CME Article #2

# Arsenic Methylation and Skin Cancer Risk in Southwestern Taiwan

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## Learning Objectives

- Review the epidemiology and clinical consequences of arsenic exposure.
- Explain the relationships found in this case-control study among cumulative arsenic exposure, primary and secondary arsenic methylation, gender, and skin cancer.
- Understand how widely—if at all—the study findings may be generalized to populations other than the Taiwanese.

## Abstract

*Arsenic is a known carcinogen, but data are especially lacking on the health effects of low-level exposure, and on the health significance of methylation ability. We conducted a case-control study (76 cases and 224 controls from 1996 to 1999) in southwestern Taiwan to explore the association among primary and secondary arsenic methylation index (PMI and SMI, respectively), cumulative arsenic exposure (CAE), and the risk of skin cancer. As compared with the controls, the skin cancer group reported more sun exposure ($P = 0.02$) and had a lower BMI ($P = 0.03$), as well as lower education level ($P = 0.01$). Skin cancer patients and controls were similar with regard to age, gender, smoking and alcohol consumption. Given a low SMI (≤5), CAE > 15 mg/L-year was associated with an increased risk of skin cancer (OR, 7.48; 95% CI, 1.65–33.99) compared to a CAE ≤2 mg/L-year. Given the same level of PMI, SMI, and CAE, men had a higher risk of skin cancer (OR, 4.04; 95% CI, 1.46–11.22) when compared to women. Subjects with low SMI and high CAE have a substantially increased risk of skin cancer. Males in all strata of arsenic exposure and methylation ability had a higher risk of skin cancer than women.* (J Occup Environ Med. *2003;45: 241–248*)

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Funding for the study was provided by the National Institute of Health grants ES 05947 and ES 00002.

David C. Christiani received an NIH-NIEHS grant for this research.

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**DOI: 10.1097/01.jom.0000058336.05741.e8**

Many epidemiologic studies have shown an association between moderate-to-high level of arsenic exposure and skin cancer. However, the association with low-level arsenic exposure remains unclear. The US Environmental Protection Agency (EPA) used a Taiwan study[1] to estimate the risks of low exposure to arsenic, utilizing extrapolation of data from high-dose exposure, which may lead to overestimation of risk. Because of the ubiquity of arsenic in the earth's crust, large populations are exposed to it in drinking water. Currently, groundwater supplies in Bangladesh, India (West Bengal), China (Inner Mongolia), and parts of the United States (California, Nevada, Alaska, and Utah) have high levels of arsenic. The EPA has estimated that about 350,000 people in the United States 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

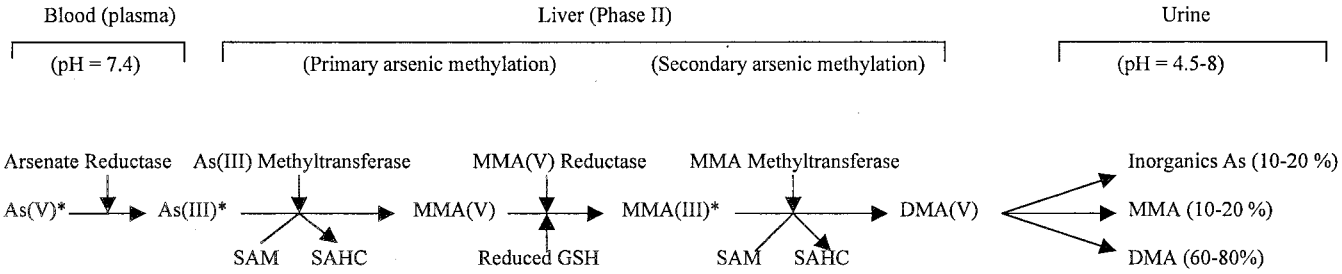


**Fig. 1.** Presumed arsenic methylation pathway in human body[12–20]. *Possible carcinogens: Methyl donors, SAM, folate, methylene, choline, and methylcobalamin ($CH_3$ $B_{12}$); Cofactors, L- cysteine, dithiothreitol, 2- mercaptoethanol, and reduced GSH; Inhibitors, methylated species in selenium (selenate, selenite, and selenide) and periodateoxidized adenosine (PAD).

