Urinary Arsenic Species and CKD in a Taiwanese Population: A Case-Control Study

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Background: Inorganic arsenic has been linked to decreased kidney function through oxidative damage. Arsenic methylation is believed to be a pathway for arsenic metabolism. Lycopene is an antioxidant that reduces oxidative stress; however, the association between urinary arsenic species, plasma lycopene level, and chronic kidney disease (CKD) has seldom been evaluated.

Study Design: Case-control study.

Setting & Participants: 125 patients with CKD and 229 controls were recruited from a hospital-based pool.

Predictor: Urinary arsenic species and plasma lycopene level.

Outcomes & Measurements: CKD was defined as estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 m^2, calculated by using the Modification of Diet in Renal Disease Study equation. Plasma lycopene was measured by means of high-performance liquid chromatography. Urinary arsenic species, including arsenite, arsenate, monomethylarsonic acid, and dimethylarsinic acid, were determined by means of high-performance liquid chromatography and hydride generator–atomic absorption spectrometry.

Results: Lycopene level was associated positively with eGFR, and participants with a high serum lycopene level had a significant, inverse association with CKD (odds ratio, 0.41; 95% confidence interval, 0.21 to 0.81). Total arsenic level was associated significantly with CKD in a dose-response relationship, especially in participants with a total arsenic level greater than 20.74 compared with 11.78 μg/g creatinine or less (odds ratio, 4.34; 95% confidence interval, 1.94 to 9.69). Furthermore, participants with a high urinary total arsenic level or participants with a low percentage of dimethylarsinic acid had a positive association with CKD when their plasma lycopene level was low.

Limitations: Because of the single spot evaluation of plasma antioxidants and urinary arsenic species and the small sample size, statistical significance should be interpreted with caution.

Conclusions: This study shows that high urinary total arsenic or low plasma lycopene level is associated positively with CKD. Results suggest that the capacity for arsenic methylation may be associated with CKD in individuals who ingest low arsenic levels in drinking water and also have a low plasma lycopene level.

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INDEX WORDS: Arsenic; arsenic methylation capacity; lycopene; chronic kidney disease.

Chronic kidney disease (CKD) now is recognized as a common condition that increases the risk of cardiovascular disease.[1] The national prevalence of CKD in Taiwanese patients with an estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 m^2 is 11.93%, but only 3.54% of participants are aware of their disorder.[2] CKD is an important public issue because Taiwan ranks first in the world in the incidence of end-stage renal disease.[3] Epidemiological and clinical evidence have shown a link between hypertension, diabetes, obesity, and metabolic syndrome and the onset and progression of CKD.[4,5]

The metalloid arsenic is a naturally occurring element in soil, food, and water. Humans are exposed to inorganic arsenic from mining and smelting metal ores, pesticide manufacturing,

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wood preservatives, and medicines. Food may contain both organic and inorganic arsenic, whereas drinking water contains primarily inorganic arsenic. Long-term exposure to inorganic arsenic has been related to risk of cancer in the skin, bladder, liver, kidney, and lung.[6] Historically, the arsenic concentration permitted in public water supplies in Taiwan was 50 μg/L. However, in 2000, a new standard of 10 μg/L was announced. Our recent study showed that individuals with an unfavorable urinary arsenic profile had increased risk of urothelial carcinoma, even at low levels of exposure.[7] We do not know whether a urinary arsenic profile within a low allowable range affects the risk of CKD.

In comparison to other metals, such as lead and cadmium, studies of arsenic-induced nephrotoxicity are rare. However, a report is available for arsenic-induced kidney damage.[8] A recent study from Michigan also has shown an increased rate of kidney disease in people exposed to arsenic-contaminated drinking water.[9] The mechanisms underlying arsenic-induced kidney toxicity are complex.

Absorbed arsenic undergoes complicated biomethylation to form monomethylarsonic acid (MMA^V [the superscript indicates an oxidation number of 5 for arsenic]) and dimethylarsinic acid (DMA^V), which are excreted by the kidneys into urine.[10] The presumed arsenic methylation pathway in the human body is shown in Fig 1.[11-15] A previously published report suggests that excessive generation of reactive oxygen species (ROS) by various metals may cause kidney damage.[16] Because arsenic also generates ROS during the metabolic activation process,[17] whether arsenic metabolites are part of the mechanism for arsenic-induced nephrotoxicity remains to be determined.

