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Urinary arsenic profile affects the risk of urothelial carcinoma even at low arsenic exposure☆

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Abstract

Arsenic exposure is associated with an increased risk of urothelial carcinoma (UC). To explore the association between individual risk and urinary arsenic profile in subjects without evident exposure, 177 UC cases and 313 age-matched controls were recruited between September 2002 and May 2004 for a case–control study. Urinary arsenic species including the following three categories, inorganic arsenic ($As^{III}+As^{V}$), monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}), were determined with high-performance liquid chromatography-linked hydride generator and atomic absorption spectrometry. Arsenic methylation profile was assessed by percentages of various arsenic species in the sum of the three categories measured. The primary methylation index (PMI) was defined as the ratio between MMA^{V} and inorganic arsenic. Secondary methylation index (SMI) was determined as the ratio between DMA^{V} and MMA^{V}. Smoking is associated with a significant risk of UC in a dose-dependent manner. After multivariate adjustment, UC cases had a significantly higher sum of all the urinary species measured, higher percent MMA^{V}, lower percent DMA^{V}, higher PMI and lower SMI values compared with controls. Smoking interacts with the urinary arsenic profile in modifying the UC risk. Differential carcinogenic effects of the urinary arsenic profile, however, were seen more prominently in non-smokers than in smokers, suggesting that smoking is not the only major environmental source of arsenic contamination since the UC risk differs in non-smokers. Subjects who have an unfavorable urinary arsenic profile have an increased UC risk even at low exposure levels.

Keywords: Bladder neoplasms; Contamination; Carcinogenesis; Synergy; Tobacco

Introduction

Urothelial carcinoma (UC) arises exclusively from the urothelium including the renal pelvis, ureter, bladder and urethra, with bladder cancer being the most common form. In most developed countries, it is among the top 10 leading cancers. The most well known risk factor for UC is cigarette smoking (Brennan et al., 2000; Zeegers et al., 2000). Current cigarette smokers have approximately a two- to three-fold risk compared to non-smokers. The mechanism by which cigarette smoking causes bladder cancer has yet to be determined. It seems likely that the risk is related to a large number of carcinogenic chemicals in cigarette smoke.

We have previously shown that chronic arsenic intoxication due to contamination of artesian well water with inorganic arsenic compounds is responsible for elevated mortality rates

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from cancers of the bladder, renal pelvis and/or ureter, lung and other organs in Taiwan (Chen et al., 1985). More specifically, we have demonstrated that bladder cancer mortality rates for patients who consumed well water with arsenic levels of 600 μg/L or higher had a mortality rate of over 30 to 60 times greater than the unexposed population (Chen et al., 1988). Evidence from elsewhere in the world also suggests that ingested inorganic arsenic very likely causes internal cancers (Morales et al., 2000; Bates et al., 2004; Smith et al., 2006). It has also been shown that combined cancer mortality rates are as high as 1 out of 100 people from drinking water containing 50 μg/L of arsenic (Smith et al., 1992). Two reports from the National Research Council (USA) have also affirmed that cancer risks might be of the order of 1 in 100 at an arsenic level of 50 μg/L (National Research Council, 1999, 2001). This estimated cancer risk is more than 100 times greater than that for any other contaminant in drinking water at the maximal contaminant level (US EPA, 1998). Thus, within the U.S., the maximum contaminant level for arsenic in public water supplies will be lowered from 50 μg/L, a level that was established in 1942, to 10 μg/L in 2006 (Smith et al., 2002). The arsenic concentration allowance in public water supplies in Taiwan was 50 μg/L; in 2000, a new standard of 10 μg/L was then announced. According to the Taipei Water Department of Taipei City Government, the average arsenic concentration of tap water in Taipei is 0.7 μg/L ranging from non-detectable to 4.0 μg/L. Whether cancer risks are higher at 50 μg/L than at 10 μg/L is still debatable. Even without confounding factors, reduction of cancer risks with the new standard of 10 μg/L will not be seen for decades. However, should it be shown that arsenic metabolic capability affects cancer risks in subjects exposed 50 μg/L of arsenic, it might still be carcinogenic for some genetically predisposed subjects.

Arsenic is usually found in drinking water in the form of arsenate (As^V) or arsenite (As^{III}) (Andreae, 1977). Inorganic arsenic is bio-transformed in humans to monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V). Previously, methylation of inorganic arsenic has always been considered a detoxification mechanism because pentavalent MMA (MMA^V) and pentavalent DMA (DMA^V) have relatively low toxicity (Yamauchi and Fowler, 1994) and are rapidly excreted in the urine (Vahter, 2002). However, recent studies have confirmed the existence of trivalent intermediates and products of monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) that are more toxic than inorganic arsenite (Thomas et al., 2001; Mass et al., 2001; Styblo et al., 2002). For a more accurate assessment of arsenic metabolism, it is therefore necessary to determine specific arsenic species in urine.

