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Arsenic methylation and bladder cancer risk in Taiwan

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Abstract

Objective: The mechanism of arsenic detoxification in humans remains unclear. Data are especially lacking for low-level arsenic exposure. We hypothesize that arsenic methylation ability, defined as the ratios of monomethylarsonic acid (MMA(V))/inorganic arsenic (primary arsenic methylation index, PMI) and dimethylarsinic acid (DMA(V))/MMA(V) (secondary arsenic methylation index, SMI), may modify the association between cumulative arsenic exposure (CAE, mg/L-year) and the risk of bladder cancer. In this study we investigated the relationship among arsenic methylation ability, CAE, and the risk of bladder cancer in a hospital-based case–control study in southwestern Taiwan.

Methods: From January 1996 to December 1999 we identified 49 patients with newly diagnosed cases of bladder cancer at the National Cheng-Kung University (NCKU) Medical Center; controls consisted of 224 fracture and cataract patients selected from the same medical center. The levels of four urinary arsenic species: arsenite (As(III)), arsenate (As(V)), MMA(V), and DMA(V)) were determined in all subjects by using the high-performance liquid chromatography hydride-generation atomic absorption spectrometry (HPLC-HGAAS). CAE was estimated by using published data collected in a survey from 1974 to 1976.

Results: Compared to a CAE $\leqq$ 2 mg/L-year, CAE > 12 mg/L-year was associated with an increased risk of bladder cancer (multivariate odds ratio (OR) 4.23, 95% confidence interval (CI) 1.12–16.01), in the setting of a low SMI ($\leqq$4.8). Compared to women, smoking men (OR 6.23, 95% CI 1.88–20.62) and non-smoking men (OR 3.25, 95% CI 0.95–11.06) had higher risks of bladder cancer. Given the same level of PMI, smoking men (OR 9.80, 95% CI 2.40–40.10) and non-smoking men (OR 4.45, 95% CI 1.00–19.84) had a higher risk of bladder cancer when compared to women. With the same level of SMI, both smoking men (OR 6.28, 95% CI 1.76–22.39) and non-smoking men (OR 3.31, 95% CI 0.84–12.97) had a higher risk of bladder cancer when compared to women.

Conclusions: Subjects with low SMI have a substantially increased risk of bladder cancer, especially when combined with high CAE levels.

Introduction

Epidemiologic studies have shown that moderate-to-high levels of arsenic exposure are consistently associated with an increased risk of bladder cancer. However, the association with low-level arsenic exposure remains unclear. The US Environmental Protection Agency (EPA) used a Taiwan study of skin cancer [1] to

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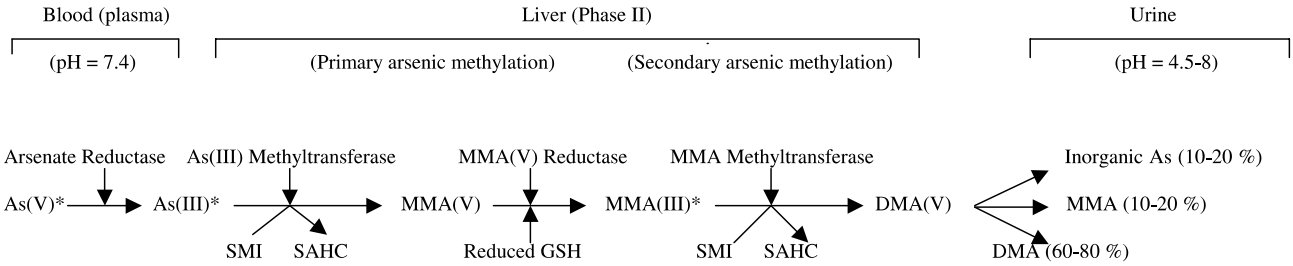
estimate the risks of low exposure to arsenic, utilizing extrapolation of data from high-dose exposure. This approach, however, may lead to overestimation of the risks of skin cancer at low-level exposures. Because of the ubiquity of arsenic in the earth's crust, large populations are exposed to it in drinking water. Groundwater supplies in Bangladesh, India (West Bengal), China (Inner Mongolia), and parts of the US (California, Nevada, Alaska, and Utah) have high levels of arsenic. The EPA has estimated that about 350,000 people in the US drink water containing more than 50 μg/L of arsenic and about 2.5 million people drink water containing more than 25 μg/L [2].

