CKD PATIENTS WHO DEVELOPED UROTHELIAL CARCINOMA HAVE HIGHER URINARY CONCENTRATIONS OF PHTHALATE METABOLITES - A PRELIMINARY STUDY FROM TAIWAN

Chiu-Ching Huang[1], Che Yi Chou[1], Chin Lin Chou[2], Joseph Kao[1], Chiz Tzung Chang[1] and Chao Jung Chen[2,3] for Urothelial Carcinoma Study Consortium, Taiwan
[1]China Medical University and Hospitals, Kidney Institute, Taichung, TAIWAN, [2]China Medical University, Proteomics Core Laboratory, Department of Medical Research, Taichung, TAIWAN, [3]China Medical University, Graduate Institute of Integrated Medicine, Taichung, TAIWAN.

Objectives:

INTRODUCTION: Increasing epidemiological and experimental evidences support the role of environmental exposures in the development of urothelial cancer (UC). Among the potential environmental factors, tobacco, inorganic arsenic, and aristolochic acid are the well-known and established risk factors for urothelial cancer. Despite the ban for importation of aristolochic acid containing Chinese herbs in 2003, Taiwan still has the highest incidence of UC in the world, particularly in CKD (chronic kidney disease) and dialysis patients. In 2011, the great DEHP plasticizer food contamination in Taiwan was disclosed. It was discovered that ilegal addition of industrial grade phthalates as food additives was widely practiced since 1980. In 2011, this issue was disclosed and had raised significant concern about its potential health hazard effects. It is well known that patients with CKD are prone to develop UC. Whether excessive phthalates exposure may contribute to the increased incidence of UC in CKD patients has not been studied before.

AIMS: To investigate if environmental phthalate exposures have a potential impact on UC risk in CKD patients.

PATIENTS: Present study is a multicenter prospective prevalent case-control study, carried out at 7 nationwide medical centers covering most of the northern, central, southern and eastern Taiwanese populations. Subjects were Taiwanese healthy adults (healthy controls), patients with CKD but without UC (disease controls) and patients with ongoing UC. UC was confirmed by standard pathology; CKD staging was defined by eGFR classification; healthy controls defined by the clinical examination and urinalysis. Blood and random spot urine specimens were collected at the time of the structured interview for demographic information.

Methods:

Urine was collected from each patient and frozen immediately after collection. For UC patients, urine and blood were collected before operation. We measured 7 urinary phthalate metabolites, e.g. mono-benzyl phthalate (MBzP), mono-isobutyl phthalate (MiBP), mono-cyclohexyl phthalate (MCHP), mono(2-ethyl-5hydroxyhexyl) phthalate(MEHHP) and mono-isononyl phthalate(MiNP), monomethyl phthalate (MMP), mono (3-carboxypropyl) phthalate(MCPP). The concentrations of the metabolites were determined using an established LC-MS/MS method. A high-performance liquid chromatographic system (Ultimate 3000 LC; Dionex, Germany) coupled with a hybrid Q-TOF mass spectrometer (maXis impact; Bruker) was used. This study was approved by the IRBs of China Medical University Hospital and other collaborative Hospitals. Mann-Whitney Rank Sum Test was used for statistical analyses when comparing urinary phthalate metabolite concentrations between healthy controls, CKD controls and UC patients. Multiple logistic regression analysis was performed to evaluate the associations between risk of UC and $\log^2$-transformed creatinine-corrected urinary phthalate concentrations.

Results:

A total of 41 UC cases, 41 CKD controls and 23 healthy controls were recruited from July 2012 to December 2013 with written informed consents. CKD control patients were age, sex and CKD-staging best matched with UC patients. The demographic and clinical data are shown in Table 1. In UC patients, 16(39%) had upper urinary tract UC (UTUC) and 25 (61%) had bladder UC. Urinary phthalate metabolites tested were expressed as microgram/gram creatinine (μg/g cr.).Among the 7 phthalate metabolites measured, only MEHHP had measurable concentrations in all patients. MIBP and MCPP had roughly measurable concentrations in 50%-60% of patients. The rest of phthalate metabolites were not detected in most paitents. The mean concentrations of MEHHP were 46.42±43.42 (healthy controls), 45.63±26.76 (CKD) and 77.16±77.90 (UC) μg/g creatinine respectively. The median concentrations of MEHHP were 30.71 (healthy controls), 40.25 (CKD controls) and 48.21 (UC) μg/g creatinine respectively. The UC patients had significantly higher urinary concentration of MEHHP than CKD control patients (p=0.002, Fig. 1).For MCPP and MIBP measurements, the differences between controls,CKD and UC patients were not statistically significant. Analysis within MEHHP tertiles showed a dose-response relationship of odds ratios, when comparing second and third tertile to the lowest tertile. The odds ratios were 1.46 (0.60-4.46) and 3.73 (1.09-9.75) respectively (Table 2).Risk of developing UC per doubling increase of MEHHP was 1.77 after adjusting for age, sex, smoking status and eGFR. No interaction between selected phthalates and smoking was found.

