between groups. This could lead to the conclusion that UPIIIa is not a marker which reflects the environmental risk. In order to obtain more information about the value of UPIIIa in chemical carcinogenesis the correlation of UPIIIa with 8-OHdG was examined. 8-OHdG is one of the markers of exposure to carcinogenic xenobiotics which generate ROS. It is a product of oxidative guanine transformation in DNA. Our previously described research on 8-OHdG level in urine of BC patients showed a positive correlation between smoking and 8-OHdG level [27]. There are many reports on increased 8-OHdG level in exposure to xenobiotics, e.g., in plasma of workers exposed to chloroaniline. The amount of 8-OHdG was higher in the smoking group than the nonsmoking one [28]. Other reports of glass production workers exposed to arsenic and some heavy metals showed increased 8-OHdG in urine [29, 30]. An interesting correlation between arsenic exposure on BC development and 8-OHdG level was observed in urine of rats.
treated with water containing dimethylarsenic acid (DMA) [31]. The research on BC showed an increased 8-OHdG level in cancer tissue [32, 33]. Prognostic value of 8-OHdG in BC was reported in another study, because a high level of 8-OHdG was related to poor prognosis [22]. Also our results indicate the prognostic value of 8-OHdG in BC. We observed higher urine expression of 8-OHdG in patients with HG than LG tumors (p<0.0129) and with MIBC than NMIBC (p<0.0011).

Glutathione S-transferases form a family of multifunctional proteins, which play an important role as detoxification enzymes in the second phase of biotransformation. Changes in expression of these proteins in some tissues, serum, or urine could be an important diagnostic detoxification index [34–36]. A higher level of GSTπ in cancer tissue in BC was previously reported [37]. Another study described a 2-fold higher GSTπ level in BC than in normal urothelium [24]. Our study shows a large increase in urine GSTπ level in BC in comparison to the control (p<0.001). It confirms intensification of detoxification processes in BC patients and supports the value of GSTπ measurement in urine as a noninvasive marker in BC diagnosis.

One of our study aims was to evaluate UPIIIαs as a potential marker of BC in the aspect of environmental exposure to carcinogenic substances (smoking). Spearman's rank correlation between smoking and markers was calculated to estimate the environmental influence. This problem has not been investigated before. The lack of correlation between UPIIIα and 8-OHdG in BC smokers suggests that UPIIIα does not reflect the exposure to carcinogens present in cigarette smoke.

7. Conclusion

The study showed the diagnostic value of urine and plasma UPIIIα in BC (good sensitivity, specificity, and predictive value). The lack of UPIIIα correlation with 8-OHdG and smoking suggests that UPIIIα does not reflect the environmental exposure. The increased level of 8-OHdG (also GSTπ level) in the invasive tumor stage indicates its value in BC monitoring.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

[1] S. A. Lewis, “Everything you want to know about the bladder epithelium but were afraid to ask,” American Journal of Physiology-Renal Physiology, vol. 278, no. 6, pp. 867–874, 2000.
[2] F. Zocher, M. L. Zeidel, A. Missner et al., “Uroplakins do not restrict CO2 transport through urothelium,” The Journal of Biological Chemistry, vol. 287, no. 14, pp. 11011–11017, 2012.
[3] T.-T. Sun, H. Zhao, J. Provot, U. Aebi, and X.-R. Wu, “Formation of asymmetric unit membrane during urothelial differentiation,” Molecular Biology Reports, vol. 23, no. 1, pp. 3–11, 1996.
[4] X. Wu, X. Kong, A. Pellicer, G. Kreibich, and T. Sun, “Uroplakins in urothelial biology, function, and disease,” Kidney International, vol. 75, no. 11, pp. 1153–1165, 2009.
[5] G. Apodaca, “The uroepithelium: Not just a passive barrier,” Traffic, vol. 5, no. 3, pp. 117–128, 2004.
[6] G. Ge Zhou, W. J. Mo, P. Sebbel et al., “Uroplakins Ia is the urothelial receptor for uropathogenic Escherichia coli: evidence from in vitro FimH binding,” Journal of Cell Science, vol. 114, no. 2, pp. 4095–4103, 2001.
[7] H. Tsumura, K. Matsumoto, M. Ikeda et al., “High expression level of preoperative serum uroplakin III is associated with biologically aggressive bladder cancer,” Asian Pacific Journal of Cancer Prevention, vol. 16, no. 4, pp. 1539–1543, 2015.
[8] J. T. Leppert, O. Shvarts, K. Kawoika, R. Lieberman, A. S. Belildegrun, and A. J. Pantuck, “Prevention of bladder cancer: A review,” European Urology, vol. 49, no. 2, pp. 226–234, 2006.
[9] B. Tétu, R. Tiguet, F. Harel, and Y. Fradel, “Immunocyt/uCyt+ improves the sensitivity of urine cytology in patients followed for urothelial carcinoma,” Modern Pathology, vol. 18, no. 1, pp. 83–89, 2005.
[10] F. Saint, H. Quintens, M. Roupert et al., “Diagnostic test for bladder cancer: the NMP22,” Progrès en Urologie, vol. 21, no. 4, pp. 245–249, 2011.
[11] Y. Lai, J. Ye, J. Chen et al., “UPK3A: A promising novel urinary marker for the detection of bladder cancer,” Urology, vol. 76, no. 2, pp. 514.e6–514.e11, 2010.
[12] A. Dlugosz, J. Gasior, and A. Guzik, “The influence of environmental risk factors on the development of bladder cancer,” Oncology, vol. 65, no. 1, pp. 35–41, 2015.
[13] P. Kumar, S. Nandi, T. Z. Tan et al., “Highly sensitive and specific novel biomarkers for the diagnosis of transitional bladder cancer,” Oncotarget, vol. 6, no. 15, pp. 13539–13549, 2015.
[14] B. Szymańska, K. J. Pawlik, E. Sawicka et al., “Evaluation of NMP22 in bladder cancer patients sensitive to environmental toxins,” Advances in Clinical and Experimental Medicine, vol. 26, no. 7, pp. 1069–1075, 2017.
[15] E. L. Winnder and R. Goldsmith, “The epidemiology of bladder cancer. A second look,” Cancer, vol. 40, no. 3, pp. 1246–1268, 1977.
[16] G. Talaska, M. Schamer, G. Casetta, A. Tizzani, and P. Vineis, “Carcinogen-DNA adducts in bladder biopsies and urothelial cells: a risk assessment exercise,” Cancer Letters, vol. 84, no. 1, pp. 93–97, 1994.
[17] G. Talaska, “Aromatic amines and human urinary bladder cancer: exposure sources and epidemiology,” Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology, vol. 21, no. 1, pp. 29–43, 2003.
[18] C. Steinmaus, Y. Yuan, M. N. Bates, and A. H. Smith, “Case-control study of bladder cancer and drinking water arsenic in the western United States,” American Journal of Epidemiology, vol. 158, no. 12, pp. 1193–1201, 2003.
[19] X.-R. Wu, J.-H. Lin, T. Walz et al., “Mammalian uroplakins: A group of highly conserved urothelial differentiation-related membrane proteins,” The Journal of Biological Chemistry, vol. 269, no. 18, pp. 13716–13724, 1994.

