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Plasma folate level, urinary arsenic methylation profiles, and urothelial carcinoma susceptibility

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Abstract

To elucidate the influence of folate concentration on the association between urinary arsenic profiles and urothelial carcinoma (UC) risks in subjects without evident arsenic exposure, 177 UC cases and 488 controls were recruited between September 2002 and May 2004. Urinary arsenic species including inorganic arsenic, monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) were determined by employing a high performance liquid chromatography-linked hydride generator and atomic absorption spectrometry procedure. After adjustment for suspected risk factors of UC, the higher indicators of urinary total arsenic levels, percentage of inorganic arsenic, percentage of MMA^V, and primary methylation index were associated with increased risk of UC. On the other hand, the higher plasma folate levels, urinary percentage of DMA^V and secondary methylation index were associated with decreased risk of UC. A dose–response relationship was shown between plasma folate levels or methylation indices of arsenic species and UC risk in the respective quartile strata. The plasma folate was found to interact with urinary arsenic profiles in affecting the UC risk. The results of this study may identify the susceptible subpopulations and provide insight into the carcinogenic mechanisms of arsenic even at low arsenic exposure.
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Keywords: Plasma folate level; Urinary arsenic species; Interaction; Urothelial carcinoma

1. Introduction

A urinary bladder cancer in Asia is considered a minor incidence cancer compared to the US and other Western countries. Urothelial carcinoma (UC) is a heterogeneous disease influenced by both environmental exposure and genetic factors. Folate is a water soluble B vitamin, and present in cells as a family of structurally related derivatives comprised of 2-amino-4-hydroxypteridine linked through a methylene carbon to *p*-amino-benzoylpolyglutamate and it is the donor of one-carbon groups in both DNA methylation and DNA synthesis (Suh et al., 2001; Stanger, 2002).

Abbreviations: SAM, *S*-adenosylmethionine; UC, urothelial carcinoma; InAs, inorganic arsenic ($As^{III} + As^V$); MMA^V, monomethylarsonic acid; DMA^V, dimethylarsinic acid; %InAs, inorganic arsenic percentage; %MMA^V, monomethylarsonic acid percentage; %DMA^V, dimethylarsinic acid percentage; PMI, primary methylation index; SMI, secondary methylation index; FFQ, food-frequency questionnaire; OR, odds ratio; CI, confidence interval.

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The epidemiologic evidence relating folate intake and the risk of bladder cancer is contradictory and limited (Bruemmer et al., 1996; Michaud et al., 2000; Zeegers et al., 2001; Schabath et al., 2005). These studies used the food-frequency questionnaire (FFQ) to estimate the folate content from food intake and to assess the relationship between folate intake and risk of bladder cancer. The estimation of folate from FFQ may influence by the recall and information bias; therefore, plasma folate of subjects used as an exposure marker is the one of methods to prevent recall bias (Szklo and Nieto, 2007). Because plasma folate reflects the dietary folate intake (Stanger, 2002), quantification of folate in biological samples may be a more reliable index for cancer risk than estimated folate from the FFQ.

Arsenic is widely distributed in nature and is spread in the environment mainly by water. Ingestion of inorganic arsenic from arterial well water increases the worldwide bladder cancer risk (Chen et al., 1985, 1992; Bates et al., 1992; Abernathy et al., 2003). The metabolism of inorganic arsenic involves reduction and oxidative methylation (Kitchin, 2001; Thomas et al., 2001, 2004; Vahter, 2002; Styblo et al., 2002). After ingestion of inorganic arsenic, the pentavalent inorganic arsenic (arsenate, As^{V}) is readily reduced to trivalent inorganic arsenic (arsenite, As^{III}) in red blood cells (Vahter, 1981) and subsequently methylated to monomethylarsonic acid (MMA^{V}), and to dimethylarsinic acid (DMA^{V}) in the liver (Buchet et al., 1981a,b). Evaluation of arsenic methylation efficiency is mainly based on the relative amounts of the different metabolites present in urine. Previous epidemiological studies from Taiwan were reported that higher cumulative arsenic exposure and less efficient methylation activities were detected in skin and bladder cancer patients than in healthy controls (Hsueh et al., 1995, 1997; Yu et al., 2000; Chen et al., 2003b, 2005).