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, ie, with sufficient evidence of human carcinogenicity.[6] An updated rank order of arsenical toxicity, defined by carcinogenesis and vascular disorders in the former arsenic endemic of southwestern Taiwan, is as follows: MMA(III) > As(III) > As(V) > MMA(V) = DMA(V).[7] Currently, the drinking water standard for arsenic (EPA) in both Taiwan and the US is 10 μg/L.

After ingestion, 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 include the 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, occurring mainly in the liver, is considered a detoxification procedure. Methylation of inorganic arsenic is opposite to most other phase-II reactions because it generally decreases 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 Fig. 1.[12–20] Factors that play an important role in arsenic methylation ability include dose, forms and routes of arsenic absorption, lifestyle (eg, diet, smoking, and alcohol consumption), possibly genetic polymorphisms in metabolism, and other sources of inter-individual variability.

Yu et al[21] reported that a higher ratio of MMA to DMA (ie, 1/SMI) in cases than in matched controls, but the difference was not statistically significant. The small sample size (26 cases) probably accounts for this phenomenon. Also, subjects with a higher percentage of MMA (>15%) had a significantly higher risk of skin cancer (OR = 5.5).

In two studies by Hsueh et al,[8,22] arsenic methylation ability was reported to be higher in women than in men, and this ability diminishes with age. They also found that an elevated proportion of MMA in total urinary arsenic level was associated with an increased risk of skin cancer. However, their study was limited in that the urinary proportion of MMA does not necessarily represent adequately arsenic methylation ability. Moreover, their sample size was small ($n$ = 16). In this study, using a case-control design with a larger sample size, we hypothesize that arsenic methylation ability modifies the association between cumulative arsenic exposure and the risk of skin 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 the southwestern Taiwan. Seventy-six newly diagnosed skin cancer patients and 224 controls (fracture and cataract patients), all over 30 years old, 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 over-matching on exposure when using community controls in this geographic region, the BFD-endemic areas.

Newly diagnosed skin cancer patients were recruited in our study and were confirmed in the following proportions: 29.2% Bowen's disease (BD), 33.3% basal cell carcinoma (BCC), and 47.2% squamous cell carcinoma (SCC). The pathologic diagnosis was performed at the NCKU Pathology Department using the International Classification of Diseases, version 9 (ICD-9), (code 173).

Based upon the mechanism of arsenic methylation described above (Fig. 1), primary and secondary arsenic methylation indexes (PMI and SMI) were defined as the ratios of urinary MMA(V)/inorganic arsenic and DMA(V)/MMA(V), respectively. Because arsenic exposure occurred mainly from the consumption

**TABLE 1**
Sociodemographic Characteristics of Skin Cancer Cases and Controls

| Variable | Skin Cancer (n = 76) | | Control Subjects (n = 224) | |
|---|---|---|---|---|
| | *N* | % | *N* | % |
| Age | | | | |
| >30–50 | 12 | 15.8 | 14 | 6.3 |
| >50–70 | 38 | 50.0 | 127 | 56.7 |
| >70 | 26 | 34.2 | 83 | 37.0 |
| Gender | | | | |
| Male | 48 | 63.2 | 131 | 58.5 |
| Female | 28 | 36.8 | 93 | 41.5 |
| Duration of sun exposure (hours/month) | | | | |
| 1st tertile | 24 | 31.6 | 102 | 45.5 |
| 2nd tertile | 28 | 36.8 | 60 | 26.8 |
| 3rd tertile | 24 | 31.6 | 62 | 27.7 |
| BMI | | | | |
| <18.5 | 6 | 7.9 | 18 | 8.0 |
| 18.5–23 | 33 | 43.4 | 83 | 37.1 |
| >23 | 27 | 35.5 | 123 | 54.9 |
| Smoking (pack-years) | | | | |
| Never | 52 | 68.4 | 131 | 58.5 |
| >0–10 | 3 | 4.0 | 17 | 7.6 |
| >10–20 | 4 | 5.3 | 15 | 6.7 |
| >20 | 17 | 22.4 | 61 | 27.2 |
| Education | | | | |
| Illiterate | 38 | 50.0 | 62 | 27.7 |
| Elementary school and above | 38 | 50.0 | 162 | 72.3 |
| Alcohol consumption | | | | |
| Yes | 18 | 24 | 53 | 24 |
| No | 58 | 76 | 169 | 76 |

of drinking water, it was necessary to estimate arsenic exposure over time. The cumulative arsenic exposure index (CAE)[5] was defined as: CAE = $\Sigma$[(average arsenic concentration of artesian well water in mg/L)$_i$ × (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.[23]

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 and urine samples were obtained on 80% and 77% of cases and controls, respectively.