The capability of metabolizing inorganic arsenic differs among individuals. We have previously shown that the capability of arsenic methylation is related to the risks of skin cancer (Hsueh et al., 1997; Chen et al., 2003a) and bladder cancer in high arsenic-exposed subjects (Chen et al., 2003b). Whether or not urinary arsenic profile affects UC risks among subjects who had no evident arsenic exposure history is an important issue. We hypothesized that, if an arsenic level of 50 μg/L in the water supply is still "unsafe", the UC risk may significantly differ between people with favorable and unfavorable arsenic methylation profile. We therefore conducted a hospital-based case–control study to investigate the association of urinary arsenic profile with the risk of UC. The possible interactions between urinary arsenic profile and cigarette smoking were also explored.

Material and methods

Study subjects and questionnaire interview. One hundred and seventy-seven patients with pathologically proven UC (age range 24 to 93 years) were recruited from the Department of Urology, National Taiwan University Hospital, between September 2002 and May 2004. Pathological verification of UC was done by routine urological practice including endoscopic biopsy or surgical resection of urinary tract tumors followed by histopathological examination by board-certified pathologists. Cytological evidence alone was not accepted as an adequate diagnosis of UC. A total of 313 age-matched control subjects with no evidence of UC or any other malignancy were accrued from a hospital-based pool, including those receiving senior citizen health examinations at Taipei Medical University Hospital and those receiving adult health examinations at Taipei Municipal Wan Fang Hospital. All three hospitals are located in Taipei approximately 200 to 300 km away from the arsenic-contaminated areas in Taiwan. No case subjects or controls came from arsenic-contaminated areas in southwestern (Chen et al., 2003b) or northeastern (Chiou et al., 2001) Taiwan.

Well-trained personnel carried out standardized personal interviews based on a structured questionnaire. Information collected included demographic and socioeconomic characteristics, general potential risk factors for malignancies such as lifestyle, alcohol consumption, cigarette smoking in quantified details, exposure to potential occupational and environmental carcinogens such as hair dyes and pesticides, chronic medication history, consumption of conventional and alternative medicines and personal and family history of urological diseases. Frequent alcohol drinkers referred to those who consumed alcohol three or more days per week, continuing for at least 6 months. Those who consumed less than this level were classified as occasional drinkers. Pesticide users were farmers who used pesticides for agricultural purposes. The Research Ethics Committee of National Taiwan University Hospital, Taipei, Taiwan, approved the study. All patients provided informed consent forms before sample and data collection. The study was consistent with the World Medical Association Declaration of Helsinki. Urine samples were stored at −20°C until further use for urinary arsenic speciation. Bladder cancer was staged into three groups: superficial (Ta, T1 and Tis), locally advanced (T2–4N0M0) and metastatic (N+ or M+). Tumor grading was based on the WHO (1999) classification system (WHO, 1999).

Determination of urinary arsenic species. It has been shown that urinary arsenic species is stable for at least 6 months when preserved at −20°C (Chen et al., 2002); thus, the urine assay was performed within 6 months post-collection. Frozen urine samples were thawed at room temperature, dispersed by ultrasonic wave, filtered through a Sep-Pak C_{18} column (Mallinckrodt Baker In., NJ) and levels for As^{III}, As^V, MMA^V and DMA^V were determined. A urine aliquot of 200 μL was used for the determination of arsenic species by high-performance liquid chromatography (HPLC) (Waters 501, Waters Associates, MA) with columns obtained from Phenomenex (Nucleosil, Torrance, CA). The contents of inorganic arsenic and their metabolites were quantified by hydride generator-atomic absorption spectrometry (HG-AAS) (Hsueh et al., 1998). The concentration of four arsenic species in standard solution, sample and sample spiked standard solution was determined by using on-line HPLC-HG-AAS respectively. Recovery rates of the four arsenic species were calculated by [(sample spiked standard solution concentration) − sample concentration]/standard solution concentration × 100 respectively. Recovery rates for As^{III}, DMA^V, MMA^V and As^V ranged between 93.8% and 102.2% with detection limits of 0.02, 0.06, 0.07 and 0.10 μg/L, respectively. Urinary concentration of the sum of inorganic arsenic, MMA and DMA was normalized against urinary creatinine levels (μg/g creatinine). The standard reference material, SRM 2670, contains 480 ± 100 μg/L inorganic arsenic and was obtained from the National

Table 1
Sociodemographic characteristics of 177 urothelial carcinoma cases and 313 age-matched non-cancer controls