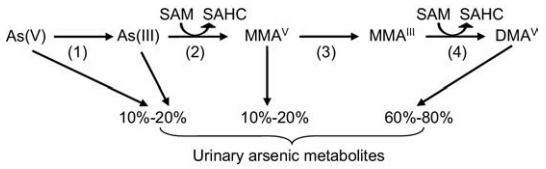

Figure 1. The presumed arsenic methylation pathway in the human body. The numbered steps are catalyzed by the following enzymes: (1) arsenate reductase or purine nucleoside phosphorylase (PNP), (2) arsenite methyl transferase (As3MT), (3) glutathione *S*-transferase omega 1 or 2 (GSTO1, GSTO2), and (4) arsenite methyl transferase (As3MT). Abbreviations: DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; SAHC, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

Lycopene is a potent carotenoid antioxidant. Lycopene most likely is involved in the scavenging ROS that contribute to defense against lipid peroxidation.[18] A recent study has shown that lycopene is able to protect against mercuric chloride–induced nephrotoxicity in rats,[19] as well as cisplastin-induced decreased kidney function and oxidative stress in rats.[20] Low plasma lycopene levels and arsenic exposure may be a risk factor for CKD. Therefore, the primary goal of the present study is to examine the association between the capacity for arsenic methylation, lycopene level, and CKD and the interaction between the capacity for arsenic methylation and lycopene level in affecting CKD.

METHODS

Study Participants, Interview Process, and Measurements

On a weekly basis from September 2005 and December 2007, patients (age range, 22 to 88 years) with clinical evidence of CKD based on urine sample collection were recruited from the Department of Internal Medicine/Nephrology of Shin Kong Wu Ho-Su Memorial Hospital in Taipei, Taiwan, resulting in 125 participants. eGFR traditionally is considered the best overall index of kidney function in health and disease. We used the 4-variable equation from the Modification of Diet in Renal Disease (MDRD) Study[1] to estimate eGFR as $186.3 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ for female})$ and defined the 5 stages of CKD according to the relevant Kidney Disease Outcomes Quality Initiative guidelines from the National Kidney Foundation. In this study, participants who were in stages 3 to 5 (eGFR < 60 mL/min/1.73 m^2) for 3 months were defined as having CKD. Age frequency–matched control participants with no evidence of CKD (eGFR ≥ 60 mL/min/1.73 m^2) in a 2:1 ratio of controls to cases were accrued weekly 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. A total of 229 control participants was obtained, and a urine sample was collected from each.

Well-trained personnel carried out standardized personal interviews based on a structured questionnaire. The information collected included demographic and socioeconomic characteristics and potential risk factors for CKD, such as lifestyle, alcohol consumption, cigarette smoking, exposure to potential occupational and environmental carcinogens (hair dyes and pesticides), medication history, consumption of conventional and alternative medicines, and personal and family histories of hypertension, diabetes, and CKD.

The Research Ethics Committee of Taipei Medical University (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.

A 10-mL blood sample was collected from participants on recruitment by use of EDTA-treated vacuum syringes and disposable needles. Plasma samples were centrifuged at 3,000 rpm for 15 minutes at room temperature, separated into aliquots, and stored at −80°C until used. Spot urine samples also were collected from all participants and immediately transferred to a −20°C freezer until further use for urinary arsenic species analysis.