In addition to its environmental ubiquity, arsenic has been used in a variety of ways. Since the nineteenth century it has been widely used in the manufacture of glass, feed additives, pigment for aniline dye, wallpaper, soap, medication, wood preservatives, pesticides, metalloids, semiconductor applications, and other materials [3]. Humans may be exposed to arsenic *via* ingestion, or inhalation (the primary means). For example, previous studies have shown that arsenic exposure may lead to cancers of the liver, kidney, bladder, prostate, lymphoid tissue, skin, lung, colon, and nasal cavity, as well as blackfoot disease (BFD), ischemic heart disease, hyperpigmentation, hyperkeratosis, diabetes, meningioma, and other health effects [4, 5]. The International Agency for Research on Cancer (IARC) has classified arsenic as a group 1 carcinogen, *i.e.* with sufficient evidence of human carcinogenicity [6]. The updated rank order of arsenical toxicity, defined by carcinogenesis and vascular disorders, is as follows: monomethylarsonous acid, MMA(III) > arsenite As(III) > arsenate As(V) > monomethylarsonic acid, MMA(V) = dimethylarsinic acid, DMA(V) [7]. Currently, the drinking water standard for arsenic is 10 μg/L in both the US and Taiwan.

After entering the human body, inorganic arsenic is taken up readily by red blood cells and then distributed primarily to the liver, kidneys, spleen, lungs, intestines, and skin [8–10]. The target organ-systems of arsenic exposure comprise respiratory, gastrointestinal (GI) tract, cardiovascular, nervous, and hematopoietic systems [8]. As(V) is reduced to As(III) in blood and then methylated to MMA(V) and DMA(V), which are less toxic forms than inorganic arsenic, and have lower tissue affinity. Therefore, methylation of inorganic arsenic, mainly in the liver, is considered a detoxification procedure and differs from most other phase II reactions because it generally decreases the water solubility and masks functional groups that might otherwise be conjugated by the phase II enzymes [11]. The presumed arsenic methylation pathway in the human body is shown in Figure 1 [12–20]. Factors that play an important role in arsenic methylation ability include dose, forms of arsenic administered and its routes of administration, lifestyle (*e.g.* diet, smoking, and alcohol consumption), genetic polymorphisms in metabolism, and probably other sources of inter-individual variability.

Several epidemiologic studies in Taiwan [5, 21–26] have related mortality, prevalence, and lifetime risk of bladder cancer to arsenic exposure. Other studies [7, 8, 13–15, 27–32] have reported the concentrations of urinary arsenic species. Arsenic methylation ability in one study [8] was reported to be higher in women than in men, and this ability diminishes with age. A cross-sectional study [27], using a population on the northeastern coast of Taiwan with low (44% <50 μg/L) to moderate (72% <300 μg/L) drinking-water arsenic



***Possible carcinogens**

Methyl donors: SMI, folate, methylene, choline, and methylcobalamin (CH_3B_{12})

Cofactors: L-cysteine, dithiothreitol, 2-mercaptoethanol, and reduced GSH

Inhibitors: methylated species in selenium (selenate, selenite, and selenide) and periodateoxidized adenosine (PAD)

Fig. 1. Presumed arsenic methylation pathway in human body (12–20).

exposure, demonstrated similar arsenic methylation ability between men and women. However, no study thus far has explored the relationship between arsenic methylation ability and the risk of bladder cancer. In this study we hypothesize that arsenic methylation ability modifies the association between cumulative arsenic exposure and the risk of bladder cancer.

Materials and methods

Study design

The research protocol was approved by the Institutional Review Boards of the Harvard School of Public Health and National Cheng-Kung University (NCKU). From January 1996 to December 1999 a hospital-based case–control study was conducted in southwestern Taiwan. Forty-nine newly diagnosed bladder cancer patients and 224 controls (fracture and cataract patients), all over 30 years old, and matched on gender and age (±5 years) were recruited from the NCKU Medical Center. The NCKU Medical Center is the main medical referral center for cancer diagnosis and treatment for residents in Tainan City and surrounding rural communities. Long-bone fracture conditions and cataract patients were chosen because these conditions are not presently known to be associated with arsenic exposure, and to avoid overestimating on exposure when using community controls in this setting.