Variables	Groups	Controls *n* (%)	UC cases *n* (%)	Age and gender adjusted OR (95% CI)
Gender	Male	201 (64.2)	124 (70.1)	1.0
	Female	112 (35.8)	53 (29.9)	0.8 (0.5–1.1) [a]
	Total	313 (100)	177 (100)	
Age (years)		64.4±0.7	63.9±0.9	0.9 (0.9–1.0) [b]
Blood type	A	80 (28.0)	47 (29.0)	1.0
	B	57 (19.9)	37 (22.8)	1.1 (0.6–1.9)
	O	133 (46.5)	64 (39.5)	0.8 (0.5–1.3)
	AB	16 (6.6)	14 (8.7)	1.6 (0.7–3.7)
	Not available	27	15	
Marital status	Single	16 (6.3)	13 (7.3)	1.0
	Married	237 (75.6)	139 (78.5)	0.8 (0.4–1.7)
	Divorced	14 (4.4)	6 (3.4)	0.6 (0.2–2.0)
	Widowed	46 (13.7)	19 (10.7)	0.6 (0.2–1.7)
				$P_{trend}=0.35$
Educational level	Elementary school or below	83 (26.7)	73 (41.3)	1.0
	High school	105 (33.8)	65 (36.7)	0.6 (0.4–0.9)
	College or above	123 (39.5)	39 (22.0)	0.3 (0.2–0.4)
				$P_{trend}<0.0001$
Paternal ethnicity	Fukien Taiwanese	177 (56.5)	147 (73.1)	1.0
	Hakka Taiwanese	28 (9.0)	19 (9.5)	8 (0.4–1.5)
	Mainland Chinese	108 (34.5)	35 (17.4)	0.4 (0.2–0.6)
				$P_{trend}<0.0001$
Maternal ethnicity	Fukien Taiwanese	180 (57.5)	31 (17.6)	1.0
	Hakka Taiwanese	28 (9.0)	15 (8.5)	0.8 (0.4–1.5)
	Mainland Chinese	103 (32.9)	35 (17.5)	0.4 (0.3–0.7)
	Not available	2 (0.6)	0 (0.0)	
				$P_{trend}=0.0001$
Alcohol drinking	Never	164 (52.4)	107 (60.5)	1.0
	Frequent	52 (16.6)	41 (23.1)	1.0 (0.6–1.7)
	Occasional	97 (31.0)	29 (16.4)	0.4 (0.2–0.7)
				$P_{trend}=0.0006$
Hair dye usage	No	188 (60.7)	122 (68.9)	1.0
	Yes	122 (39.3)	55 (31.1)	0.7 (0.5–1.1)
Pesticide usage	No	299 (95.8)	150 (85.2)	1.0
	Yes	13 (4.2)	26 (14.8)	4.0 (2.0–8.0)

[a] Age-adjusted odds ratio by logistic regression.
[b] Gender-adjusted odds ratio by logistic regression.

Institute of Standards and Technology (NIST, Gaithersburg, MD). SRM 2670 was used as a quality standard and analyzed along with urine samples. The mean value of SRM 2670 determined by our system was 507±17 (SD) μg/L ($n=4$). Arsenic methylation index was assessed by percentages of various urinary arsenic species in the sum of inorganic arsenic, MMA and DMA. The primary methylation index (PMI) was defined as the ratio between MMA^V and inorganic arsenic ($As^{III}+As^V$) levels and the secondary methylation index (SMI) was defined as the ratio between DMA^V and MMA^V (Tseng et al., 2005).

Statistical analysis. Continuous variables are expressed as mean±standard error. Student's *t*-test was used to compare differences in the urinary arsenic profile between case subjects and controls. ANOVA and Student–Newman–Keuls multiple comparison correction was applied to compare urinary arsenic profiles between varied exposure strata. Multiple logistic regression models were used to estimate the multivariate adjusted odds ratio (OR) and the 95% confidence interval (CI). Cutoff points for continuous variables were the respective tertiles of the controls. Significance tests for linear trend among ORs across exposure strata were calculated by categorizing exposure variables and treating scored variables as continuous. For the joint effect analysis, the cutoff points for the percentages of arsenic species, PMI and SMI were the respective medians of the controls. The joint effects of urinary arsenic species and cigarette smoking on the UC risk were evaluated on both multiplicative and additive scales. The binary interaction terms were calculated by multiplying the indicators for two explored risk factors and were added to the main effect models. Their significance was then tested by the likelihood ratio statistic based on a multiplicative model. The synergy index proposed by Rothman was computed to assess the empirical deviation from the additive interaction relationship (Rothman, 1986). The synergy index is equal to the calculation of $[OR(AB)-1]/[(OR(Ab)-1)+(OR(aB)-1)]$, where A and B denote the presence of the two risk factors and a and b are designated as the absence of the risk factors, respectively. An observed synergy index value that departs substantially from the expected additive null, *i.e.*, synergy index not equal to 1, suggests an additive interaction effect. The OR values and their variance covariance matrix were then used to calculate values for synergy index and 95% CIs (Hosmer and Lemeshow, 1992). SAS version 8.2 was used for all statistical analyses.