Determination of Urinary Arsenic Species

It has been shown that urinary arsenic species are stable for at least 6 months when preserved at −20°C.[21] Therefore, the urine assay was performed within 6 months after collection. Frozen urine samples were thawed at room temperature, dispersed by using ultrasonication, filtered through a Sep-Pak C_{18} column (Mallinckrodt Baker Inc, Phillipsburg, NJ) and levels of arsenite (As[III]), arsenate (As[V]), MMA^V, and DMA^V were determined. A urine aliquot of 200 μL was used for determination of arsenic species by using high-performance liquid chromatography (HPLC; Waters 501; Waters Associates, Milford, MA) with columns obtained from Phenomenex (Nucleosil, Torrance, CA). Inorganic arsenic and its metabolites were quantified by using hydride generator–atomic absorption spectrometry.[22] A standard solution of 4 arsenic species was prepared in our laboratory; the sample and sample-spiked standard solution were determined by using online HPLC–hydride generator–atomic absorption spectrometry. Recovery rates of the 4 arsenic species were calculated by using the following formula: [(sample-spiked standard solution concentration − sample concentration)/(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. The urinary concentration of the sum of inorganic arsenic, MMA^V, and DMA^V was normalized against urinary creatinine levels (micrograms per gram of creatinine). The colorimetric assay automatically determined by the Roche Modular P800 instrument (Roche Inc, Mannheim, Germany) was used to calculate creatinine level by measuring the creatinine–picric acid complex formed by the reaction of creatinine and picric acid. The standard reference material, SRM 2670, contains 480 ± 100 μg/L of inorganic arsenic and was obtained from the National Institute of Standards and Technology (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 μg/L (n = 4). The arsenic methylation indices were assessed by the percentages of various urinary arsenic species present in the sum of inorganic arsenic, MMA^V, and DMA^V. The primary methylation index was defined as the ratio of MMA^V to levels of inorganic arsenic, ie, As(III) + As(V), and the secondary methylation index was defined as the ratio of DMA^V to MMA^V.[23]

Determination of Plasma Antioxidant Micronutrient Level

Levels of β-carotene, lycopene, α-tocopherol, and retinol in plasma samples were measured by using HPLC according to the procedure described previously.[24] Analysis was carried out by using reversed-phase HPLC (Hitachi Inc, Tokyo, Japan) with a mobile phase consisting of methanol:acetonitrite:chloroform (47:47:6) and multiwave length monitoring. Retinol was detected at 325 nm; α-tocopherol, at 280 nm; and lycopene and β-carotene, at 466 nm. Plasma samples for each case and control set were thawed from −80°C in dim light at room temperature and assayed on the same day to ensure that temporal variability in laboratory assays would affect cases and controls equally. All laboratory personnel were unaware of the disease status of participants from whom plasma samples were tested. Recovery rates for β-carotene, lycopene, α-tocopherol, and retinol were 90% to 100% at the highest concentration and 90% to 107% at the lowest concentration of the standard solution. The precision (coefficient of variance) of β-carotene, lycopene, α-tocopherol, and retinol was 1.0% to 6.0%. We also used an internal control (α-tocopherol acetate) to reduce systematic error; the coefficient of variance for α-tocopherol acetate was 2.5%.

Statistical Analysis

Continuous variables are expressed as mean ± SE. Student *t* test was used to compare differences in urinary arsenic profiles between case participants and controls. Analysis of variance and Scheffe multiple comparison correction were applied to compare urinary arsenic profiles between the varied exposure strata. Unconditional logistic regression models were used to estimate multivariate-adjusted odds ratio (OR) and 95% confidence interval (CI). Cutoff values for continuous variables were the respective tertiles of controls. Significance tests for linear trend among ORs across exposure strata were calculated by categorizing exposure variables and treating scored variables as continuous. For joint-effect analysis, cutoff values for plasma lycopene, urinary arsenic species percentage, or arsenic methylation indices were the respective medians of the controls. The synergy index proposed by Rothman[25] was computed to assess the additive interaction relationship between lycopene levels and urinary arsenic species percentages or arsenic methylation indices on CKD risk. An observed synergy index value that departs substantially from the expected additive null, ie, a synergy index not equal to 1, suggests an additive interaction effect. ORs and variance covariance matrixes then were used to calculate values for synergy index and 95% CIs.[26]

RESULTS

Participants who had higher educational levels had a significantly lower risk of CKD than those with lower educational levels. Participants with diabetes or hypertension had a significantly greater CKD risk than those without diabetes (OR, 4.00; 95% CI, 2.04 to 7.76) or those with normal blood pressure (OR, 2.23; 95% CI, 1.34 to 3.70). Alco-