## Laboratory Analysis

A spot urine sample was collected from each subject and was preserved 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 (and 95% CIs) of skin cancer associated with arsenic methylation ability (PMI & SMI) and CAE. We also accessed whether gender modified the association between arsenic methylation ability and the risk of skin cancer. To control for potential confounding, we adjusted for the following risk factors for skin cancer in the multivariate models: age, gender, body-mass index (BMI, kg/m$^2$), CAE, cigarette smoking, the use of hair dye, and education.

# Results

As compared with the controls, the skin cancer group reported more sun exposure ($P = 0.02$) and had a lower BMI ($P = 0.03$), as well as lower education level ($P = 0.01$) (Table 1). Skin cancer patients and controls were similar with regard to age, gender, smoking, and alcohol consumption (Table 1).

Cases had significantly higher mean CAE level than controls (mean = 15.33 versus 8.14, $P = 0.002$; Table 2). All other variables did not differ significantly between cases and controls. Cases had higher As(III)%, MMA%, and PMI than controls, but these differences were not statistically significant. Men had both lower mean PMI (3.40, $P = 0.04$) and mean SMI (10.02, $P = 0.32$) than women (PMI = 5.66, SMI = 11.71).

High urinary As(III)% was significantly associated with a higher risk of skin cancer (Table 3; 3$^{rd}$ versus 1$^{st}$ tertile; multivariate OR, 2.39; 95% CI, 1.02 to 5.60). All other proportions of urinary arsenic species were

**TABLE 2**
Percentage of Urinary Arsenic Species, Arsenic Methylation Ability, and Cumulative Arsenic Exposure of Skin Cancer Cases and Controls

| | Cases | | | Controls | | | |
|---|---|---|---|---|---|---|---|
| **Variable** | ***N*** | **Mean** | **SD[a]** | ***N*** | **Mean** | **SD[a]** | ***P*** |
| Total As[b] (μg/L) | 73 | 43.03 | 24.03 | 215 | 43.71 | 29.34 | 0.86 |
| % of As(III) | 73 | 2.59 | 2.99 | 215 | 2.01 | 2.47 | 0.10 |
| % of As(V) | 73 | 5.03 | 5.10 | 215 | 5.33 | 6.54 | 0.72 |
| % of As(III) + As(V) | 73 | 7.62 | 5.86 | 215 | 7.34 | 6.83 | 0.75 |
| % of MMA(V) | 73 | 13.85 | 11.35 | 215 | 12.67 | 10.81 | 0.42 |
| % of DMA(V) | 73 | 78.52 | 24.31 | 215 | 79.99 | 12.55 | 0.39 |
| PMI[c] | | | | | | | |
| Men | 44 | 3.51 | 5.46 | 121 | 3.36 | 4.65 | 0.86 |
| Women | 29 | 6.84 | 14.27 | 80 | 5.23 | 11.95 | 0.56 |
| Total | 73 | 4.83 | 9.98 | 201 | 4.10 | 8.38 | 0.55 |
| SMI[d] | | | | | | | |
| Men | 43 | 11.80 | 15.77 | 121 | 9.38 | 12.85 | 0.32 |
| Women | 25 | 8.65 | 10.73 | 75 | 12.73 | 13.39 | 0.17 |
| Total | 68 | 10.64 | 4.12 | 196 | 10.66 | 13.13 | 0.99 |
| CAE (mg/L-year)[e] | | | | | | | |
| Men | 48 | 14.67 | 20.64 | 120 | 7.63 | 15.65 | 0.02* |
| Women | 27 | 16.50 | 18.52 | 85 | 8.85 | 15.30 | 0.03* |
| Total | 68 | 15.33 | 19.80 | 205 | 8.14 | 15.48 | 0.002* |