The evidence for nutritional regulation of arsenic methylation and excretion in humans is limited and rarely considered as a disease risk. A case–control study in West Bengal showed a modestly increased risk of arsenic related skin lesions for individuals with the lowest quintiles of dietary folate intake than those with higher quintiles (Mitra et al., 2004). A recent study found that high plasma folate levels were associated with efficient arsenic methylation pattern (Gamble et al., 2005). These studies all focused on subjects who had high arsenic exposure. The arsenic concentration allowance in public water supplies in Taiwan was 50 μg/L and a new standard of 10 μg/L was announced in 2000. We designed a case–control study to assess the association between individual plasma folate levels and arsenic methylation capability on UC risk among a population having no evident arsenic exposure in Taiwan.

2. Materials and methods

2.1. Study subjects

One hundred and seventy-one patients, age range 24–93 years, with pathologically proven UC 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 methods including endoscopic biopsy or surgical resection of urinary tract tumors followed by histopathological examination by board-certified pathologists. A total of 488 control subjects with no evidence of UC or any other malignancy were recruited from a hospital-based pool, including those receiving senior citizen health examinations at Taipei Medical University Hospital and those receiving health examinations at Taipei Municipal Wan Fang Hospital. These three hospitals are medical center and their clinical clients' bases are similar and located in Taipei approximately 200–300 km away from the arsenic-contaminated areas in Taiwan. In this study, no case subjects or controls have lived in the arsenic-contaminated areas in southwestern (Chen et al., 2003b) or northeastern Taiwan (Chiou et al., 2001). Although we only collected tap water from 37 UC cases and determined the total arsenic levels, the mean ± standard error was 17.14 ± 0.55 μg/L. However, urinary total arsenic levels in cases and controls were 24.47 ± 2.56 μg/L and 24.85 ± 1.06 μg/L, respectively (p-value is 0.89 for Student's t-test). These results may indicate no difference in arsenic exposure between cases and controls.

2.2. Questionnaire interview and specimens collection

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, quantified details of alcohol consumption, cigarette smoking, 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. Regular alcohol drinkers referred to those who consumed alcohol three or more days per week, continuing for at least six months. The Research Ethics Committee of National Taiwan University Hospital, Taipei Medical University Hospital and Taipei Municipal Wan Fang Hospital approved the study. All subjects provided informed consent forms before specimen's collection and questionnaire interview. The study was consistent with the World Medical Association Declaration of Helsinki.

After the questionnaire interview, a 10-mL blood sample was drawn into an EDTA-treated tube and centrifuged at 3000 rpm for 15 min at room temperature after collection. Plasma was separated and stored at −80 °C until analysis. Urine samples were collected simultaneously and drawn into a 1% nitric acid rinsed PE bottle, and stored at −20 °C until used for urinary arsenic speciation. Because questionnaire and biospecimens were obtained before UC cases' acceptance with surgery, radiotherapy, or chemotherapy, any influence of treatment is unlikely.

2.3. Plasma folate assays

Plasma folate levels were determined using a competitive immunoassay kit (Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's instructions. All plasma samples were processed under dim yellow light. Laboratory personnel were unaware of the case–control status. The coefficient of variation was used to test the reliability and the mean coefficient of variation for 23 pairs of replicate plasma samples was 8.8%.

2.4. Determination of urinary arsenic species

It has been shown that urinary arsenic species are stable for at least six months when preserved at −20 °C (Chen et al., 2002); thus, the urine assay was performed within six months post-collection. Frozen urine samples were thawed at room temperature, dispersed by ultrasonication, filtered through a Sep-Pak C18 column (Mallinckrodt Baker Inc., NJ) and the levels of As^{III}, As^{V}, MMA^{V} and DMA^{V} were determined. A 200 μL aliquot of urine was used for the determination of arsenic species by high performance liquid chromatography (Hitachi 7110, Naka, Japan) using columns obtained from Phenomenex (Nucleosil, Torrance, CA). The contents of inorganic arsenic and their metabolites were quantified by

Table 1
Demographic characteristics, urothelial carcinoma risk variables, plasma folate level, and odds ratio of urothelial carcinoma in UC patients and controls