Newly diagnosed bladder cancer patients recruited in our study had confirmed transitional cell carcinoma (TCC) (82%), with 18% missing specific cell-type information. The pathologic diagnosis was performed at the NCKU Pathology Department using the *International Classification of Diseases*, version 9 (ICD-9), (code 188).

Based upon the mechanism of arsenic methylation described above (Figure 1), primary and secondary methylation index were defined as the ratios of urinary MMA(V)/inorganic arsenic and DMA(V)/MMA(V), respectively. Since arsenic exposure has occurred mainly from consumption of drinking water, it was necessary to estimate arsenic exposure over time. The cumulative arsenic exposure index (CAE) [5] was defined as: $\mathrm{CAE} = \sum[(\text{Average arsenic concentration of artesian well water in mg/L})_i \times (\text{Duration of consuming artesian well water in years})_{i:\ \text{unit of village}}]$. The average arsenic concentration of artesian well water was estimated from questionnaire data based upon the village in which they lived 30 years ago and the average arsenic level in well water for each village, obtained from the Taiwan Provincial Institute of Environmental Sanitation survey of 83,656 wells between 1974 and 1976 [33].

At the time of urine collection, trained interviewers administered a questionnaire to each subject, and all interviewers were blinded to exposure status and study hypotheses. Information collected from the questionnaire included demographic information (gender, age, ethnicity, height, body weight, education, and working experience); personal habits (cigarette smoking, consumption of alcohol, tea, and coffee, and use of hair dye); disease history; other relevant questions (history and duration of each residence, sources of drinking water, medication usage, and occupations); and diet information recalled over the past year. Persons who failed to complete or refused to answer the questionnaire were excluded. Completed questionnaires were obtained on 74% of eligible subjects. To verify parts of the questionnaire, lists of names and addresses of residents in the study area were obtained from local household registration offices where socio-demographic characteristics (*e.g.* gender, age, educational level, marital status, and occupation) of all residents are registered and updated annually.

Laboratory analysis

A spot urine sample was collected from each subject and was stored in a −20 °C freezer at the NCKU Medical Center. The urinary levels of four arsenic species: (As(III), As(V), MMA(V), and DMA(V)) were determined in all subjects by using the HPLC-HGAAS [8]. Detection limits for As(III), As(V), MMA(V), and DMA(V) were 0.45, 0.23, 0.53, and 0.61 μg/L, respectively. Total arsenic species in urine was defined as the sum of As(III), As(V), MMA(V), and DMA(V).

Statistical analysis

We used multiple logistic regression models to estimate the multivariate odds ratios (OR) (and 95% confidence intervals (CI)) of bladder cancer associated with arsenic methylation ability and CAE. We also assessed whether smoking status modified the association between arsenic methylation ability and the risk of bladder cancer by stratifying by arsenic methylation ability. To control for potential confounding we adjusted for the following risk factors in the multivariate models: age, gender, body mass index (BMI), CAE, cigarette smoking, the use of hair dye, and education.

Results

When compared with the controls, bladder cancer patients tended to have higher CAE ($p = 0.36$), higher

primary arsenic methylation index (PMI) ($p = 0.30$), lower secondary arsenic methylation index (SMI) ($p = 0.41$), more males ($p = 0.002$), more smokers and ex-smokers ($p = 0.003$), and lower education level ($p = 0.13$) (Table 1). Bladder cancer patients and controls were similar with regard to age, hair dye usage, and BMI.

When compared with subjects with low CAE ($\leqq 2$ mg/L-year), subjects with medium CAE (>2 to $\leqq 12$ mg/L-year) had an OR of 0.57 (95% CI 0.18–1.83) and subjects with high CAE (>12 mg/L-year) had an OR of 2.01 (95% CI 0.84–4.77) (p for trend = 0.24).