| | Men | | | Women | | | |
|---|---|---|---|---|---|---|---|
| **Variable** | ***N*** | **Mean** | **SD[a]** | ***N*** | **Mean** | **SD[a]** | ***P*** |
| PMI | 165 | 3.40 | 4.86 | 109 | 5.66 | 12.56 | 0.04* |
| SMI | 164 | 10.02 | 13.66 | 100 | 11.71 | 12.85 | 0.32 |

[a] SD denotes standard deviation.
[b] Total As is the sum of As(III), As(V), MMA(V), and DMA(V).
[c] PMI denotes primary arsenic methylation ability calculated from urinary MMA(V)/inorganic arsenic.
[d] SMI denotes secondary arsenic methylation ability calculated from urinary DMA(V)/MMA(V).
[e] CAE denotes cumulative arsenic exposure and this data was collected from a questionnaire.
* *P*-value $<0.05$.
Note: For subjects with zero level of MMA(V) or DMA(V), we assume his/her PMI or SMI is missing, respectively. Therefore some of the case number in this table is smaller than the number of subjects mentioned in the study design (n = 76). So do the controls.

not significantly associated with the risk of skin cancer. As compared with subjects with low CAE (≦2 mg/L-year), subjects with medium (>2 to ≤15 mg/L-year) and high (>15 mg/L-year) CAE had multivariate ORs of 1.87 (95% CI, 0.79–4.45) and 2.99 (95% CI, 1.30–6.87), respectively ($p$ for trend = 0.007). Methylation ability alone did not predict skin cancer risk. As compared with subjects who had PMI ≤ 1, subjects with 1<PMI ≤ 3 and PMI >3 had multivariate ORs of 0.93 (95% CI, 0.41–2.14) and 1.30 (95% CI, 0.58–2.94), respectively ($p$ for trend = 0.53). As compared with subjects who had SMI ≤ 5, subjects with 5 < SMI ≤ 9.4 and SMI >9.4 had multivariate ORs of 1.20 (95% CI, 0.52–2.77) and 0.90 (95% CI, 0.38–2.14), respectively ($p$ for trend = 0.81). Given the same level of PMI, SMI, and CAE, men had a higher risk of skin cancer (OR, 4.04; 95% CI, 1.46–11.22) when compared to women.

There is no evidence to suggest a two-way interaction between CAE and PMI for the risk of skin cancer (Table 3). Given low PMI (≤1), high CAE (>15 mg/L-year) was associated with an increased risk of skin cancer (multivariate OR, 2.95; 95% CI, 0.69 to 12.60) as compared with subjects who had low CAE (≤2 mg/L-year) (Table 3). A not significant increase of the skin cancer risk was observed in this subgroup of subjects who had high CAE (>15 mg/L-year) and high PMI (>1) (multivariate OR, 2.59; 95% CI, 0.86–7.85) as compared with low CAE (≤2 mg/L-year). Because of the small sample size, subjects with medium CAE (>2–15 mg/L-year) had a not significant risk of skin cancer at all level of PMI. As compared with low CAE (≤2 mg/L-year), subjects who had medium CAE (>2 to 15 mg/L-year) did not have significant risks of skin cancer when PMI was low (≤1) (multivariate OR, 2.11; 95% CI, 0.38–11.71). A similar phenomenon was observed when PMI was high (>1) (multivariate OR, 1.89; 95% CI, 0.64–5.61).

