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Biochemical Evaluation between Urothelial bladder cancer and Upper Tract Urothelial Carcinomas

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Abstract: Background: Urothelial carcinomas (UC) can be either in the upper or in the lower urinary tract or both. Urothelial bladder cancer (UBC) is more common than upper tract urothelial carcinoma (UTUC). This research was designed to study the biochemical difference between UBC and UTUC through the comparison between Catalase and Nitric oxide and the measurements of CK20 and CD44 by flow cytometry. **Methods:** To study the discrepancy between UBC and UTUC. A prospective trial was carried out for 28 radical cystectomy and 18 nephrouretrectomy specimens of UBC and UTUC patients, respectively. The activity of Catalase and concentration of Nitric oxide were assessed in in normal adjacent tissues, UTUC and UBC spectrophotometrically. CK20 and CD44 also were assessed in in normal adjacent tissues, UTUC and UBC by flow cytometry. **Results:** Comparison between UBC and UTUC regarding Catalase had significant difference (*P* value < 0.05) and comparison between UTUC and UBC regarding nitric oxide had significant difference (*P* value < 0.05), regarding CK20 in UTUC was positive (73.6%) and in UBC was positive CK20 (62.4%) and regarding CD44 in UTUC was positive (65.5%) and in UBC was negative CD44 (52.2%). **Conclusions:** Even though UTUC and UBC have the same origin, there is clear evidence that there is a biochemical difference between them. This biochemical difference could be the reason that UTUC is more aggressive than UBC.

keywords: Urothelial carcinoma, Catalase, Nitric oxide, CD44, CK20

1.Introduction

Urothelium is the specific epithelial lining of the upper urinary tract that includes the renal pelvis and ureters and lower urinary tract that includes bladder and urethra [1]. Urothelial carcinomas (UC) can be in the upper and/or the lower urinary tract. Urothelial cancers are the most abundant histologic subtype of bladder cancer (BC) with nearly 90% of bladder tumors being urothelial. Upper tract urothelial carcinoma (UTUC) is uncommon, accounting for only 5–10% of all UC [2].

Both UC of the bladder and upper tract are considered as a disparate twin. Both originates from the same tissue type, however both have discrepancies in diagnosis, treatment and prognosis [3]. Both arise from distinct embryonic tissues and have overlapping genomic features [4].

Though UCB and UTUC are two separate diseases, smoking is the most well-known risk factor for both. Current smoking increases the risk of developing UCB and UTUC by as much as fourfold and sixfold, respectively [5]. Cigarette smoking increases the formation of free radicals e.g. nitric oxide (NO) which results in increases the oxidative stress of plasma proteins [6]. Some defense mechanisms in the body prevent the development of free radicals and the damage they cause. One of the most important antioxidant enzymes is catalase

(CAT), which removes hydrogen peroxide (H_2O_2) that results from dismutation of superoxide anion [7].

Studies report that UTUC is more aggressive and invasive than UBC [8]. CD44 is a widely distributed cell surface adhesion molecule that is important in a variety of biological activities, including cell motility and cell–matrix interaction [9]. Tumor invasion and metastasis are connected to changes in CD44 expression patterns. CD44 expression and its relationship to tumor biological features, on the other hand, varies depending on the kind and origin of the tumor [9].

Cytokeratins are intermediate filaments expressed in epithelial cells. One of these, CK20, is higher in urothelial tumor than in non-neoplastic hematuria patients, hence it can be used as a marker of urothelial differentiation [10].

Several studies had compared between UBC and UTUC in epidemiology, etiology, staging, risk factors, prognosis, and management. There are no enough biochemical studies to compare between UBC and UTUC [1, 2, 11]. Therefore, this study was designed to investigate the biochemical characteristics in the two types of UC, through shedding the light on the oxidative stress in both tumors by measuring the activity of catalase and the level of nitric oxide. Also, by comparing between CD44 and CK20 in both tumors.

2. Patients and methods:

- **Patients:**

This prospective study was undertaken after Institutional Review Board (IRB) approval at Mansoura Urology and Nephrology center (ID number RP.19.07.38) and informed consent was taken from all patients. The study was performed on pathological specimens of 31 subjects with UBC who underwent cystectomy and 19 with UTUC who underwent radical nephroureterectomy with bladder cuff excision. In each group samples were taken from the malignant tissue and non-tumor tissue of the same specimen. All tumors were graded using the 2004 WHO classification and staged according to the 1997 TNM classification.