Table 1. Sociodemographic Characteristics of the CKD Group and Healthy Controls

Variables	CKD Group	Healthy Controls	Odds Ratio* (95% confidence interval)	*P*
Sex				
Men	59 (47.20)	91 (39.74)	1.00	
Women	66 (52.80)	138 (60.26)	0.77 (0.49-1.20)†	0.3
Age (y)	58.81 ± 13.96	60.61 ± 13.09	0.99 (0.97-1.00)‡	0.2
Educational level				
Illiterate/elementary school	60 (48.00)	63 (27.75)	1.00	
Junior/senior high school	40 (32.00)	69 (30.40)	0.45 (0.25-0.78)	0.005
≥College	25 (20.00)	95 (41.85)	0.13 (0.07-0.27)	<0.001
Cigarette smoking				
No	100 (80.65)	178 (77.73)	1.00	
Yes	24 (19.35)	51 (22.27)	0.65 (0.35-1.21)	0.2
Alcohol consumption				
Never	103 (82.40)	147 (64.19)	1.00	
Frequency	12 (9.60)	32 (13.97)	0.37 (0.17-0.79)	0.01
Occasional	10 (8.00)	50 (21.83)	0.20 (0.10-0.44)	<0.001
Diabetes				
No	81 (75.70)	210 (92.11)	1.00	
Yes	26 (24.30)	18 (7.89)	4.00 (2.04-7.76)	<0.001
Hypertension				
No	64 (59.81)	174 (76.32)	1.00	
Yes	43 (40.19)	54 (23.68)	2.23 (1.34-3.70)	0.002
Analgesic use				
No	97 (77.60)	173 (75.88)	1.00	
Yes, routinely	14 (11.20)	9 (3.95)	3.00 (1.24-7.27)	0.02
Yes, as the need arises	14 (11.20)	46 (20.18)	0.53 (0.28-1.01)	0.05

Note: Values expressed as number (percent) or mean ± SE unless noted otherwise.
Abbreviation: CKD, chronic kidney disease.
*Adjusted for age and sex, except where indicated.
†Adjusted only for age.
‡Adjusted only for sex.

hol consumption was related to a significantly lower CKD risk than for nondrinkers. Cigarette smoking was not associated with CKD risk. A significantly greater risk was shown in analgesic users than nonusers; however, analgesic use on an as-needed basis had a significantly lower CKD risk than in nonusers (Table 1). Coffee consumption, pesticide exposure, and paint or dye use did not affect risk of CKD (data not shown).

The CKD group had a significantly lower eGFR (28.40 ± 1.41 mL/min/1.73 m^2; n = 125) than controls (80.17 ± 1.21 mL/min/1.73 m^2; n = 229; $P < 0.001$; Fig 2). Patients with CKD had a significantly greater urinary total arsenic level, greater MMA^V percentage, lower DMA^V percentage, and lower plasma lycopene level than controls (Table 2).

Plasma lycopene level was positively associated and urinary total arsenic level was negatively associated with eGFR (both associations were statistically significant; Fig 3), whether adjusted for age and sex or multiple covariates. When eGFR was adjusted for multiple covariates, greater MMA^V percentages correlated with significantly lower eGFRs (ie, inverse correlation), and greater DMA^V percentages correlated with significantly greater eGFRs (data not shown).

Compared with men, women had lower MMA^V percentages, but significantly greater total arsenic levels. Cigarette smoking, alcohol consumption, and habitual analgesic use did not influence the arsenic profile (Table 3).

By performing trend analysis on urinary total arsenic level, percentage of arsenic species, or plasma lycopene strata in tertiles, total urinary arsenic level was associated significantly with the CKD OR in a dose-response relationship, as listed in Table 4. This was especially true in participants with a total arsenic level greater than 20.74 μg/g creatinine, in whom the OR of CKD was increased 4-fold compared with those with a total arsenic