There is evidence to suggest a two-way interaction between CAE and SMI on the risk of skin cancer. Given a low SMI (≤5), a high CAE (>15 mg/L-year) was associated with an statistically significantly increased risk of skin cancer (multivariate OR, 7.48; 95% CI, 1.65–33.99) as compared with low SMI subjects who had low CAE (≤2 mg/L-year) (Table 3). A corresponding reduction in the risk of skin cancer was observed in the subgroup of subjects who had high CAE (>15 mg/L-year) and high SMI (>5) (multivariate OR, 1.89; 95% CI, 0.60–6.01). Because of small sample size, subjects with medium CAE (>2–15 mg/L-year) had a not significant risk of skin cancer at all levels of SMI as compared with subjects who had low CAE (≤2 mg/L-year). Given a high SMI(>5), subjects who had medium CAE (>2 to 15 mg/L-year) had higher risk of skin cancer (multivariate OR, 1.68; 95% CI, 0.52–5.39) as compared with subjects who had low CAE(≤2 mg/L-year). A similar phenomenon was observed when SMI was low (≤5) (multivariate OR, 2.53; 95% CI, 0.54–11.85).

**TABLE 3**
Odds Ratios for Skin Cancer in Relation to Percentage of Urinary Arsenic Species, Cumulative Arsenic Exposure, Arsenic Methylation Ability, and Cumulative Arsenic Exposure Stratified by Arsenic Methylation Ability (Interaction)

| Variable | Group | Number of cases | Number of controls | Multivariate adjusted OR (95% CI) | $P_{Trend}$ |
|---|---|---|---|---|---|
| Urinary As(III)% | 1st tertile | 16 | 80 | 1.00 | |
| | 2nd tertile | 27 | 69 | 2.25 (0.88–5.72) | |
| | 3rd tertile | 30 | 66 | 2.39 (1.02–5.60)* | 0.09 |
| Urinary As(V)% | 1st tertile | 22 | 74 | 1.00 | |
| | 2nd tertile | 24 | 72 | 1.07 (0.41–2.76) | |
| | 3rd tertile | 27 | 69 | 1.24 (0.47–3.31) | 0.96 |
| Urinary MMA(%) | 1st tertile | 20 | 76 | 1.00 | |
| | 2nd tertile | 27 | 69 | 1.78 (0.77–4.12) | |
| | 3rd tertile | 26 | 70 | 1.41 (0.59–3.38) | 0.48 |
| Urinary DMA(%) | 1st tertile | 24 | 72 | 1.00 | |
| | 2nd tertile | 31 | 65 | 2.02 (0.90–4.52) | |
| | 3rd tertile | 18 | 78 | 0.76 (0.32–1.85) | 0.63 |
| CAE (mg/L-year)[a] | 0–2 mg/L-year | 14 | 32 | 1.00 | |
| | >2–15 mg/L-year | 22 | 25 | 1.87 (0.79–4.45) | |
| | >15 mg/L-year | 39 | 145 | 2.99 (1.30–6.87)* | 0.007* |
| PMI[b] | ≤1 | 24 | 67 | 1.00 | |
| | >1–3 | 23 | 69 | 0.93 (0.41–2.14) | |
| | >3 | 26 | 65 | 1.30 (0.58–2.94) | 0.53 |
| SMI[c] | ≤5 | 25 | 63 | 1.00 | |
| | >5–9.4 | 22 | 65 | 1.20 (0.52–2.77) | |
| | >9.4 | 21 | 68 | 0.90 (0.38–2.14) | 0.81 |
| Gender[d] | Male | 48 | 131 | 4.04 (1.46–11.22)* | |
| | Female | 29 | 93 | 1.00 | |
| Low PMI[b] (≤1) | | | | | |
| Low CAE[a] | ≤2 mg/L-year | 14 | 56 | 1.00 | |
| Medium CAE[a] | >2–15 mg/L-year | 3 | 4 | 2.11 (0.38–11.71) | |
| High CAE[a] | >15 mg/L-year | 11 | 19 | 2.95 (0.69–12.60) | 0.14 |
| High PMI[b] (>1) | | | | | |
| Low CAE[a] | ≤2 mg/L-year | 25 | 89 | 1.00 | |
| Medium CAE[a] | >2–15 mg/L-year | 3 | 9 | 1.89 (0.64–5.61) | |
| High CAE[a] | >15 mg/L-year | 19 | 25 | 2.59 (0.86–7.85) | 0.07 |
| Low SMI[c] (≤5) | | | | | |
| Low CAE[a] | ≤2 mg/L-year | 16 | 60 | 1.00 | |
| Medium CAE[a] | >2–15 mg/L-year | 3 | 6 | 2.53 (0.54–11.85) | |
| High CAE[a] | >15 mg/L-year | 13 | 15 | 7.48 (1.65–33.99)* | 0.008* |
| High SMI[c] (>5) | | | | | |
| Low CAE[a] | ≤2 mg/L-year | 23 | 83 | 1.00 | |
| Medium CAE[a] | >2–15 mg/L-year | 3 | 7 | 1.68 (0.52–5.39) | |
| High CAE[a] | >15 mg/L-year | 17 | 29 | 1.89 (0.60–6.01) | 0.22 |

* P-value $< 0.05$.