- **Methods:**

- **Determination of oxidative stress markers**

Catalase activity and Nitric oxide concentration were determined by colorimetric method using commercially available kits provided by Bio-Diagnostic Company, Giza, Egypt [12, 13].

- **Flow cytometry**

Immunomagnetically enriched samples were subjected to flow cytometry. Cells were prepared using fixation/permeabilization kit (Beckman Coulter, Brea, USA) before the addition of anti-cytokeratin 20- FITC and CD44-FITC. Briefly, cells and both antibodies were incubated in the dark then cells were washed and centrifuged. Labeled cells were detected by using BD Accuri C6 flow cytometry [14].

- **Statistical analysis**

Statistical analysis was performed with the use of the IBM SPSS Statistics ver. 22.0 for Windows (IBM Corp., Armonk, NY, USA). In the normally distributed variables, student t-test was used for comparison between groups. Chi-square and Fisher exact tests were used for comparing categorical data of both groups. P value ≤ 0.05 was considered statistically significant.

3. Results

Twenty-eight subjects with UBC and 18 subjects with UTUC were included in this study. **Table1** shows the patient demographic characteristics of the two groups. Groups were matched in age, sex, BMI, and renal function. Moreover, co-morbidities in terms of liver disease, diabetes mellitus and chronic kidney disease were not significantly different among both groups.

Oncologic comparison between both groups is shown in **Table 1.** UTUC had significantly lower tumor stage with 89.5% having T1. On the hand lymphovasular invasion was significantly higher in UTUC 77.8% versus 39.3% in UBC. Meanwhile, both groups were comparable regarding tumor grade, lymph node status and pathological cell type.

Table 1:Demographic and oncologic features of UTUC and UBC

	UTUC	UBC	*P* value
Age, years(mean ± SD)	63.89 ± 10.21	61.9 ± 10.64	0.517
BMI(mean ± SD)	28.48 ± 6.9	31.24 ± 7.63	0.334
Gender, no. (%) – Male – Female	15 (83.3%) 3 (16.7%)	22 (78.6%) 6 (21.4%)	0.691
Co-morbidities, no. (%) Liver disease – Yes – No Diabetes mellitus – Yes – No CKD – Yes – No	0 18 (100%) 1 (5.6%) 17 (94.4%) 1 (5.6%) 17 (94.4%)	4 (14.3%) 24 (85.7%) 10 (35.7%) 18 (64.3%) 3 (10.7%) 25 (89.3%)	0.093 0.019 0.545
Serum creatinine, mg/dL(mean ± SD)	1.35 ± 0.6	1.34 ± 1.27	0.969
T stage, no. (%) – T1 – T2 – T3 – T4	16 (89.5%) 1 (5.3%) 1 (5.3%) 0	6 (21.4%) 7 (25%) 12(42.8%) 3 (10.7%)	<0.001*
N stage, no. (%) – N0/x – N1 (single) – N2 (multiple) – N3	 15 (83.3%) 3 (16.6%) 0 0	 17 (60.7%) 4 (14.2%) 6 (21.4%) 2 (7.1%)	0.107
Cell type final pathology, no. (%) – Pure TCC – TCC with squamous differentiation – TCC with micropapillary component	18 (100%) 0 0	20 (71.4%) 7 (25.0%) 1 (3.6%)	0.06
Grade, n (%) – Low grade – High grade	0 18 (100%)	1 (3.6%) 27 (96.4%)	0.429
Lymphovasular invasion final pathology, no. (%) – Yes – No	14 (77.8%) 4 (22.2%)	11 (39.3%) 17 (60.7%)	0.228
* $p \leq 0.05$			

- **Determination of Catalase activity**

Catalase activity was detected spectrophotometery in tumor tissue of Upper urinary tract the adjacent normal. **Table (2)** showed comparison between CAT activity in normal and tumor upper urinary tract tissue. CAT activity was significantly increased in normal tissue more than tumor tissue with p value < 0.05. The comparison between CAT activity in normal and tumor urinary bladder tissue. CAT activity was significantly increased in normal tissue more than tumor tissue with p value < 0.001.

Table 2: Catalase activity in normal and tumor Upper urinary tract

		CAT (U/g) (Mean ± SD)	*p* value
Upper urinary tract	**Normal**	19.43 ± 9.81	< 0.05
	Tumor	11.51 ± 6.14	
Bladder	**Normal**	19.3 ± 2.15	<0.001
	Tumor	15.96 ± 2.36	

CAT activity was compared between both tumors of upper urinary tract and urinary bladder. **Table (3)** showed the comparison between CAT activity in upper urinary tract

tumor and urinary bladder tumor. CAT activity was significantly increased in upper urinary tract tumor than bladder tumor with p value < 0.001.