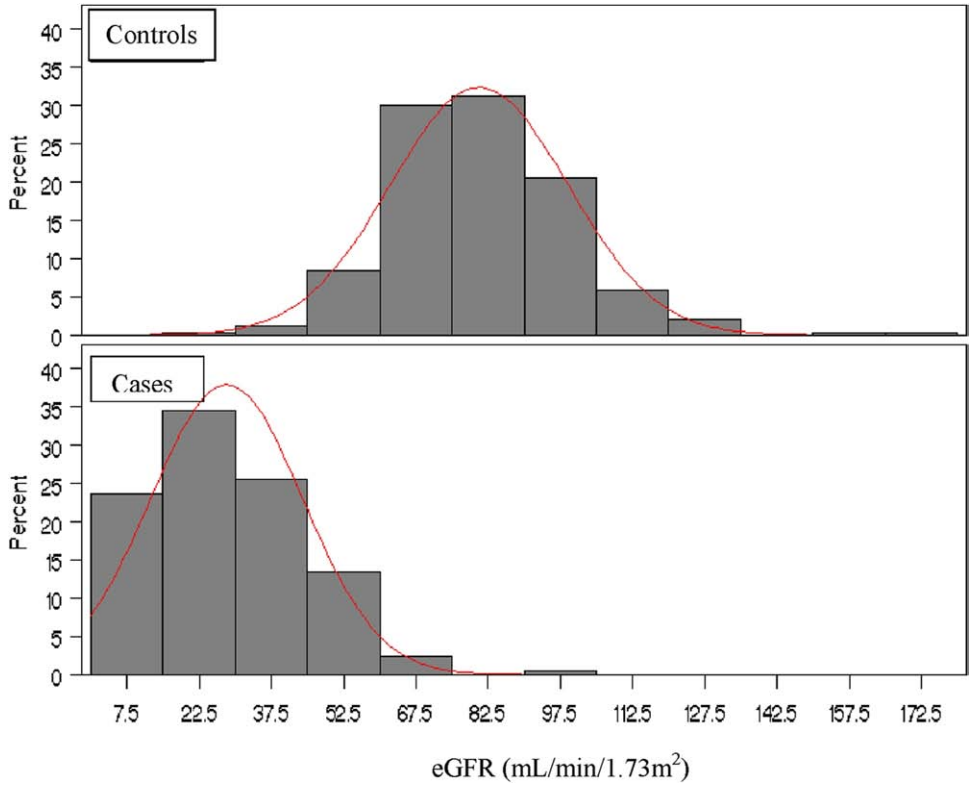

Figure 2. The distribution of estimated glomerular filtration rate (eGFR) in the chronic kidney disease group and controls.

level of 11.78 μg/g creatinine or less. Other arsenic species indices were not related to the CKD OR. Plasma lycopene level was related inversely to CKD in a dose-response relationship (participants with a plasma lycopene level $>$ 18.64 μg/dL compared with $\leq$ 8.29 μg/dL; OR, 0.41; 95% CI, 0.21 to 0.81). Plasma retinol level was associated significantly with CKD risk (data not shown), whereas other micronutrients were not related to CKD (data not shown).

Additional analyses were carried out to assess the joint effects of the following pairs of factors on CKD risk: lycopene and total arsenic levels, lycopene level and percentage of arsenic species, or lycopene level and arsenic methylation indices (Fig 4). Trend analysis showed progressively

Table 2. Differences in Urinary Total Arsenic, Percentages of Arsenic Species, and Arsenic Methylation Indices Between the CKD Group and Healthy Controls

	CKD Group		Healthy Controls		
Variables	No. Tested	Value	No. Tested	Value	*P*
Total arsenic (μg/g creatinine)	124	31.95 $\pm$ 2.59	229	20.71 $\pm$ 1.10	$<$0.001
Arsenic species (%)					
Inorganic arsenic	125	7.50 $\pm$ 1.04	229	6.67 $\pm$ 0.62	0.5
DMA	125	82.02 $\pm$ 2.05	229	87.04 $\pm$ 0.83	0.03
MMA	125	10.49 $\pm$ 1.77	229	6.29 $\pm$ 0.49	0.02
Primary methylation index	120	2.97 $\pm$ 0.55	219	2.37 $\pm$ 0.42	0.4
Secondary methylation index	97	29.68 $\pm$ 5.58	164	26.03 $\pm$ 3.63	0.6
Lycopene (μg/dL)	125	6.22 $\pm$ 1.43	229	10.40 $\pm$ 0.89	$<$0.001

Note: Values expressed as mean $\pm$ SE. Total arsenic indicates inorganic arsenic + MMA +DMA.
Abbreviations: CKD, chronic kidney disease; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid.