Methylation ability alone did not predict bladder cancer risk. As compared with subjects with low PMI ($\leqq 0.9$), subjects with medium PMI (>0.9 to $\leqq 2.7$) had an OR of 1.24 (95% CI 0.54–2.88) and subjects with high PMI (>2.7) had an OR of 0.53 (95% CI 0.21–1.35) (p for trend = 0.29) (data not shown). When compared with subjects with low SMI ($\leqq 4.8$), subjects with medium SMI (>4.8 to $\leqq 9.4$) had an OR of 0.79 (95% CI 0.36–1.76) and subjects with high SMI (>9.4) had an OR of 0.62 (95% CI 0.26–1.49) (p for trend = 0.19) (data not shown).

When compared with the reference group of subjects who had low CAE ($\leqq 2$ mg/L-year), high CAE (>12 mg/L-year) was associated with an increased risk of bladder cancer (OR 2.04, 95% CI, 0.43–9.68), given PMI $\leqq 0.9$ (Table 2). A non-significant increase of the risk of bladder cancer was observed in the subgroup of subjects who had high CAE (>12 mg/L-year) and PMI > 0.9 (OR 2.27, 95% CI 0.63–8.23). When compared with low CAE ($\leqq 2$ mg/L-year), subjects who had medium CAE (>2 to 12 mg/L-year) do not have a statistically significant increased risk of bladder cancer when PMI was low ($\leqq 0.9$) (OR 1.10, 95% CI 0.16–7.35). A similar phenomenon was observed when PMI was high (>0.9) (OR 0.40, 95% CI 0.08–2.03).

When compared with the reference group of subjects who had low CAE ($\leqq 2$ mg/L-year), high CAE (>12 mg/L-year) was associated with an increased risk of bladder cancer (OR 4.23, 95% CI 1.12–16.01), given a low SMI ($\leqq 4.8$) (Table 2). When SMI is high (>4.8), a

Table 1. Characteristics of subjects with bladder cancer and controls

Variable	Bladder cancer (n = 49)		Controls subjects (n = 224)	
	No.	Percent	No.	Percent
Age (years)				
>40–60	11	22.4	48	21.4
>60–70	23	46.9	92	41.1
>70	15	30.6	84	37.5
Gender				
Male	41	83.7	131	58.4
Female	8	16.3	93	41.5
BMI				
<18.5	2	4.1	17	7.6
18.5-23	28	57.1	84	37.5
>23	19	38.8	123	54.9
Smoking (pack-years)				
Never	17	34.7	131	58.5
>0-10	4	8.2	17	7.6
>10-20	5	10.2	15	6.7
>20	23	46.9	61	27.2
Hair dye				
Yes	15	30.6	76	33.9
No	34	69.4	149	66.5
Education				
Illiterate	10	20.4	62	26.7
Elementary	24	49.0	105	46.9
High school and above	15	6.7	57	25.4
Average CAE (mg/L-year)	10.1		8.1	
PMI	5.9		4.6	
SMI	8.24		11.5	

[a] CAE denotes the cumulative arsenic exposure and these data were collected from a questionnaire.
[b] PMI denotes primary methylation index calculated from urinary MMA(V)/inorganic arsenic.
[c] SMI denotes secondary methylation index calculated from urinary DMA(V)/MMA(V).

Table 2. Odds ratios for bladder cancer by cumulative arsenic exposure and arsenic methylation ability

Variable	Group	Number of cases	Number of controls	Multivariate adjusted odds ratio (95% confidence interval)	p_{trend}	$p_{continuous}$
PMI[a]						
Low (≦0.9)						
Low CAE[b]	0–2 mg/L-year	13	55	1.00	0.39	0.34
Medium CAE[b]	>2–12 mg/L-year	2	9	1.10 (0.16–7.35)		
High CAE[b]	>12 mg/L-year	5	13	2.04 (0.43–9.68)		
High (>0.9)						
Low CAE[b]	0–2 mg/L-year	17	90	1.00	0.47	0.59
Medium CAE[b]	>2–12 mg/L-year	2	22	0.40 (0.08–2.03)		
High CAE[b]	>12 mg/L-year	5	13	2.27 (0.63–8.23)		
SMI[c]						
Low (≦4.8)						
Low CAE[b]	0–2 mg/L-year	14	58	1.00	0.06	0.09
Medium CAE[b]	>2–12 mg/L-year	2	12	0.54 (0.09–3.33)		
High CAE[b]	>12 mg/L-year	7	8	4.23 (1.12–16.01)*		
High (>4.8)						
Low CAE[b]	0–2 mg/L-year	16	87	1.00	0.85	0.72
Medium CAE[b]	>2–12 mg/L-year	2	19	0.49 (0.10–2.50)		
High CAE[b]	>12 mg/L-year	3	18	1.12 (0.26–4.77)		

All models adjusted for age, gender, BMI, cumulative arsenic exposure, cigarette smoking, hair dye usage, and education.