Results

The sociodemographic characteristics of cases and controls are shown in Table 1. Subjects who had higher educational levels had a lower risk than those with lower educational levels. Mainland Chinese had a significantly lower UC risk than the Fukien Taiwanese. Age, ABO blood type, marital status or use of hair dye did not affect the UC risk. Occasional alcohol drinkers had a significantly lower UC risk than non-drinkers and frequent drinkers. Pesticide users had a significantly higher UC risk than non-users.

Comparing cases and controls in terms of smoking duration, daily smoking amount and cumulative smoking in pack-years,

Table 2
Comparison of detailed smoking behavior between cases and controls [a]

Variables	Controls *n* (%)	UC cases *n* (%)	Age and gender adjusted OR (95% CI)	Multivariate adjusted OR [b] (95% CI)
Non-smoker	196 (62.6)	85 (48.0)	1.0	1.0
Former smoker	66 (21.1)	65 (36.7)	2.6 (1.5–4.3)	2.6 (1.5–4.7)
Current smoker	51 (16.3)	27 (15.3)	1.3 (0.7–2.3)	1.3 (0.6–2.5)
Duration of cigarette smoking (years)				
0	196 (65.1)	86 (51.5)	1.0	1.0
0.1–33	50 (16.6)	34 (20.4)	1.4 (0.8–2.6)	1.4 (0.7–2.7)
>33	55 (18.3)	47 (28.1)	2.2 (1.3–3.9)	2.4 (1.2–4.6)
			$P_{trend}=0.005$	$P_{trend}=0.01$
Amount of cigarette smoking (pack/day)				
0	196 (65.1)	86 (51.5)	1.0	1.0
0.1–0.75	49 (16.3)	23 (13.8)	1.0 (0.6–2.0)	1.5 (0.8–3.0)
>0.75	56 (18.6)	58 (34.7)	2.5 (1.5–4.3)	2.2 (1.2–4.2)
			$P_{trend}=0.0007$	$P_{trend}=0.01$
Cumulative cigarette smoking (pack-years) [c]				
0	196 (65.1)	85 (50.9)	1.0	1.0
0.1–22	49 (16.3)	28 (16.8)	1.4 (0.7–2.5)	1.1 (0.6–2.3)
>22	56 (18.6)	54 (34.7)	2.4 (1.4–4.0)	2.5 (1.3–4.7)
			$P_{trend}=0.002$	$P_{trend}=0.004$

[a] Detailed quantified smoking history was unavailable in twelve (3.8%) controls and ten (5.6%) case subjects.
[b] Multivariate adjusted ORs: adjusted for age, gender, education, paternal and maternal ethnicity, alcohol drinking and pesticide usage.
[c] Cumulative smoking=(amount in pack/day)×(duration in years).

cigarette smokers had a significantly higher UC risk than non-smokers in a dose-dependent manner (Table 2). Heavy smokers who smoked for more than 33 years, 0.75 packs per day and 22 pack-years had a 2.4-, 2.2- and 2.5-fold risk, respectively, compared to non-smokers by multivariate adjusted logistic regression. Modest dose smokers, however, did not have a significantly increased risk compared to non-smokers.

Table 3 compares the urinary arsenic profile between varied exposure strata. Among non-cancer controls, male subjects had a lower sum of inorganic arsenic, MMA and DMA level, higher MMA percentage and lower DMA percentage than females. Current smokers had a higher MMA percentage and lower DMA percentage than non-smokers. No significant differences were found in urinary arsenic profiles between subjects consuming varied amount of alcohol. Pesticide users had an insignificantly higher MMA percentage than non-users.

UC cases had a significantly higher sum of inorganic arsenic, MMA and DMA levels, higher MMA percentages, lower DMA percentages and higher PMI levels than controls (Table 3). Inorganic arsenic percentage was marginally higher in cases than in controls ($P=0.052$). To examine if various cancer stages affect urinary arsenic profile, we performed an analysis showed that urinary arsenic profile did not differ between patients of various tumor stages or grades in case subjects (Table 3). With trend analysis on exposure strata in tertiles, all urinary arsenic parameters, with the exception of inorganic arsenic percentage, were found to be significantly associated with the UC risk after the multivariate analysis (Table 4).

Since both smoking and urinary arsenic indices affect the UC risk, further analyses were carried out to assess joint effects of the two risk factors (Table 5). Trend analysis revealed progressively increased risks through exposure to no risk factor, either one of the factors, or both of the two risk factors.