All models controlled for age, gender, BMI, cumulative arsenic exposure, sun exposure, cigarette smoking, alcohol consumption, and education.

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

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

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

[d] Model also controlled for PMI and SMI.

## Discussion

We observed a statistically significant interaction between the SMI and the level of CAE in relation to the risk of skin cancer. The predominant function of SMI is to produce nontoxic or less toxic metabolites. These findings support our hypothesis that a higher SMI decreases the detrimental effect of high CAE on the risk of skin cancer. In addition, we found that men had a significant risk of skin cancer than women for all strata of CAE and arsenic methylation abilities (PMI and SMI). This suggests that different behavioral and perhaps other genetic factors may lead to a higher risk of skin cancer among men.

For studies in the BFD-endemic area in southwestern Taiwan, Hsueh et al[22] reported that urinary MMA(V)% were significantly associated with skin cancer (BCC and

BD), a finding not seen in our study. Yu et al[21] found a higher urinary MMA: DMA ratio (ie, 1/SMI) among cases (BCC, BD, hyperpigmentation, and hyperkeratosis) than controls, a finding that is consistent with our results. They also found that MMA(V)% was significantly associated with skin cancer. Of note is that their cases had a lower arsenic exposure level (0.77 mg/L) than controls (0.98 mg/L), a finding contrary to almost all other studies. Because of small case numbers for both studies ($n$ = 16 and 26, respectively), Hsueh and Yu et al. failed to find the significant interaction among CAE, arsenic methylation abilities, and the risk of skin cancer that was observed in our study. Furthermore, urinary MMA(V)% alone may not be a good indicator of arsenic methylation ability. In Hsueh's study, 69% of cases had high CAE (>17.7 mg/L-year), similar to Yu et al study. Our study was larger, and 63% of cases ($n$ = 76) had a CAE <10.6 mg/L-year (ie, < 0.05 mg/L), much lower than both the Hsueh et al and Yu et al studies.

Del Razo et al[14] reported that exposed individuals (arsenic exposure: 0.408 mg/L) bearing cutaneous signs had a significantly longer time of exposure, higher urinary concentrations and percent of MMA and MMA/inorganic arsenic values (ie, PMI), as well as significantly lower DMA/MMA (ie, SMI) than exposed individuals without cutaneous signs in four villages in Mexico. Because they examined both exposed and nonexposed individuals, and arsenic-related cutaneous signs instead of skin cancer, their study design is different from ours and, thus, not comparable. Moreover, Del Razo et al studied a population with current higher arsenic exposure among exposed subjects (0.408 mg/L) than most of our subjects. Finally, the small number of cases ($n$ = 35) in their study is another limitation.

Sun exposure is an important risk factor for skin cancer. We found that the second tertile of sun exposure had significantly higher risk of skin cancer as compared to the lowest tertile (multivariate OR, 2.66; 95% CI, 1.18–6.02). But this phenomenon was not apparent when we compared the highest tertile to the lowest one (multivariate OR, 1.74; 95% CI, 0.76–3.99). A possible explanation is that the people at high risk have stronger skin reaction to sunlight and, therefore, tend to stay indoors.