* p-Value < 0.05.

[a] PMI denotes primary methylation index calculated from urinary MMA(V)/inorganic arsenic.

[b] CAE denotes the cumulative arsenic exposure and those data were collected from a questionnaire.

[c] SMI denotes secondary methylation index calculated from urinary DMA(V)/MMA(V).

reduction in the risk of bladder cancer was observed in subjects who had high CAE (>12 mg/L-year) (OR 1.12, 95% CI 0.26–4.77).

Given the same level of PMI, both smoking men (OR 9.8, 95% CI 2.40–40.10) and non-smoking men (OR 4.45, 95% CI 1.00–19.84) had a higher risk of bladder cancer when compared to women (model 1) (Figure 2). With the same level of SMI, both smoking men (OR 6.28, 95% CI 1.76–22.39) and non-smoking men (OR 3.31, 95% CI 0.84–12.97) had a higher risk of bladder cancer when compared to women (model 2) (Figure 2). Overall, smoking men (OR 6.23, 95% CI 1.88–20.62) and non-smoking men (OR 3.25, 95% CI 0.95–11.06) had higher risks of bladder cancer than women (model 3) (Figure 2).

Discussion

We observed a statistically significant interaction between the SMI and the level of CAE in relation to the risk of bladder cancer. Since the predominant function of SMI is to produce non-toxic or less toxic metabolites, this finding is consistent with the hypothesis that a higher SMI decreases the detrimental effect of high CAE on the risk of bladder cancer. In addition, we found a significant interaction between smoking status and CAE in relation to the risk of bladder cancer.

It is unlikely that the statistically significant interactions found are due to chance, since similar results have been found in our study in this region using a larger sample size and different disease outcome (skin cancer). The odds ratios for the CAE alone or for the methylation phenotype alone are close to unity, whereas the combined odds ratio for SMI and CAE is 4.23. Thus, our data suggest that the increase in bladder cancer risk can be attributed to an interaction between SMI and CAE, and is not due solely to an increase in the CAE. In addition, our data confirm that an increase in the risk of bladder cancer is attributed to smoking status.

Several biomarkers, such as toenails, hair, and blood, have been used to estimate arsenic exposure, but none of these markers assesses arsenic methylation. Urinary arsenic levels may reflect exposure to arsenic within the past 48 hours [34], but are variable and do not reflect arsenic methylation ability. Therefore, we used phenotypic indicators (MMA(V)/inorganic arsenic and DMA(V)/MMA(V)), to estimate the arsenic methylation ability for subjects exposed to arsenic 30 years ago. Diet (especially DMA(V), arsenobetaine, arsenosugars, and arsenocholine in seafood) might complicate the use of urine as an exposure biomarker [35, 36]. However, a

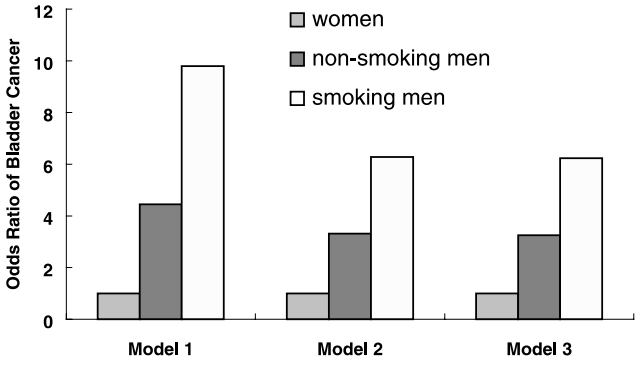


Fig. 2. Multivariate odds ratio of bladder cancer to arsenic methylation ability and smoking status. All models adjusted for age, gender, BMI, CAE, cigarette smoking, hair dye, and education. Model 1: additionally adjusted for PMI. Model 2: additionally adjusted for SMI. Model 3: without adjusting for arsenic methylation ability. Because of the small number of smoking women we combined smoking and non-smoking women as one group and divided men into two groups by smoking status.

study in southwestern Taiwan indicated that no increases were observed for urinary inorganic arsenic, MMA(V), or DMA(V) for subjects who ate seafood [35], possibly because different kinds of seafood may contain different arsenic species.