Dicussions:

In past 20 years, the incidence of UC in US declined gradually, while the escalating trend was still observed in Taiwan (Fig.2). In this study, we observed 5 times higher mean urinary levels of phthalate metabolites in Taiwanese healthy controls when compared to US residents (46.42 vs. 8.99 μg/g cr of US residents, 2015 Fourth National Report on Human Exposure to Environmental Chemicals, p388-393), indicating much higher DEHP exposures in Taiwanese residents. In US, the mean urinary concentration of MEHHP declined from 25.5(2005-2006) to 8.99 μg/g cr (2011-2012).
MEHHP, the metabolite of DEHP, is a potential novel and modifiable risk factor for UC. Prospective research is needed to enable causal inference and determine the generalizability of these findings to other populations with a different range of phthalate exposures.

Conclusions:

In this multi-center, cross-sectional study of Taiwan, higher urinary concentrations of MEHHP showed statistically significant correlation with increasing risk of UC. Our findings would suggest high environmental exposure to phthalates may contribute to the development of UC in CKD patients. Future research with more patients and longitudinal study will be required to provide

Table 1. The demographic and clinical data of study subjects in Taiwan UC study

	Healthy controls (N=23)	UC (N=41)	CKD controls (N=41)
Age (years)	40.00 ± 12.37 (20-75)	63.39 ± 11.12 (38-79)	61.24 ± 10.57 (35-78)
Sex : Male/Female	4 / 19	33 / 8	34 / 7
% Male	17.39%	80.49%	82.93%
Smoking (%)			
No	21(91.30)	19(46.34)	23(56.10)
Yes	2(8.70)	8(19.51)	8(19.51)
Quit	0	14(34.15)	10(24.39)
Serum Cr.	-	1.87 ± 1.92 (0.60-9.74)	1.79 ± 1.56 (0.65-7.81)

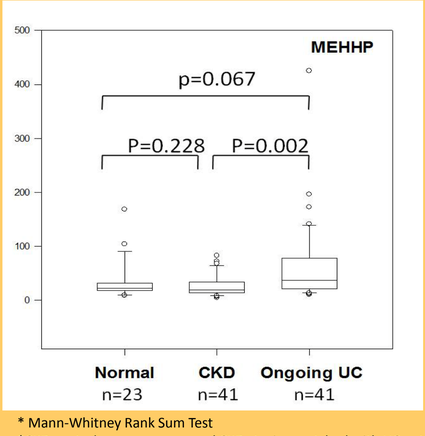
*Age and serum creatinine expressed as mean±SD

Table 2. The hazard ratios (95% confidence interval) of UC by the concentrations of urinary phthalates MEHHP in participants of Taiwan UC study.

	Model 1	Model 2	Model 3
MEHHP (μg/g)			
Tertile 1 (<17.5)	Ref	Ref	Ref
Tertile 2 (17.5-36.9)	1.75 (0.62-4.99)	1.75 (0.60-5.08)	1.46 (0.48-4.46)
Tertile 3 (> 36.9)	4.22 (1.41-12.7)	4.19 (1.36-12.9)	3.73 (1.15-12.1)
per doubling increase of MEHHP	1.77 (1.19-2.62)	1.79 (1.20-2.66)	1.77 (1.19-2.63)

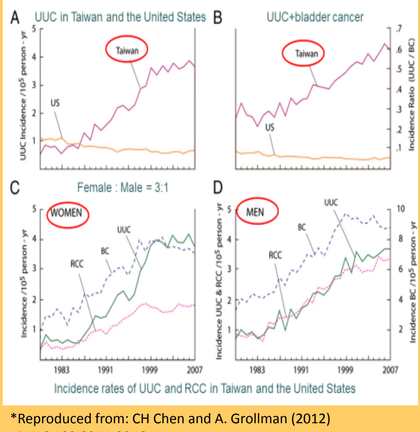
C/NC: Case/Non case
Model 1: Univariate analysis
Model 2: Further adjusted for age, sex, and smoking status
Model 3: Further adjusted for estimated glomerular filtration rate(eGFR)

Figure 1. Comparison of urinary phthalate metabolite concentrations (MEHHP) among healthy controls, CKD controls and UC patients.



* Mann-Whitney Rank Sum Test
*CKD controls were age, sex and CKD staging matched with UC patients

Fig. 2 Comparison of age-adjusted incidence rate of urothelial cancer (UC) between Taiwan and the United States (Year 1980-2007).



*Reproduced from: CH Chen and A. Grollman (2012) PNAS 109:8241-8246

References:

1. Chen et.al. (2012) Aristolochic acid-associated urothelial cancer in Taiwan. PNAS 109:8241-8246
2. Fourth National Report on Human Exposure to Environmental Chemicals February 2015), by CDC, USA

Acknowledgement:

We would like to thank Dr. Chao-Hsiang Chang, Dr. Hao-Zhong He, Dr. Hung-Chun Chen, Dr. Kuo-Hsiung Shu, Dr. Tzen-Wen Chen, Dr. Ming-Chang Wang, Dr. Bang-Gee Hsu, Dr. Chia-Chu Chang, and Dr. Chien-Lung Chen for recruiting patients.