Table 3
Comparison of urinary arsenic species between varied exposure strata

Variables	*n*	Sum of inorganic arsenic, MMA and DMA (μg/g creatinine)	Inorganic arsenic percentage	MMA percentage	DMA percentage	Primary methylation index	Secondary methylation index
Non-cancer controls							
Gender							
Male	189	23.3±1.2	5.8±0.4	9.4±0.6	84.8±0.8	2.9±0.8	12.8±1.7
Female	112	27.9±1.8	5.1±0.7	5.7±0.5	89.2±0.8	1.7±0.2	16.6±3.1
*P**		0.03	0.39	<0.0001	<0.0001	0.14	0.29
Cigarette smoking							
Non-smoker	196	25.9±1.4	5.3±0.5	6.9±0.5‡,§	87.8±0.7‡	1.8±0.2	16.2±2.2
Former smoker	59	25.1±2.2	4.8±0.6	10.2±1.1§	85.0±1.4	5.1±2.4	12.4±2.9
Current smoker	46	21.3±1.8	7.3±1.2	9.9±1.4‡	82.7±1.6‡	1.8±0.3	8.2±1.2
P†		0.27	0.11	0.002	0.003	0.03	0.18
Alcohol drinking							
Very little	161	25.0±1.4	5.3±0.5	7.2±0.6	87.4±0.7	1.8±0.2	15.7±2.6
Occasional	89	24.9±2.4	6.4±0.9	9.7±1.1	83.9±1.4	2.2±0.3	10.5±1.9
Frequent	115	25.2±1.9	5.4±0.7	8.6±0.8	86.1±1.1	3.7±1.6	15.6±2.2
P†		0.99	0.61	0.09	0.08	0.21	0.50
Pesticide usage							
No	288	24.9±1.1	5.5±0.4	7.9±0.4	86.6±0.6	2.4±0.5	14.5±1.7
Yes	12	28.1±1.9	5.2±0.8	11.8±2.5	83.0±2.7	2.4±0.9	9.5±2.8
*P**		0.15	0.76	0.07	0.22	0.99	0.14
UC cases¶							
Tumor stage							
Superficial	100	38.7±3.6	6.7±0.7	13.4±1.4	79.9±1.7	3.2±0.5	11.2±4.2
Locally advanced	38	42.2±9.2	7.0±1.2	13.4±1.7	79.6±1.8	7.4±2.9	9.8±2.4
Metastatic	20	36.5±6.5	7.0±1.3	13.4±4.1	79.7±4.0	9.0±6.7	4.9±1.2
P†		0.91	0.67	0.99	0.94	0.19	0.77
Tumor grade							
I	29	30.6±3.1	6.0±1.1	13.2±3.2	80.7±3.6	2.6±0.7	10.4±3.3
II	65	38.9±5.0	7.1±0.9	12.8±1.1	80.1±1.5	2.7±0.5	8.0±2.5
III	72	41.4±5.5	6.7±0.8	13.8±1.9	79.5±2.0	7.6±2.5	13.7±5.8
P†		0.63	0.75	0.96	0.99	0.17	0.78
UC cases and non-cancer controls							
Controls	313	25.0±1.0	5.5±0.4	7.9±0.4	86.6±0.6	2.4±0.5	14.3±1.5
Cases	177	38.7±3.1	6.7±0.5	13.5±1.0	79.9±1.1	4.8±1.1	10.5±2.6
*P**		<0.0001	0.052	<0.0001	<0.0001	0.04	0.22

*Student's *t*-test.
†ANOVA.
‡,§$P<0.05$ by Student–Newman–Keuls multiple comparisons.
¶Information of tumor staging and grading was not available in 16 and 9 UC patients, respectively.