Several biomarkers have been used to estimate the amount of arsenic exposure (such as toenails, hair, and blood) but none of these markers can assess arsenic methylation. Urinary arsenic levels may reflect exposure to arsenic within the past 48 hours,[24] but are variable and do not reflect arsenic methylation ability. Therefore, we used phenotypic indicators (urinary MMA(V)/inorganic arsenic and DMA(V)/MMA(V)) to estimate the arsenic methylation ability for subjects exposed to arsenic 30 years ago. The similarity in total urinary arsenic level, but different arsenic methylation ability, between cases and controls may indicate that arsenic methylation ability, rather than urinary arsenic level, is an indicator of past arsenic exposure. Diet (especially DMA(V), arsenobetaine, arsenosugars, and arsenocholine in seafood) might complicate the use of urine as an exposure biomarker.[25,26] Lin et al[25] found that diet (especially DMA(V) in seafood) did not increase urinary inorganic arsenic, MMA(V), or DMA(V) for subjects who ate seafood in southwestern Taiwan, because of species of seafood different from those in other places.

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 will accumulate MMA(III) in the keratin of skin, thus increase the risk of skin cancer.[16,17] This hypothesis is consistent with our observations. SMI plays a more important role in lowering, but not eliminating, the risk of skin cancer, while PMI exhibits a weak effect in the opposite direction that needs to be replicated in other studies. Populations in a nonarsenic contaminated environment excrete 10 to 20% inorganic arsenic, 10 to 20% MMA(V), and 60 to 80% DMA(V) in urine. Our population had an average PMI (4.8 for cases and 4.1 for controls) that was similar to other arsenic exposed populations. In Taiwan the methylation profiles have been reported to be: PMI = 2.3 to 5.2.[5,22] Other areas in the world (China, Chile, Nevada, and California) have reported urinary PMI of 0.4 to 2.3.[27] Our study also has a high average SMI (10.6 for cases and 10.7 for controls) compared to others which reported the SMI values from 2.3 to 4.9.[5,22,27] Because of the better detoxification ability of second arsenic methylation process (ie, higher SMI), our population had a lower risk of skin cancer than other populations, given the same level of CAE.

Our study has several strengths. Although several studies determined arsenic methylation ability by the level of arsenic species,[7,14,15,28] this is the first study that detected a statistically significant association between arsenic methylation ability and the risk of skin cancer. It is worth noting that the average total urinary arsenic in our study was much lower than other studies in Taiwan (the BFD-endemic area: 206.13 to 325.11 μg/L;[8] northeast coast of Taiwan: 173 μg/L[28]). However, this apparent difference can be explained by the fact that the total arsenic measured by using HGAAS in the above studies actually contains organic arsenic species other than MMA(V) and DMA(V) that are either not methylated from inorganic arsenic or nontoxic and of no interest in this study. The total arsenic done by HGAAS has led to difficulty in estimating the arsenic methylation ability. Hence we used the sum of As(III), As(V), MMA(V), and DMA(V) as the total arsenic to estimate arsenic methylation ability.

MMA(III) has a very short half-life and converts to MMA(V) in a very short time;[29] 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.[29–32] However, this technique was still under development when we conducted this study. Because arsenic-exposure information was collected from questionnaires by area of residence (the average arsenic level of well water in each village), our exposure estimation might lead to a nondifferential misclassification, resulting in an underestimation of the association between CAE and the risk of skin cancer. However, having found a statistically significant association between SMI and skin cancer, the ORs are likely underestimates. Selection bias is unlikely in this study because the NCKU Medical Center, a referral center, covers 80% of all disease requiring specialists in the region and our cases are likely to be representative of skin cancer affecting the general community. Recall bias is a potential confounder for all case-control studies. To verify part of the questionnaire, lists of names and addresses of residents in the study area were obtained from local household registration (ie, census) offices where socio-demographic characteristics (eg, gender, age, educational level, marital status, and occupation) of all residents are registered and updated annually. Besides arsenic ingestion via drinking water, other minor sources including diet, inhalation, and hand-to-mouth for children were not to estimated in this study.

Almost all previous studies from Taiwan have reported on high arsenic exposure. Our study may be generalizable to populations with high and low levels of CAE, but not to those with medium CAE because of sample size limitations. Additional studies are needed to evaluate the roles of nutrition, genetic polymorphisms, tumor cell-type,[33] and individual arsenic exposure data.[34] The large populations currently exposed to high levels of drinking-water arsenic in Bangladesh, West Bengal and Inner Mongolia, as well as in regions of North and South America represent important opportunities and challenges for epidemiologic research on the effects of arsenic on human health.

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