Table 3: Comparison between Catalase activity in upper urinary tract tumor and urinary bladder tumor

	Upper Tumor	**Bladder Tumor**	***P* value**
CAT (U/g) (Mean ± SD)	11.51 ± 6.14	15.96 ± 2.27	< 0.05

- **Determination of Nitric acid**

Nitric oxide concentration was detected spectrophotometery in tumor tissue of Upper urinary tract the adjacent normal. **Table (4)** showed comparison between NO concentration in normal and tumor upper urinary tract tissue. NO concentration was significantly increased in normal tissue more than tumor tissue with p value < 0.001. The comparison between NO concentration in normal and tumor urinary bladder tissue. NO concentration was significantly increased in normal tissue more than tumor tissue with p value = 0.001

Table 4: Nitric oxide activity in normal and urinary bladder tumor

		NO (µmol/g) (Mean ± SD)	***p* value**
Upper urinary tract	**Normal**	2.88 ± 1.7	< 0.001
	Tumor	6.68 ± 2.4	
Bladder	**Normal**	0.31 ± 0.26	< 0.05
	Tumor	0.53 ± 0.27	

NO concentration was compared between both tumors of upper urinary tract and urinary bladder. **Table (5)** showed the Comparison between NO concentration in upper urinary tract tumor and urinary bladder tumor. NO concentration was significantly increased in upper urinary tract tumor than bladder tumor with p value < 0.001.

Table 5: Comparison between Nitric oxide concentration in upper urinary tract tumor and urinary bladder tumor

	Upper Tumor	**Bladder Tumor**	**P value**
NO(µmol/g) (Mean ± SD)	6.68 ± 2.45	0.53 ± 0.27	< 0.001

- **Flow cytometry:**

Flow cytometric analysis showed negative in both CK20 (89.5%) in normal upper tract cells and positive in both CK20 (73.6%) in UTUC, **(Figure 1)** and negative CK20 (90.1%) in normal bladder cells and positive CK20 (62.4%) in UBC **(Figure 2).**

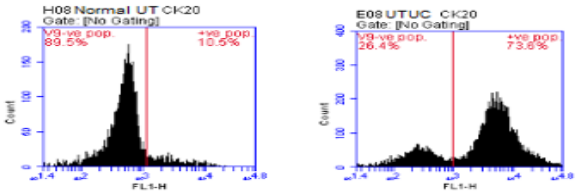

Fig 1: Flow cytometry of CK20 in Upper tract urothelial

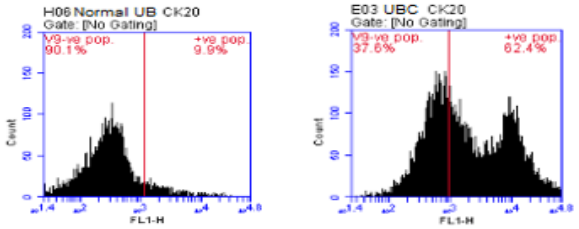

Fig 2: Flow cytometry of CK20 in Bladder

Flow cytometric analysis also showed negative CD44 (95.3%) in normal upper tract cells and positive CD44 (65.5%) in UTUC **(Figure 3)** and negative CD44 (90%) in normal bladder cells negative CD44 (52.2%) in UBC **(Figure 4).**

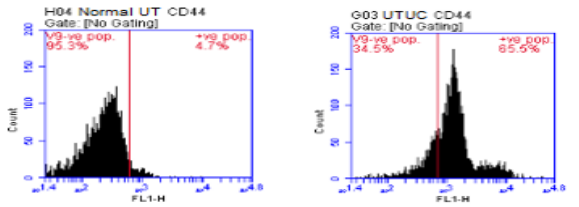

Fig 3: Flow cytometry of CD44 in Upper tract urothelial

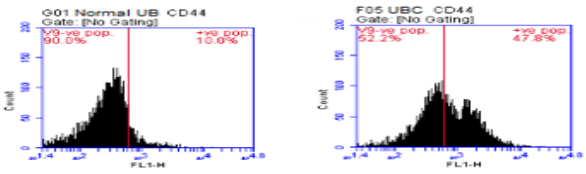

Fig 4: Flow cytometry of CD44 in Bladder

4. Discussion

As Upper tract urothelial carcinoma (UTUC) and Urothelial bladder cancer (UBC) are considered as distinct tumor entities, a biochemical study was required to clarify the

difference between the two tumors and to understand the behavior of each one of them.