Table 4
Urinary arsenic profiles of 177 UC patients and 313 non-cancer controls

Variables	Controls *n* (%)	UC cases *n* (%)	Age and gender adjusted OR (95% CI)	Multivariate adjusted OR[a] (95% CI)
Sum of inorganic arsenic, MMA and DMA (μg/g creatinine)				
≤15.4	104 (33.2)	24 (13.6)	1.0	1.0
15.5–26.4	104 (33.2)	44 (24.8)	1.9 (1.1–3.4)	1.6 (0.8–3.0)
>26.4	105 (33.6)	109 (61.6)	5.3 (3.1–9.0)	3.2 (1.8–5.9)
			P_{trend}<0.0001	P_{trend}<0.0001
Inorganic arsenic percentage				
≤2.4	93 (29.7)	40 (22.6)	1.0	1.0
2.5–5.2	113 (36.1)	56 (31.6)	1.2 (0.7–1.9)	1.6 (0.9–2.7)
>5.2	107 (34.2)	81 (45.8)	1.7 (1.1–2.7)	1.2 (0.7–2.0)
			P_{trend}=0.024	P_{trend}=0.70
MMA percentage				
≤3.0	89 (28.4)	37 (20.9)	1.0	1.0
3.1–9.2	111 (35.5)	36 (20.3)	0.8 (0.4–1.3)	0.9 (0.5–1.9)
>9.2	113 (36.1)	104 (58.8)	2.2 (1.3–3.5)	2.8 (1.6–4.8)
			P_{trend}=0.0002	P_{trend}<0.0001
DMA percentage				
≤85.0	114 (36.4)	105 (59.3)	1.0	1.0
85.1–92.5	102 (32.6)	44 (24.9)	0.5 (0.3–0.7)	0.6 (0.4–0.9)
>92.5	97 (31.0)	28 (15.8)	0.3 (0.2–0.5)	0.4 (0.2–0.7)
			P_{trend}<0.0001	P_{trend}=0.0004
Primary methylation index				
≤0.3	91 (29.1)	42 (23.7)	1.0	1.0
0.4–2.0	113 (36.1)	53 (29.9)	1.0 (0.6–1.6)	1.3 (0.7–2.2)
>2.0	109 (34.8)	82 (46.4)	1.6 (1.0–2.6)	3.1 (1.7–5.6)
			P_{trend}=0.029	P_{trend}<0.0001
Secondary methylation index				
≤4.8	98 (31.3)	84 (47.5)	1.0	1.0
4.9–12.7	111 (35.5)	70 (39.5)	0.7 (0.5–1.1)	0.9 (0.5–1.4)
>12.7	104 (33.2)	23 (13.0)	0.3 (0.2–0.4)	0.3 (0.2–0.6)
			P_{trend}<0.0001	P_{trend}=0.001

[a] Multivariate adjusted OR: adjusted for age, gender, education, father and mother ethnicity, alcohol drinking, and pesticide usage.

Although cigarette smoking tended to interact additively with urinary arsenic species in modifying the UC risk, the interactions were all statistically insignificant as shown by the absence of a substantial deviation from 1 in the synergy index. Likelihood ratio tests, however, revealed that smoking effects interact synergistically with PMI (P=0.003).

Since both PMI and SMI levels were associated with an altered UC risk, they were jointly analyzed. Subjects with a PMI ≤1.1 and an SMI >8.2 served as the referent group. A significant trend of progressively increased risks was observed in subjects who had none, either one, or both of the two unfavorable indices (Table 6). More specifically, subjects with both high PMI and low SMI were associated with a significantly increased risk compared to the referent group by logistic regression analysis. Interestingly, the association was statistically significant in both non-smokers and smokers. The UC risk in non-smokers who had a high PMI and a low SMI was 6.6-fold higher (95% CI 2.5–17.4) than that of the referent group.

Discussion

Our data are consistent with other published reports that smoking is associated with an increased risk of UC in a dose-dependent manner (Zeegers et al., 2004). Of particular note, our data showed that those who were medium-dose smokers had an insignificantly elevated risk compared to non-smokers. Only those who were heavy smokers had a significantly increased risk compared to non-smokers. It has been reported that exposure to certain carcinogens in cigarette smoke may contribute to DNA damage, including DNA adducts from arylamines (Vineis et al., 1996). The mechanism of arsenic intoxication also involves the formation of DNA adducts (Tezuka et al., 1993) and DNA damage (Schwerdtle et al., 2003b). The additive and even synergistic interaction between the two risk factors may be a consequence of shared mechanisms of intoxication. Indeed, the following factors have been reported and may explain the synergistic carcinogenic effects observed between benzo(a)pyrene, major component of cigarette smoking and arseniasis: (1) both benzo(a)pyrene and arsenic co-treatment increase benzo(a)pyrene DNA adducts (Evans et al., 2004); (2) arsenite and MMA^{III} increase benzoapyrene-7,8-diol,9,10-expoxide (BPDE)-DNA adduct formation and diminish repair (Schwerdtle et al., 2003a); (3) arsenite inhibits DNA adduct excision induced by different DNA-damaging agents (Chien et al., 2004); and (4) arsenic inhibits the repair of DNA damage induced by benzo(a)pyrene (Tran et al., 2002).

A recent study showed that mortality from bladder cancer declined gradually after improving the supply system of drinking water in southwest Taiwan to eliminate arsenic exposure from artesian well water (Yang et al., 2005). This finding substantiates the association between arsenic exposure and bladder cancer risk. A former study evaluating the association between inorganic arsenic and UC risk focused on the estimated total arsenic ingested from drinking water (Chiou et al., 2001). It would be more relevant if the proportion of urinary arsenic species was used as an indicator of arsenic metabolism. In the study, we demonstrated that urinary arsenic profile indices are significantly associated with the risk of UC. A higher PMI and lower SMI indicate accumulation of MMA by an increased upstream input and reduced downstream output of the arsenic methylation pathway. Recently, MMA^{III} and DMA^{III} have been identified in human urine (Le et al., 2000; Mandal et al., 2001). Many studies have demonstrated that these trivalent methylated arsenic species are more toxic than the inorganic compounds (Petrick et al., 2000; Mass et al., 2001). In addition, MMA^{III} is a potent and irreversible inhibitor of thioredoxin reductase (Lin et al., 2001) that regulates cellular response to oxidative stress in rat hepatocytes. However, the trivalent methylated arsenic metabolites are not stable. Whether they can be detected or not depends on the conditions and temperature of sample storage and concentrations in the urine. The reason why we did not observe any trivalent methylated metabolites in the study is that the analytical method used lacks the requisite specificity. In general, arsenic methylation is