	Cases ($N = 171$)	Controls ($N = 488$)	Odds ratio (95% CI)	
Gender, *n* (%)				
Male	116 (67.84)	286 (58.61)	1.00	
Female	55 (32.16)	202 (41.39)	0.67 (0.46–0.97)[*]	
Education, *n* (%)				
Elementary school	78 (45.61)	136 (27.87)	1.00	
High school	60 (35.09)	171 (35.04)	0.61 (0.41–0.92)[*]	
University	33 (19.30)	181 (37.09)	0.32 (0.20–0.51)[**]	
Smoking, *n* (%)				
Non-smokers	93 (54.39)	335 (68.65)	1.00	
Light smokers (<22 pack-years)	21 (12.28)	77 (15.78)	0.98 (0.58–1.68)	
Heavy smokers (⩾22 pack-years)	57 (33.33)	76 (15.57)	2.70 (1.79–4.08)[**]	
Alcohol consumption, *n* (%)				
Never	111 (64.91)	288 (59.02)	1.00	1.00
Occasional	22 (12.87)	136 (27.87)	0.42 (0.25–0.69)[**]	
Regular	38 (22.22)	64 (13.11)	1.54 (0.97–2.43)[***]	1.89 (1.21–2.96)[**]
Age				
Mean ± SE	64.60 ± 0.99	63.51 ± 0.69	$p = 0.37$[a]	
Folate level (ng/mL)				
Mean ± SE	7.31 ± 0.41	12.29 ± 0.25	$p < 0.01$[a]	

SE: standard error.
[*] $p < 0.05$.
[**] $p < 0.01$.
[***] $0.1 > p > 0.05$.
[a] *p*-Value for student's *t*-test.

Table 2
Distribution of the plasma folate level and urinary arsenic profile among subgroups of demographic characteristics

	N	Folate level (ng/mL)	Urinary arsenic level (μg/g creatinine)	InAs (%)	MMA (%)	DMA (%)
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Gender						
Male	402	10.75 ± 0.30	27.25 ± 1.34	6.21 ± 0.36	9.96 ± 0.49[a]	83.83 ± 0.63[a]
Female	257	11.41 ± 0.38	31.01 ± 1.57	5.60 ± 0.51	6.48 ± 0.48	87.92 ± 0.72
Education						
Elementary school	214	10.91 ± 0.43	34.94 ± 1.57	5.1 ± 0.38	8.31 ± 0.55	86.59 ± 0.71
High school	231	11.23 ± 0.39	27.63 ± 1.84[b]	6.58 ± 0.61	9.45 ± 0.59	83.97 ± 0.91
University	214	10.86 ± 0.40	23.65 ± 1.78[b]	6.18 ± 0.51	7.99 ± 0.72	85.83 ± 0.86
Smoking status						
Non-smokers	428	11.21 ± 0.28[c]	28.96 ± 1.29	5.46 ± 0.35	7.69 ± 0.42[c]	86.86 ± 0.56
Light smokers (<22 pack-years)	98	12.13 ± 0.67[c]	24.63 ± 1.52	6.58 ± 0.83	9.21 ± 1.11	84.21 ± 1.30
Heavy smokers (⩾22 pack-years)	133	9.53 ± 0.49	30.94 ± 2.68	7.17 ± 0.72	11.12 ± 0.78	81.71 ± 1.19
Alcohol consumption						
Never	399	11.07 ± 0.29	29.51 ± 1.50	5.72 ± 0.38	8.26 ± 0.47	86.02 ± 0.63
Occasional	158	11.65 ± 0.51[d]	26.90 ± 1.43	5.56 ± 0.57	8.86 ± 0.76	85.58 ± 1.04
Regular	102	9.75 ± 0.63	28.41 ± 2.00	7.58 ± 0.81	9.57 ± 0.80	82.86 ± 1.06

SE: standard error.
[a] *p*-Value for student's *t*-test, $p < 0.0001$.
[b] Significant different ($p < 0.05$) from elementary school group by ANOVA and Scheffe's test.
[c] Significant different ($p < 0.05$) from heavy smokers group by ANOVA and Scheffe's test.
[d] Significant different ($p < 0.05$) from regular alcohol consumption group by ANOVA and Scheffe's test.

hydride generator-atomic absorption spectrometry (Hsueh et al., 1998). The concentrations of the four arsenic species in a standard solution, a sample, and a sample spiked standard solution were determined by using on-line HPLC-HG-AAS, respectively. Recovery rates of the four arsenic species were estimated according to the following calculation: [(sample spiked standard solution concentration) − sample concentration]/

Table 3
Multivariate-adjusted ORs and 95% CI for associations of plasma folate levels and arsenic methylation capability with the risk of urothelial carcinoma