[20] D. Murtas, F. Piras, L. Minerba et al., “Nuclear 8-hydroxy-2′-deoxyguanosine as survival biomarker in patients with cutaneous melanoma,” Oncology Reports, vol. 23, no. 2, pp. 329–335, 2010.

[21] T. Akçay, I. Saygili, G. Andican, and V. Yakın, “Increased formation of 8-hydroxy-2′-deoxyguanosine in peripheral blood leukocytes in bladder cancer,” Urologia Internationalis, vol. 71, no. 3, pp. 271–274, 2003.

[22] J. Yu, M. Manabe, X.-R. Wu, C. Xu, B. Surya, and T.-T. Sun, “Uroplakin I: A 27-kD protein associated with the asymmetric unit membrane of mammalian urothelium,” The Journal of Cell Biology, vol. 111, no. 3, pp. 1207–1216, 1990.

[23] H.-I. Chen, S.-H. Liou, and S.-F. Ho, “Oxidative DNA damage estimated by plasma 8-hydroxydeoxyguanosine (8-OHdG): influence of 4, 4′-methylenebis(2-chloroaniline) exposure and smoking,” Journal of Occupational Health, vol. 49, no. 5, pp. 389–398, 2007.

[24] T. Lin, C.-C. Wu, J.-D. Wu, and C.-H. Wei, “Oxidative DNA damage estimated by urinary 8-hydroxy-2′-deoxyguanosine and arsenic in glass production workers,” Toxicology & Industrial Health, vol. 28, no. 6, pp. 513–521, 2012.

[25] M. Wei, H. Wani, N. Tanji, A. Ozawa et al., “Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors,” Carcinogenesis, vol. 23, no. 8, pp. 1387–1397, 2002.

[26] J. L. Koyner, V. S. Vaidya, M. R. Bennett et al., “Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury,” Clinical Journal of the American Society of Nephrology, vol. 5, no. 12, pp. 2154–2165, 2010.

[27] B. Szymańska, E. Sawicka, A. Guzik, R. Zdrojowy, and A. Długosz, “The diagnostic value of nuclear matrix proteins in bladder cancer in the aspect of environmental risk from carcinogens,” BioMed Research International, vol. 2017, Article ID 9643139, 11 pages, 2017.

[28] B. Ketterer, “Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis,” Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis, vol. 202, no. 2, pp. 343–361, 1988.

[29] S. Tsučkida and K. Sato, “Glutathione transferases and cancer,” Critical Reviews in Biochemistry and Molecular Biology, vol. 27, no. 4, pp. 337–384, 1992.

[30] A. F. Howie, L. M. Forrester, M. J. Glancey et al., “Glutathione s-transferase and glutathione peroxidase expression in normal and tumour human tissues,” Carcinogenesis, vol. 11, no. 3, pp. 451–458, 1990.

[31] M. Wei, H. Wani, N. Tanji, A. Ozawa et al., “Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors,” Carcinogenesis, vol. 23, no. 8, pp. 1387–1397, 2002.

[32] T. Akçay, I. Saygili, G. Andican, and V. Yakın, “Increased formation of 8-hydroxy-2′-deoxyguanosine in peripheral blood leukocytes in bladder cancer,” Urologia Internationalis, vol. 71, no. 3, pp. 271–274, 2003.