Table 5
Interaction between urinary arsenic indices and cigarette smoking[a]

Variables		*n* of controls/UC cases	Age and gender adjusted OR (95% CI)	Synergy index (95% CI)	Likelihood ratio test
Cigarette smoking	Sum of inorganic arsenic, MMA and DMA (μg/g creatinine)				
No	≤20.3	95/17	1.0	1.35 (0.70–2.66)	χ^2 0.021; P=0.88
No	>20.3	101/68	4.4 (2.3–8.5)	Estimated OR 0.83	
Yes	≤20.3	56/21	2.9 (1.3–6.5)		
Yes	>20.3	49/61	8.2 (3.8–17.8)		
			P_{trend}<0.0001		
Cigarette smoking	Inorganic arsenic percentage				
No	≤3.7	99/39	1.0	1.21 (0.36–4.05)	χ^2 0.038; P=0.84
No	>3.7	97/46	1.1 (0.7–1.9)	Estimated OR 1.01	
Yes	≤3.7	44/27	2.0 (1.0–4.3)		
Yes	>3.7	61/55	2.4 (1.3–4.7)		
			P_{trend}=0.007		
Cigarette smoking	MMA percentage				
No	≤6.1	105/25	1.0	0.86 (0.42–1.77)	χ^2 3.34; P=0.07
No	>6.1	34/20	3.3 (1.4–7.6)	Estimated OR 0.96	
Yes	≤6.1	34/20	3.3 (1.4–7.6)		
Yes	>6.1	71/62	4.7 (2.3–9.5)		
			P_{trend}<0.0001		
Cigarette smoking	DMA percentage				
No	>89.1	101/24	1.00	1.09 (0.53–2.24)	χ^2 2.08; P=0.15
No	≤89.1	95/61	2.9 (1.7–5.2)	Estimated OR 1.03	
Yes	>89.1	37/16	2.9 (1.2–6.9)		
Yes	≤89.1	68/66	5.1 (2.5–10.5)		
			P_{trend}<0.0001		
Cigarette smoking	Primary methylation index				
No	≤1.1	103/29	1.0	0.93 (0.43–2.03)	χ^2 11.78; P=0.003
No	>1.1	93/56	2.2 (1.3–3.8)	Estimated OR 0.88	
Yes	≤1.1	39/27	2.8 (1.3–6.0)		
Yes	>1.1	66/55	3.8 (1.9–7.5)		
			P_{trend}=0.0002		
Cigarette smoking	Secondary methylation index				
No	>8.2	106/30	1.0	1.31 (0.60–2.85)	χ^2 2.14; P=0.16
No	≤8.2	90/55	2.6 (1.5–4.5)	Estimated OR 0.97	
Yes	>8.2	43/18	2.1 (0.9–4.9)		
Yes	≤8.2	62/64	4.5 (2.3–8.9)		
			P_{trend}<0.0001		

[a] Yes includes current and former smokers.

considered a detoxification process where MMA^V and DMA^V are generally considered non-toxic. In fact, inhibition of the secondary methylation process, resulting in higher MMA^V and lower DMA^V percentages in the urine, has been reported to be associated with an increased risk of skin cancers in Mexico (Hopenhayn-Rich et al., 1996). In summary, DMA is the end methylation and detoxification product. UC cases have lower DMA percentages and lower SMI, suggesting weaker detoxification capabilities that lead to a higher cancer risk.

Seaweed contains appreciable amounts of arsenosugars. Consuming arsenosugars present in seaweed (Ma and Le, 1998) or synthetic forms of arsenosugars (Francesconi et al., 2002) may increase urinary DMA concentration. Our previous study, however, has shown that the frequencies of fish, shellfish and seaweed intake do not significantly correlate with urinary arsenic species in Taipei residents (Hsueh et al., 2002). In addition, a study based on people living in southwestern Taiwan revealed that there were no increases in urinary inorganic arsenic, MMA^V or DMA^V levels in subjects who ate seafood (Lin, 1986). This evidence suggests that seafood or seaweed intake does not influence urinary arsenic profiles in our population.