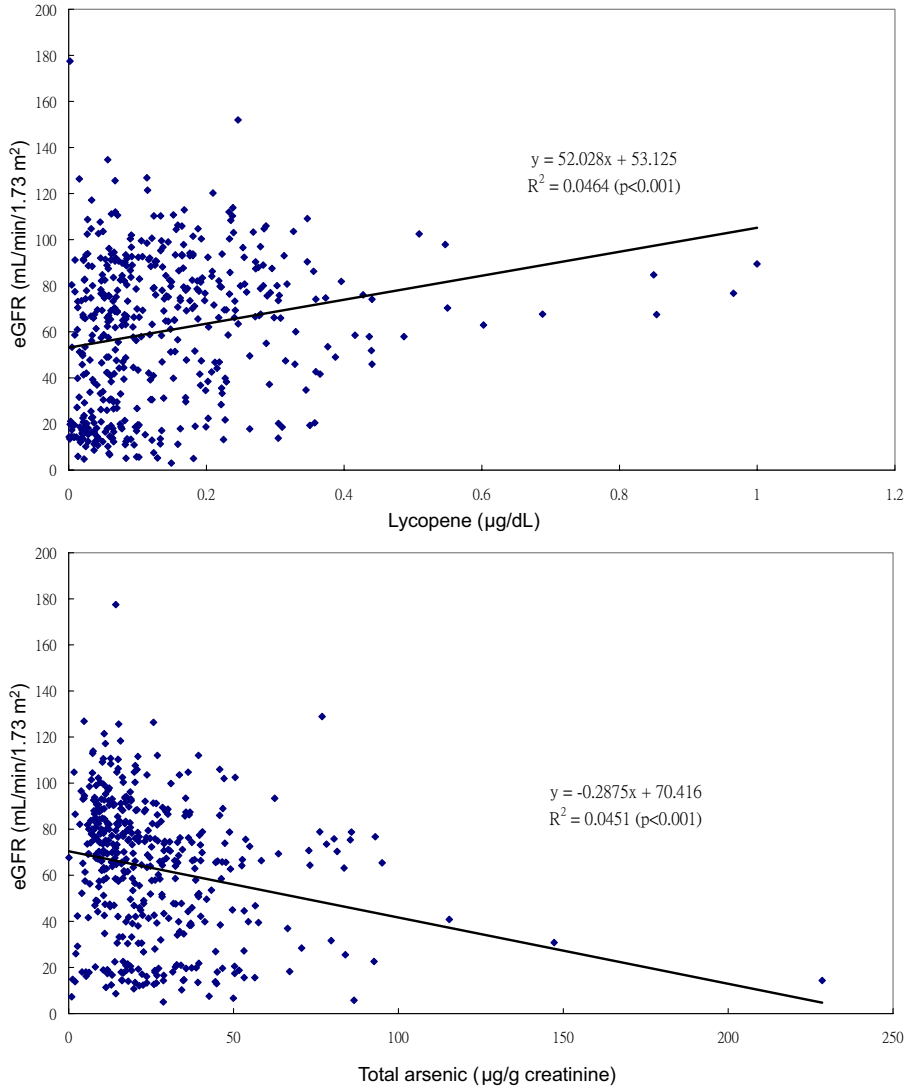

Figure 3. The association between estimated glomerular filtration rate (eGFR) and plasma lycopene or urinary total arsenic level.

increased risks through exposure to no risk factor, 1 risk factor, or both 2 risk factors. Although plasma lycopene level tended to interact additively with total urinary arsenic level, percentage of inorganic arsenic, primary methylation index, and secondary methylation index in modifying CKD risk, the interactions were all statistically insignificant, as shown by the absence of a substantial deviation from 1 in the synergy index. We also assessed the interaction as a departure from joint multiplicative effects by using the product term of 2 risk factors and showed that total arsenic level and DMA percentage significantly interacted with lycopene level (Fig 4).

DISCUSSION

The present study showed that patients with CKD compared with control individuals had a significantly greater total urinary arsenic level, greater MMA^V percentage, and lower DMA^V percentage, indicating a less efficient capacity to methylate inorganic arsenic to DMA^V. In addition, it was found that only total urinary arsenic level was related to CKD risk in a dose-response

Table 3. Distribution of Urinary Total Arsenic, Percentages of Arsenic Species, and Arsenic Methylation Index According to Sex, Cigarette Smoking, Alcohol Consumption and Analgesic Use