Because of the high toxicity of MMA(III), SMI plays an important role in transforming MMA(III) into the less toxic metabolite, DMA(V). Assuming equal MMA(V) reductase activity, subjects with high PMI or low SMI could accumulate MMA(III) in the body and prolong the contact of MMA(III) with bladder epithelium, thus increasing the risk of bladder cancer [16, 17]. This hypothesis is consistent with our observations. SMI plays a more important role in lowering, but not eliminating, the risk of bladder cancer, while PMI exhibits a weak effect in the opposite direction that needs to be replicated in other studies. Populations in a non-arsenic-contaminated environment excrete 10–20% inorganic arsenic, 10–20% MMA(V), and 60–80% DMA(V) in urine. Our population had average PMI (4.8 for cases and 4.1 for controls) that was similar to other arsenic-exposed populations. In Taiwan the methylation profiles MMA(V)/inorganic arsenic have been reported to range from 2.3 to 5.2 [5, 37]. Other areas in the world (China, Chile, Nevada, and California) have reported urinary MMA(V)/inorganic arsenic values of 0.4–2.3 [28].

Our study has several strengths. Although several studies determined arsenic methylation ability by the level of arsenic species [7, 14, 15, 27], this is the first study to our knowledge to explore the association between arsenic methylation ability and the risk of bladder cancer. The average total urinary arsenic in our study was much lower than other studies in Taiwan (the BFD-endemic area: 206.13–325.11 μg/L [8]; northeastern coast of Taiwan: 173 μg/L [27]). However, this apparent difference can be explained by the fact that the total arsenic measured using HGAAS in the above studies actually contains organic arsenic species other than MMA(V) and DMA(V). This has led to difficulty in estimating arsenic methylation ability because some of the organic arsenic species are not methylated from inorganic arsenic in the human body and are excreted in urine unchanged.

Our study has several limitations that need to be considered when interpreting our results. MMA(III) has a very short half-life and converts to MMA(V) in a very short time [38]; thus, it appears in trace amounts in urine. More and more attention has been paid on developing the techniques to determine the level of MMA(III) in urine [38–41]. However, this technique was still under development when we conducted this study. In addition, our study lacks the statistical power to determine the effect of arsenic methylation ability on the risk of bladder cancer for subjects in the medium CAE group. In addition, arsenic exposure information was collected from questionnaires by area of residence, and by using the average arsenic level of well water in each village. This estimation does not permit an evaluation of individual dose–response relationships between arsenic exposure and the risk of bladder cancer. Hence, our exposure estimation might lead to non-differential misclassification of exposure, resulting in an underestimation of the association between CAE and the risk of bladder cancer. However, having found a statistically significant association between SMI and bladder cancer, the odds ratios are likely underestimates. Selection bias is unlikely in this study because the NCKU Medical Center, a referral center, covers 80% of all diseases requiring specialists in the region, and our cases are likely to be representative of bladder cancer affecting the general community. Recall bias is a potential confounder for all case–control studies. Therefore, we validated most of the information obtained from questionnaires (*e.g.* gender, age, occupation, and residence) from the household registration (*i.e.* census) office.

Almost all previous studies from Taiwan have reported high arsenic exposure. Our study may be generalizable to populations with high and low levels of CAE but not to those with medium CAE, due to sample size limitations. Additional studies are needed to evaluate the roles of nutrition, genetic polymorphisms, tumor cell-type [21], and individual arsenic exposure data [42]. In addition, CAE is a risk factor for other diseases, such as Blackfoot disease (BFD), skin cancer,

and kidney cancer. More research is needed to explore the long-term health consequences of low-level arsenic exposure and the role of individual variation in arsenic metabolism.

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