All our cases and controls were intentionally recruited from geographical regions away from the two arsenic-contaminated areas in Taiwan. Thus, the exposure levels should be fairly low in both cases and controls. The sum of

Table 6
Interaction of primary and secondary methylation indices stratified by cigarette smoking status

Primary methylation index	Secondary methylation index	*n* of controls/ UC cases	Age and gender adjusted OR (95% CI)	Multivariate adjusted OR [a] (95% CI)
All subjects (case n=177, control n=313)				
≤1.1	>8.2	70/21	1.0	1.0
>1.1	>8.2	88/31	1.2 (0.6–2.3)	2.0 (1.0–4.3)
≤1.1	≤8.2	77/41	1.9 (1.0–3.3)	1.5 (0.7–3.0)
>1.1	≤8.2	78/84	3.6 (2.0–6.5)	5.0 (2.5–9.9)
			$P_{trend}<0.0001$	$P_{trend}<0.0001$
Non-smokers (case n=85, control n=196)				
≤1.1	>8.2	53/11	1.0	1.0
>1.1	>8.2	53/19	1.8 (0.8–4.2)	2.5 (0.9–7.2)
≤1.1	≤8.2	50/18	1.7 (0.7–4.4)	1.4 (0.5–3.7)
>1.1	≤8.2	40/37	4.7 (2.1–10.6)	6.6 (2.5–17.4)
			$P_{trend}<0.0001$	$P_{trend}=0.0004$
Current and former smokers (case n=92, control n=117)				
≤1.1	>8.2	17/10	1.0	1.0
>1.1	>8.2	35/12	0.5 (0.2–1.5)	1.1 (0.4–3.7)
≤1.1	≤8.2	27/23	1.3 (0.5–3.4)	1.0 (0.3–2.9)
>1.1	≤8.2	38/47	2.1 (0.9–5.1)	3.1 (1.1–8.6)
			$P_{trend}=0.004$	$P_{trend}=0.01$

[a] Multivariate adjusted OR: adjusted for age, gender, education, paternal and maternal ethnicity, alcohol drinking, and pesticide usage.

inorganic arsenic, MMA and DMA levels of UC cases and non-cancer controls in this study were 26.6±1.4 and 22.1±1.8 μg/L (data not shown), respectively. These values were significantly lower than those in our previous study, where the levels for skin cancer patients and healthy controls from the arsenic-contaminated area in Taiwan were 104.1±15.2 and 89.5±7.2 μg/L, respectively (Hsueh et al., 1997). Taken together, these values indicate that our cases and controls were subjects of low exposure. Nonetheless, we still observed an elevated UC risk in subjects with unfavorable urinary arsenic profile. More strikingly, unfavorable PMI and SMI levels confer an increased risk in non-smokers, suggesting that undetectable or negligible environmental arsenic still plays an important role.

Although the allowed arsenic levels in drinking water were not greater than 50 μg/L prior to year 2000, there may be minor differences in arsenic levels between various regions in Taiwan. According to the Taipei Water Department of the Taipei City Government, the average arsenic concentration in Taipei tap water is 0.7 μg/L and ranges from non-detectable to 4.0 μg/L. We do not know whether differential exposure within a low allowable range affects UC risk. However, we have demonstrated that differential urinary arsenic profile in subjects with no arsenic exposure history who had consumed low allowable levels of arsenic in water significantly affected UC risk. This finding suggests that low arsenic levels in drinking water may still be unsafe for susceptible subjects.

Smoking was found to interact with urinary arsenic profile in affecting the UC risk. The synergy indices of the two factors ranged from 0.86 to 1.35, although they were all statistically insignificant. The likelihood ratio test, however, revealed significant synergistic interactions between smoking and inorganic arsenic and PMI. This finding is similar to a recent study that demonstrated a significant interaction between smoking and SMI on the risk of bladder cancer (Chen et al., 2005). These data indicate that subjects who have unfavorable urinary arsenic profile are at a greater risk if they smoke.

Our previous study has shown that as concentrations of urinary selenium and serum alpha-tocopherol increased, percent inorganic arsenic significantly decreased and percent DMA increased (Hsueh et al., 2003). Bangladesh study found that the plasma folate was positively and negatively associated with the DMA and MMA percentage, respectively (Gamble et al., 2005). Several antioxidants, including *N*-acetylcysteine, zinc, methionine and cysteine, when used in conjunction with standard chelating agents, can improve the mobilization and excretion of arsenic compounds (Patrick, 2003). Heavy medications especially those which induce or inhibit cellular glutathione contents, glutathione *S*-transferase, multi-drug resistance-1, multi-drug resistance associated protein-1, etc. may all significantly affect arsenic metabolism. For the vast majority (>95%) of our patients, however, the chance of getting interfered by medications in case subjects was no different from that of controls. Understanding the arsenic methylation pathway has important clinical implications. Chemoprevention for high-risk populations is thus feasible by facilitating the arsenic methylation process and increasing the excretion of toxic metabolites.

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