	Quartiles				p-Value for trend[a]
	Q1	Q2	Q3	Q4	
Folate (ng/mL)					
Range	<7.89	7.90–11.49	11.50–15.99	⩾16.00	
Case/control	104/121	33/120	24/123	10/124	
OR (95% CI)[b]	1.00	0.33 (0.20–0.54)***	0.22 (0.13–0.38)***	0.09 (0.04–0.19)***	<0.0001
Total arsenic (μg/g creatinine)					
Range	<13.09	13.10–20.29	20.30–30.59	⩾30.60	
Case/control	13/121	21/121	47/122	90/123	
OR (95% CI)[b]	1.00	1.48 (0.69–3.12)	3.22 (1.62–6.27)***	6.26 (3.21–12.22) ***	<0.0001
Percentage of inorganic arsenic (%)					
Range	<1.49	1.50–3.69	3.70–6.29	⩾6.30	
Case/control	24/121	40/122	41/122	66/123	
OR (95% CI)[b]	1.00	1.67 (0.93–3.00)	1.67 (0.93–3.01)	2.52 (1.44–4.41)**	0.002
Percentage of MMA (%)					
Range	<0.89	0.9–5.89	5.90–10.89	⩾10.90	
Case/control	25/121	27/122	39/121	80/124	
OR (95% CI)[b]	1.00	0.98 (0.53–1.82)	1.41 (0.79–2.51)	2.75 (1.61–4.71)**	<0.0001
Percentage of DMA (%)					
Range	<81.89	81.90–89.19	89.20–94.39	⩾94.40	
Case/control	73/121	49/122	33/121	16/124	
OR (95% CI)[b]	1.00	0.66 (0.42–1.05)	0.46 (0.27–0.76)**	0.22 (0.12–0.42)***	<0.0001
Primary methylation index					
Range	<0.29	0.30–1.39	1.40–2.79	⩾2.80	
Case/control	73/121	49/122	33/121	16/124	
OR (95% CI)[b]	1.00	1.05 (0.57–1.93)	1.44 (0.87–2.57)	1.99 (1.13–2.48)*	0.0063
Secondary methylation index					
Range	<6.59	6.60–10.59	10.60–19.29	⩾19.30	
Case/control	26/105	32/106	44/106	57/106	
OR (95% CI)[b]	1.00	0.51 (0.30–0.85)**	0.32 (0.18–0.57)**	0.28 (0.15–0.51)**	<0.0001

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
[a] p-Value for trend for category variables.
[b] Adjusted for age, sex, educational attainment, smoking status (pack-year), and alcohol consumption.

standard solution concentration × 100. 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^{V} and DMA^{V} was normalized against urinary creatinine levels (μg/g creatinine). The tap water was digested by 65% nitric acid and determined the total arsenic by HG-AAS. The standard reference material, SRM 2670, containing 480 ± 100 μg/L inorganic arsenic was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD), and was used as a quality standard and analyzed along with urine samples. The mean value of arsenic of SRM 2670 determined by our system was 507 ± 17 (SD) μg/L ($n = 4$). Arsenic methylation indices were assessed as the percentages of various urinary arsenic species in the sum of inorganic arsenic, MMA^{V} and DMA^{V}. 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} (Hsueh et al., 1998; Vahter, 2002).

3. Statistical methods

Student's *t*-test was used to compare the differences in continuous variables between UC cases and controls. Logistic regression models were used to estimate the univariate and multivariate-adjusted odds ratio (OR) and the 95% confidence interval (CI). Cutoff points for continuous variables were the respective quartiles of the controls. For the joint effect analysis, the cutoff points for the plasma folate levels and arsenic methylation indices were the medians of the controls, respectively. The joint effects of cigarette smoking and plasma folate and urinary arsenic methylation indices, or plasma folate levels and urinary arsenic methylation indices on the UC risk were evaluated by estimating the synergy index. An observed synergy index value that departs substantially from the expected additive null, i.e., synergy index greater than 1, suggests an additive interaction effect. The OR values and their variance covariance matrix were then used to calculate synergy index and 95% CIs (Hosmer and Lemeshow, 1992). SAS version 8.2 was used for all statistical analyses.

4. Results

The UC risks were significantly influenced by gender, education level, cigarette smoking status, alcohol consumption and plasma folate levels strata (Table 1). Males or

lower education or regular alcohol drinkers or lower plasma folate levels subjects had significantly higher UC risk than females or higher education or alcohol non-drinkers or higher plasma folate levels subjects. There was no significant difference of the mean age of cases at 64.60 years and controls at 63.51 years. Folate (7 μmol/L) in plasma was recommended as normal standard by Institute of Medicine (1998). In this study, the proportion under 7 μmol/L plasma folate levels was 57% (98/171) and 18% (88/488) in UC cases and controls, respectively.