In the present study we investigated the activity of catalase (CAT) and nitric oxide (NO) on both UTUC and UBC as they play important role in cancer biology.

Catalase (CAT) is an antioxidant enzyme that catalyzes the breakdown of hydrogen peroxide to water and oxygen in all cells. Both tumors and their surrounding normal tissue had their CAT activity assessed. In comparison to the adjacent normal, activity was low in UTUC (P value = 0.002) and low in UBC (P value = 0.000). This result is consistent with the result reported that CAT activity was lower in UBC tissue than the adjacent normal [15]. When CAT activity was compared between UTUC and UBC, CAT activity was considerably higher in UBC (P value = 0.000).

Nitric oxide (NO) is a short-lived free radical that is important in a variety of biological activities. NO has been proposed to play a key role in tumor biology, with both facilitatory and inhibitory effects on tumor growth [16]. NO activity was recorded in both tumors and the normal tissue surrounding them. UTUC had higher concentration than the surrounding normal (P value = 0.000), while UBC had higher concentration than the adjacent normal (P value = 0.001). It was reported that NO concentration was higher in UBC than in normal tissue [16]. When NO concentration at UTUC and UBC was compared, NO concentration in UTUC was significantly higher than in UBC (P value = 0.000).

The significant increase in NO and decrease in CAT in UTUC could explain its aggressive attitude than UBC [8].

In the present study we used flow cytometry technique to assess CD44 and CK20. In UTUC, CK20 was positive (73.6%) in compared to normal upper tissue (negative (89.5%)). This result agrees with study that reported CK20 was overexpressed in UTUC [17]. In UBC, CK20 was positive (62.4%) in compared to normal bladder tissue (negative (90.1%)). This results agrees with study that reported CK20 was overexpressed in UBC [18]. In the comparison between UTUC and UBC, CK20 is more positive in UTUC than UBC.

In UTUC, CD44 was positive (65.5%) in compared to normal upper tissue (negative (95.3%)). This result agrees with study that reported CD44 was overexpressed in UTUC

[19]. In UBC, CD44 expression was higher (negative (52.2%)) in compared to normal bladder tissue (negative (90%)). This result agrees with study that reported CD44 was overexpressed in UBC [20]. In the comparison between UTUC and UBC, CK20 is more positive in UTUC than UBC.

The significant expression of CK20 and CD44 in UTUC could explain its aggressive and invasive attitude than UBC [8]. The Results of this study showed that CK20 and CD44 are very likely to be involved in the occurrence and development of UTUC. Our study provided references for the molecular etiology of the aggressiveness and invasiveness of UTUC.

5. Conclusion:

Results of the present study showed clear evidence that there is a biochemical difference between UTUC and UBC despite they share the same origin. This biochemical difference could be the reason that UTUC is more aggressive than UBC.

6. References:

1. Szarvas, T., O. Modos, A. Horvath, and P. Nyirady, (2016) Why are upper tract urothelial carcinoma two different diseases? Transl Androl Urol. **5(5)**: p. 636-647.
2. Aragon-Ching, J.B., (2017) Challenges and advances in the diagnosis, biology, and treatment of urothelial upper tract and bladder carcinomas. Urol Oncol**. 35(7):** p. 462-464.
3. Green, D.A., M. Rink, E. Xylinas, S.F. Matin, A. Stenzl, M. Roupret, et al., 2013 Urothelial carcinoma of the bladder and the upper tract: *disparate twins. J Urol*. **189(4):** p. 1214-21.
4. Necchi, A., R. Madison, S.K. Pal, J.S. Ross, N. Agarwal, G. Sonpavde, et al., (2020) Comprehensive Genomic Profiling of Upper-tract and Bladder Urothelial Carcinoma. Eur Urol Focus.
5. Crivelli, J.J., E. Xylinas, L.A. Kluth, M. Rieken, M. Rink, and S.F. Shariat, (2014) Effect of Smoking on Outcomes of Urothelial Carcinoma: A Systematic