Variables	No. of Participants	Total Arsenic (μg/g creatinine)	Arsenic Species (%)					
			Inorganic Arsenic	MMA	DMA	PMI	SMI	Lycopene (μg/dL)
Sex								
Men	150	21.72 ± 1.55	6.50 ± 0.56	9.22 ± 1.12	84.28 ± 1.32	2.44 ± 0.46	25.63 ± 4.24	13.88 ± 1.09
Women	204	26.84 ± 1.71	7.31 ± 0.85	6.71 ± 0.91	85.99 ± 1.24	2.69 ± 0.47	29.01 ± 4.45	14.83 ± 0.91
P		0.03	0.4	0.08	0.4	0.7	0.6	0.5
Cigarette smoking								
No	277	24.79 ± 1.30	6.89 ± 0.58	7.27 ± 0.79	85.84 ± 1.00	2.62 ± 0.41	27.35 ± 3.43	15.06 ± 0.77
Yes	75	24.23 ± 2.88	7.26 ± 1.41	9.63 ± 1.59	83.12 ± 2.19	2.45 ± 0.46	27.75 ± 6.95	12.25 ± 1.64
P		0.9	0.8	0.2	0.2	0.8	0.9	0.1
Alcohol consumption								
No	249	24.89 ± 1.93	7.08 ± 0.65	7.76 ± 0.91	85.16 ± 1.15	2.83 ± 0.45	30.18 ± 3.98	14.75 ± 0.86*,†
Yes	44	23.96 ± 2.89	5.30 ± 0.48	7.64 ± 2.06	87.06 ± 2.13	2.09 ± 0.63	30.98 ± 10.60	10.26 ± 1.20
Occasional	60	24.22 ± 4.50	7.71 ± 1.73	7.90 ± 0.93	84.39 ± 1.91	1.92 ± 0.26	14.45 ± 1.65	16.13 ± 1.78
P		0.9	0.5	0.9	0.7	0.5	0.1	0.06
Analgesic use								
No	269	25.12 ± 1.45	7.00 ± 0.56	7.47 ± 0.80	85.53 ± 0.99	2.49 ± 0.38	26.65 ± 3.19	13.66 ± 0.70
Yes, routinely	23	25.82 ± 3.74	10.35 ± 4.34	7.64 ± 3.38	82.01 ± 5.66	2.40 ± 0.93	46.12 ± 23.30	19.25 ± 3.84
Yes, as needed	60	22.37 ± 2.12	5.53 ± 1.15	9.29 ± 1.73	85.25 ± 2.09	3.11 ± 0.91	24.37 ± 7.18	15.46 ± 2.15
P		0.8	0.3	0.8	0.8	0.9	0.5	0.2

Note: Values expressed as mean ± SE. Total arsenic indicates inorganic arsenic + MMA + DMA. Cigarette smoking history and analgesic use data were unavailable for 1 and 2 participants, respectively.

Abbreviations: CKD, chronic kidney disease; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; PMI, primary methylation index; SMI, secondary methylation index.

*Significantly different from those who consume alcohol, $P < 0.05$.

†Significantly different from occasional drinker, $P < 0.05$.

relationship adjusted for age and sex or separately adjusted for multiple risk factors. Patients with CKD had significantly lower plasma lycopene levels, indicating lower antioxidant capabilities than controls.

Upon entering the body, arsenic targets ubiquitous enzyme reactions and affects nearly all organ systems.[27] Several trace elements, including arsenic, cadmium, lead, and mercury, have been implicated in the decrease in kidney function.[28] A study in Utah has shown increased rates of nephritis and nephrosis in people drinking arsenic-contaminated well water.[29] According to animal studies, vacuolation of renal tubular epithelium was observed in a case of low-dose arsenic exposure, whereas pathologically moderate glomerular sclerosis and severe tubular necrosis were shown in the case of exposure to high doses of arsenic.[30] However, a case report by Prasad and Rossi[31] showed that tubulointerstitial nephritis is associated with increased urinary arsenic concentration.