The distribution of the plasma folate levels and urinary arsenic profiles among subgroups of gender, education, cigarette smoking, and alcohol consumption was shown in Table 2. Male had a higher urinary $\%MMA^V$ and a lower $\%DMA^V$ than female. The higher total urinary arsenic levels were observed for subjects who had education level of elementary school than those had high school and university. Compared to heavy smokers, non-smokers had a higher folate levels and a lower $\%MMA^V$. Light smokers had a higher folate levels than heavy smokers. Occasional alcohol drinkers had a higher folate levels than regular alcohol drinkers. The results of Table 2 suggested that male, lower education level, heavy smokers, and regular alcohol drinkers may have an inefficient methylation process of metabolizing arsenic to DMA^V.

Table 3 presents the multivariate-adjusted ORs for the associations between plasma folate levels or arsenic methylation indices and UC risk. In general, there was a dose–response association between the quartile of plasma folate levels or arsenic methylation indices and UC risk after adjustment for suspected UC risk factors. The plasma folate levels appeared to have an inverse association with the risk of UC having OR of quartiles strata of 1.0, 0.33, 0.22, and 0.09, respectively ($p < 0.0001$ for the trend test). The creatinine-adjusted total arsenic levels appeared to have an increased UC risk, the OR of quartiles strata were 1.0, 1.48, 3.22, and 6.26, respectively ($p < 0.0001$ for the trend test). Subjects with either lower $\%MMA^V$, or lower %InAs, or lower PMI, or higher $\%DMA^V$ or higher SMI were suggested a more efficient capacity to methylate inorganic arsenic to DMA^V, and the more efficient capacity was the less risk of UC.

Table 4 examined the joint effects of the plasma folate levels in combination with various arsenic methylation profiles. The folate concentrations were divided into two categories based on the median values of controls. Subjects with a higher plasma folate levels and possessing efficient arsenic methylation profiles were the reference group. The OR was 5.24 (95% CI, 1.93–14.20) for individuals with a higher plasma folate levels and a higher total arsenic levels. The OR was 5.93 (95% CI, 2.19–16.01) for individuals with a lower folate levels and lower total arsenic levels as compared to those with a higher folate and a lower total arsenic levels. The highest risk group occurred in those with a lower folate levels and a higher total arsenic levels having an adjusted OR 19.58 and 95% CI, 7.63–50.23.

Table 4
Joint effects of plasma folate level and arsenic methylation capability index on urothelial carcinoma risk

Arsenic methylation profiles	Folate (ng/mL)				S index (95% CI)
	⩾11.5		<11.5		
	Case/control	OR (95% CI)	Case/control	OR (95% CI)	
Total arsenic (μg/g creatinine)					
<20.30	5/118	1.00	29/125	5.93 (2.19–16.01)**	2.02 (1.24–3.28)**
⩾20.30	29/129	5.24 (1.93–14.20)**	108/116	19.58 (7.63–50.23)***	
Percentage of inorganic arsenic (%)					
<3.70	10/124	1.00	54/119	5.78 (2.77–12.03)***	1.18 (0.71–1.98)
⩾3.70	24/123	2.37 (1.07–5.23)*	83/122	8.30 (4.02–17.12)***	
Percentage of MMA (%)					
<5.90	17/122	1.00	35/121	2.12 (1.10–4.08)*	4.43 (1.18–16.57)*
⩾5.90	12/125	0.92 (0.44–1.91)	102/120	5.61 (3.10–10.16)**	
Percentage of DMA (%)					
⩾89.20	19/105	1.00	39/106	3.10 (1.54–6.24)**	2.51 (1.25–5.03)**
<89.20	12/106	1.68 (0.97–3.57)	89/106	7.98 (4.15–15.35)***	
Primary methylation index					
<1.40	19/105	1.00	39/106	2.10 (1.12–3.96)*	4.64 (0.74–29.07)
⩾1.40	12/106	0.60 (0.27–1.33)	89/106	4.25 (3.38–7.58)***	
Secondary methylation index					
⩾10.60	13/98	1.00	29/89	2.40 (1.16–4.98)*	3.77 (1.29–10.96)*
<10.60	14/90	1.19 (0.52–2.71)	91/96	7.00 (3.58–13.67)**	

Adjusted for age, sex, educational attainment, smoking status (pack-year), and alcohol consumption.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Furthermore, the interaction between plasma folate levels and urinary total arsenic levels were statistically significant on the additive scale (S index 2.02, $p < 0.01$). The phenomena were identical to the %InAs, %MMA^V, %DMA^V, PMI and SMI. Except for %InAs and PMI, interactions between arsenic methylation profiles and folate on additive scale were statistically significant.