Review of the Literature. European Urology. **65(4)**: p. 742-754.
6. Islam, M.O., T. Bacchetti, and G. Ferretti, (2019) Alterations of antioxidant enzymes and biomarkers of nitro-oxidative stress in tissues of bladder cancer. Oxidative medicine and cellular longevity. 2019: p. 1-10.
7. Pirinççi, N., I. Geçit, M. Güneş, M.B. Yüksel, M. Kaba, S. Tanık, et al.,(2012) Serum adenosine deaminase, catalase and carbonic anhydrase activities in patients with bladder cancer. Clinics (Sao Paulo, Brazil). **67(12):** p. 1443-1446.
8. Kim, M., C.W. Jeong, C. Kwak, H.H. Kim, and J.H. Ku, 2015 Are urothelial carcinomas of the upper urinary tract a distinct entity from urothelial carcinomas of the urinary bladder? Behavior of urothelial carcinoma after radical surgery with respect to anatomical location: a case control study. BMC Cancer. 15: p. 149.
9. Miyake, H., I. Hara, S. Kamidono, and H. Eto, (2004) Multifocal transitional cell carcinoma of the bladder and upper urinary tract: molecular screening of clonal origin by characterizing CD44 alternative splicing patterns. *J Urol*. **172(3)**: p. 1127-9.
10. Siracusano, S., B. Niccolini, R. Knez, A. Tiberio, E. Benedetti, S. Bonin, et al., (2005) The simultaneous use of telomerase, cytokeratin 20 and CD4 for bladder cancer detection in urine. Eur Urol. **47(3)**: p. 327-33.
11. Krabbe, L.-M., Y. Lotan, A. Bagrodia, B.A. Gayed, O.M. Darwish, R.F. Youssef, et al., 2014 Prospective Comparison of Molecular Signatures in Urothelial Cancer of the Bladder and the Upper Urinary Tract—Is There Evidence for Discordant Biology? *The Journal of Urology*. **191(4):** p. 926-931.
12. Sherif, I.O., (2018) The effect of natural antioxidants in cyclophosphamide-induced hepatotoxicity: Role of Nrf2/HO-1 pathway. International Immunopharmacology. **61**: p. 29-36.
13. Almasaudi, S.B., N.A. El-Shitany, A.T. Abbas, U.A. Abdel-dayem, S.S. Ali, S.K. Al Jaouni, et al.,(2016) Antioxidant, Anti-inflammatory, and Antiulcer Potential of Manuka Honey against Gastric Ulcer in Rats. Oxidative Medicine and Cellular Longevity. 2016: p. 1-10.
14. Awadalla, A., H. Abol-Enein, M.M. Gabr, E.T. Hamam, and A.A. Shokeir, (2020) Prediction of recurrence and progression in patients with T1G3 bladder cancer by gene expression of circulating tumor cells. Urologic Oncology: Seminars and Original Investigations. **38(4):** p. 278-285.
15. Bayraktar, N., S. Kilic, M.R. Bayraktar, and N. Aksoy, (2010)Lipid peroxidation and antioxidant enzyme activities in cancerous bladder tissue and their relation with bacterial infection: a controlled clinical study. *Journal of clinical laboratory analysis*. **24(1):** p. 25-30.
16. Gecit, İ., R. Eryılmaz, S. Kavak, İ. Meral, H. Demir, N. Pirinççi, et al., (2017) The Prolidase Activity, Oxidative Stress, and Nitric Oxide Levels of Bladder Tissues with or Without Tumor in Patients with Bladder Cancer. *The Journal of membrane biology.* **250(5):** p. 455-459.
17. Jung, M., B. Kim, and K.C. Moon, 2019 Immunohistochemistry of cytokeratin (CK) 5/6, CD44 and CK20 as prognostic biomarkers of non-muscle-invasive papillary upper tract urothelial carcinoma. Histopathology. **74(3)**: p. 483-493.
18. Yikilmaz, T.N., A. Dirim, E.S. Ayva, H. Ozdemir, and H. Ozkardes, (2016) Clinical Use of Tumor Markers for the Detection and Prognosis of Bladder Carcinoma: A Comparison of CD44, Cytokeratin 20 and *Survivin. Urology Journal***. 13(3):** p. 2677-2683.
19. Hagiwara, M., E. Kikuchi, T. Kosaka, S. Mikami, H. Saya, and M. Oya, (2016) Variant isoforms of CD44 expression in upper tract urothelial cancer as a predictive marker for recurrence and mortality. Urologic Oncology: Seminars and Original Investigations. **34(8)**: p. 337.e19-337.e26.
20. Asgari, M., M. Nabi Maybodi, and M. Abolhasani, (2016) Differential diagnosis of urothelial carcinoma in situ from non-neoplastic urothelia: Analysis of CK20, CD44, P53 and Ki67. Medical *journal of the Islamic Republic of Iran.* **30**: p. 400-400.