Table 5 examined the joint effects of cigarette smoking and plasma folate levels or cigarette smoking and various arsenic methylation indices for UC risk. The reference group was the non-smokers or light smokers with a high plasma folate levels or an efficient arsenic methylation profiles. Heavy smokers with a higher plasma folate levels had 2.52-fold (95% CI, 1.08–5.85) risk of UC. A 4.51-fold UC risk (95% CI, 2.71–7.51) for non-smokers or light smokers with a lower plasma folate levels, and the risk increased to 8.25 (95% CI, 1.23–16.09) for heavy smokers with a lower plasma folate levels. Similar results were obtained for the various arsenic methylation profiles. For example, non-smokers or light smokers with the higher total arsenic levels had an OR of 4.27 (95% CI, 2.39–7.31), heavy smokers with the lower total arsenic levels had an OR of 2.95 (95% CI, 1.32–6.61). The highest risk was found in heavy smokers with the higher total arsenic levels (adjusted OR 8.89; 95% CI, 4.38–18.03). The joint effect was shown statistically insignificant between cigarette smoking and plasma folate levels or between cigarette smoking and urinary arsenic indices. Comparing Tables 4 and 5, the interaction was statistically significant between the plasma folate levels and the arsenic methylation indices on UC risk but not between the plasma folate levels and cigarette smoking on UC risk.

5. Discussion

High folate levels and efficient arsenic methylation profiles are associated with a decreased risk of UC. Both factors exhibited a strong interaction on UC risk. More interestingly, the marked interaction between folate and arsenic methylation indices was greater than that between cigarette smoking and folate or between cigarette smoking and arsenic methylation indices. This study demonstrated that the UC risk is enhanced by a synergistic interaction between low plasma folate levels and inefficient arsenic methylation capability among a population having no evident arsenic exposure in Taiwan.

Numerous epidemiological studies had conflicting results between folate intake and bladder cancer risk. In a folate supplement case–control study, total folate intake was inversely related with bladder cancer, OR 0.54, 95% CI, 0.31–0.93 for the highest quartile compared to the

Table 5
Joint effects of smoking status, and plasma folate level or arsenic methylation capability index on urothelial carcinoma risk

Folate and arsenic methylation profiles	Smoking status				S index (95% CI)
	Non-smokers or light smokers (<22 pack years)		Heavy smokers (>22 pack years)		
	Case/control	OR (95% CI)	Case/control	OR (95% CI)	
Plasma folate level (ng/mL)					
⩾11.50	23/123	1.00	11/34	2.52 (1.08–5.85)*	1.44 (0.72–2.87)
<11.50	91/199	4.51 (2.71–7.51)***	46/42	8.25 (1.23–16.09)***	
Total arsenic (μg/g creatinine)					
<20.30	20/104	1.00	14/39	2.95 (1.32–6.61)**	1.51 (0.76–2.99)
⩾20.30	94/208	4.27 (2.49–7.31)***	43/37	8.89 (4.38–18.03)***	
Percentage of inorganic arsenic (%)					
<3.70	46/211	1.00	18/32	2.16 (1.05–4.45)*	1.32 (0.46–3.74)
⩾3.70	68/201	1.55 (1.00–2.93)*	39/44	3.26 (1.76–6.03)***	
Percentage of MMA (%)					
<5.90	37/217	1.00	15/26	2.79 (1.25–6.24)*	1.03 (0.44–2.42)
⩾5.90	77/195	2.25 (1.43–3.54)***	42/50	4.16 (2.24–7.73)***	
Percentage of DMA (%)					
⩾89.20	26/216	1.00	13/29	2.33 (1.02–5.37)*	1.34 (0.58–3.08)
<89.20	78/196	2.44 (1.52–3.89)***	44/47	4.75 (2.52–8.90)***	
Primary methylation index					
<1.40	37/183	1.00	21/28	3.21 (1.53–6.72)**	0.70 (0.28–1.75)
⩾1.40	67/167	1.94 (1.21–3.11)**	34/45	3.22 (1.69–6.14)***	
Secondary methylation index					
⩾10.60	34/163	1.00	8/24	1.38 (0.53–3.63)	1.97 (0.68–6.71)
<10.60	65/146	2.24 (1.37–3.65)**	40/40	4.22 (2.19–8.11)***	

Adjusted for age, sex, educational attainment, and alcohol consumption.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

lowest quartile (Sharp and Little, 2004; Schabath et al., 2005). Other studies did not observe any association between folate and bladder cancer risk (Michaud et al., 2002; Holick et al., 2005). The evidence for a relationship between folate and bladder cancer from epidemiological studies is limited and mainly focused on the folate ingested from dietary food rather than the concentration in the body. Plasma folate is a precise marker to reflect dietary folate intake (Stanger, 2002) and the result of this study found that the UC risk was raised with the plasma folate levels decreasing. Other nutrition such as methionine, vitamins B-6 and B-12, which interacted metabolically with folate in the one-carbon metabolism processes, may also influence cancer risk (Bailey, 2003). Dark-green vegetables and certain other fruits and vegetables are rich sources of folate and B vitamins (Gebhardt et al., 2007), and epidemiological study suggested that high fruits and vegetables intake reduced the risk of bladder cancer (Steinmaus et al., 2000).

Besides nutrition, life styles such as cigarette smoking or regular alcohol drinking also influenced the bioavailability of folate. In accordance with previous studies (Piyathilake et al., 1994; Schabath et al., 2005), reduced plasma folate level was observed with either heavy cigarette smoking or regular alcohol drinking in our study. Recently a study showed that cigarette smoking might result in localized deficiencies of folate coenzymes, tetrahydrofolate and 5,10-methylenetetrahydrofolate (Gabriel et al., 2006). On the other hand, alcohol drinking also affected the folate-dependent metabolism including inhibition of enzymes central to one-carbon metabolism (methionine synthase, methylenetetrahydrofolate reductase, methionine adenosyltransferase 1A, glycine *N*-methyltransferase, and *S*-AdoHcyst hydrolase), and stimulation of serine synthesis and inhibition of thymidine synthesis (Mason and Choi, 2005). These evidences suggested that the folate bioavailability was influenced by cigarette smoking and alcohol drinking.

A recent study showed that bladder cancer mortality declined gradually after eliminating arsenic exposure from artesian well water by improving the drinking water supply system in southwest Taiwan (Yang et al., 2005). This finding substantiates the association between arsenic exposure and bladder cancer risk. A previous study evaluated the relationship between UC risk and arsenic exposure by focusing on the total arsenic levels in drinking water (Chiou et al., 2001). It would be more relevant if urinary arsenic species were used as indicators of arsenic metabolism (Francesconi and Kuehnelt, 2004; Steinmaus et al., 2005). The order of toxicity in arsenic species by oral exposure in mice was $As^{III} > DMA^{V} > MMA^{V}$ (Shiomi, 1994). Humans excreted appreciable amounts of MMA^{V} in the urine compared with other mammals. The methylated metabolites were virtually undetectable in the urine of marmoset monkeys that were administered with inorganic arsenic (Vahter et al., 1995; Vahter, 2002). Studies indicated that marmoset, tamarin monkeys and guinea-pigs were deficient in methyltransferase activity (Zakharyan et al., 1996; Healy et al., 1997). Rat revealed significantly high methylating activity and it is the only species that excreted significant amounts of TMAO (Aposhian, 1997; Cohen et al., 2006). The variation in the metabolism of inorganic arsenic between human and other animals may result in variant susceptibility to inorganic arsenic carcinogenesis.

This study demonstrated that the profile of urinary arsenic metabolites was significantly associated with the risk of UC. A high PMI and low SMI indicated an accumulation of MMA^{V} by an increased upstream input and a reduced downstream output of the arsenic methylation pathway metabolites. In addition to bladder cancer, our previous study reported that skin cancer patients had higher indicators of %InAs and %MMA^{V}, and had lower indicators of %DMA^{V}, and PMI than healthy controls (Hsueh et al., 1997). These results are compatible with the study of Chen et al. (2003a,b) that showed skin and bladder cancer patients had a lower SMI in high chronic arsenic exposure area. Arsenic methylated metabolites in urine have been reported to be biomarkers for disease states and disease susceptibility in other ethnicities (Valenzuela et al., 2005). The key metabolic intermediates, MMA^{III} and DMA^{III}, have been identified in human urine (Mandal et al., 2001), and these trivalent methylated arsenicals are more toxic than inorganic arsenic compounds (Styblo et al., 2000; Petrick et al., 2001). Levels of trivalent methylated metabolites in the urine are expected to be significantly low, since these metabolites have short half-lives and, therefore, were considered not to be suitable markers for arsenic methylation at the present time (Mass et al., 2001; Gong et al., 2001; Nesnow et al., 2002; Francesconi and Kuehnelt, 2004). The arsenic methylation pattern may remain stable over time and be influenced by factors such as methylation related enzymes, genes, environmental exposure, smoking habits and diet (Francesconi and Kuehnelt, 2004; Steinmaus et al., 2005).

This study discovered the synergy indices of the plasma folate and urinary methylation profiles ranged from 1.18 to 4.64, revealing significant synergistic interactions between folate and total arsenic levels, %MMA^{V}, %DMA^{V}, or SMI. Another study also reported that %DMA^{V} was significantly positive and %MMA^{V} was negatively related to the plasma folate levels (Gamble et al., 2005). Recently, a clinical study showed that folate supplementation improved the arsenic methylation efficiency, such as a low urinary %MMA^{V} and a high %DMA^{V} in the supplemented group compared to the placebo group (Gamble et al., 2006). These observations suggested that subjects with low folate levels were at a high disease risk especially when they had low arsenic methylation capability. Indeed, the arsenic methylation profiles may be altered by the folate status in the body.

Previous animal studies provided evidence that folate can influence arsenic methylation, excretion, and toxicity. (Kim, 2000; Moyers and Bailey, 2001; Townsend et al.,

2004). Folate is essential for methylation in the human body and its metabolism is important for the biosynthesis of *S*-adenosylmethionine (SAM), a substrate for methylation including DNA methylation (Choi and Mason, 2000; Gregory and Quinlivan, 2002; Choi and Friso, 2005) and arsenic methylation (Vahter, 1981, 2002). Inorganic arsenic is enzymatically methylated and consumes SAM in the biotransformation process. The molecular mechanism of arsenic carcinogenesis may cause DNA damage or alter the methylation status of DNA (Zhao et al., 1997; Okoji et al., 2002; Huang et al., 2004; Reichard et al., 2007); this process is similar to folate deficiency. In human colon epithelial cells, a folate deficiency increased uracil mis-incorporation 2–3-fold and lowered the cells' capacity for DNA repair in response to oxidation or alkylation (Choi et al., 1998; Duthie et al., 2000b). Uracil mis-incorporation or DNA strand breakage was significantly increased in rat lymphocytes after 4–8 weeks or 10 weeks of folate deficient diet intake (Duthie et al., 2000a,b,c). The individual susceptibility may result from the differences in the genes controlling the metabolism of xenobiotics, DNA repair, cell transport, immune responses, antioxidant defenses and cell cycle control (Huang et al., 2004). Several animal studies reported that folate binding or transport gene knock-out mice or rabbits decreased biotransformation and excretion of arsenic, and these animals were more susceptible to arsenic and induced the defects (Vahter and Marafante, 1987; Spiegelstein et al., 2003, 2005a,b; Spuches et al., 2005). Based on these observations, the interaction between folate and the arsenic methylation pathways may cause UC risk through the DNA methylation or DNA repair systems.

One limitation of this study is that the UC cases are prevalent cases and we cannot rule out the possibility that folate and/or arsenic methylation patterns changed after the participants became UC cases. Dietary habits lacked from the questionnaires are another limitation of this study; however, the plasma folate levels are a good biomarker to reflect the dietary folate intake (Piyathilake et al., 1994; Stanger, 2002).

Inorganic arsenic is a human carcinogen; however, a good animal model has not yet been found. The arsenic methylation process may be less efficient and leads to more severe toxicity in humans than in several other species (Cohen et al., 2006). In this study, we established the dose–response relationships among two credible markers, plasma folate levels and arsenic methylation indices, and UC risk. It was indicated that folate interacted with urinary arsenic profiles in affecting the UC risk. These results may identify the susceptible subpopulations and provide insight into the carcinogenic mechanisms of arsenic even at low arsenic exposure.

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