According to the Taipei Water Department of the Taipei City Government, average arsenic concentration in Taipei tap water is 0.7 μg/L and ranges from undetectable to 4.0 μg/L. However, the concentration range of urinary arsenic of study participants of approximately 20 to 30 μg/g creatinine in this study possibly resulted from exposure to some foods. Although our study participants drank tap water with no evidence of arsenic contamination, we also found that total urinary arsenic level and MMA^V percentage were associated significantly with decreased eGFR in this study. However, the precise mechanism of arsenic-induced nephrotoxicity may be difficult to assess because of the complex biological chemistry associated with arsenic.[32]

Absorbed arsenic is excreted mainly through urine, suggesting that the kidney is a primary target for arsenic toxicity. Kidney arsenic toxicity may be complicated by methylation of inorganic arsenic to the less toxic MMA^V and DMA^V, which are excreted rapidly by the kidney.[10] MMA^{III} and DMA^{III} have been identified in human urine.[33,34] Many studies have shown that these trivalent methylated arsenic species are more toxic than inorganic compounds.[35,36] How-

Table 4. Dose-Response Relationship Between CKD Risk and Urinary Total Arsenic, Percentages of Arsenic Species, Arsenic Methylation Indices, and Plasma Lycopene

Variables	CKD Group/Healthy Controls	Odds Ratio* (95% confidence interval)	Odds Ratio† (95% confidence interval)
Total arsenic (μg/g creatinine)		$P_{trend} < 0.001$	$P_{trend} < 0.001$
≤11.78	19/75	1.00	1.00
11.78-20.74	30/78	1.73 (0.89-3.40)	1.41 (0.62-3.19)
>20.74	76/76	5.66 (2.96-10.85)‡	4.34 (1.94-9.69)‡
Arsenic species (%)			
Inorganic arsenic		$P_{trend} = 0.8$	$P_{trend} = 0.6$
≤2.75	40/75	1.00	1.00
2.75-5.86	40/78	0.86 (0.50-1.51)	1.01 (0.52-1.98)
>5.86	45/76	0.99 (0.58-1.72)	1.20 (0.61-2.36)
MMA		$P_{trend} = 0.9$	$P_{trend} = 0.7$
≤1.29	39/75	1.00	1.00
1.29-7.60	43/77	1.03 (0.60-1.78)	0.63 (0.32-1.23)
>7.60	43/77	0.97 (0.56-1.68)	0.87 (0.45-1.71)
DMA		$P_{trend} = 0.7$	$P_{trend} = 0.5$
≤85.62	45/76	1.00	1.00
85.62-93.40	44/77	1.00 (0.59-1.70)	0.58 (0.30-1.13)
>93.40	36/76	0.88 (0.50-1.53)	0.79 (0.40-1.55)
PMI		$P_{trend} = 0.6$	$P_{trend} = 0.7$
≤0.28	40/83	1.00	1.00
0.28-1.86	42/73	1.12 (0.65-1.93)	0.75 (0.38-1.49)
>1.86	43/73	1.14 (0.66-1.96)	0.88 (0.46-1.69)
SMI		$P_{trend} = 0.8$	$P_{trend} = 0.4$
≤8.44	61/119	1.00	1.00
8.44-17.18	26/54	0.90 (0.51-1.58)	0.48 (0.24-0.99)§
>17.18	38/56	1.31 (0.78-2.20)	0.79 (0.42-1.50)
Plasma lycopene (μg/dL)		$P_{trend} < 0.001$	$P_{trend} = 0.003$
≤8.29	74/76	1.00	1.00
8.29-18.64	24/76	0.31 (0.18-0.55)‡	0.33 (0.17-0.64)‖
>18.64	27/77	0.35 (0.21-0.61)‖	0.41 (0.21-0.81)‖

Note: Total arsenic indicates inorganic arsenic + MMA + DMA.

Abbreviations: CKD, chronic kidney disease; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; PMI, primary methylation index; SMI, secondary methylation index.

*Adjusted for age and sex.

†Adjusted for age, sex, educational level, paternal and maternal ethnicity, cigarette smoking, coffee drinking, analgesic use, hypertension, and diabetes history.

‡$P < 0.001$.

§$P < 0.05$.

‖$P < 0.01$.

ever, trivalent methylated arsenic metabolites have a short half-life. Whether they can be detected depends on the conditions and temperature of sample storage and concentrations in urine. The reason we did not observe trivalent methylated metabolites in the study is that the analytical method used lacks the requisite specificity. In general, arsenic methylation is considered a detoxification process in which MMA^V and DMA^V generally are considered nontoxic. Few studies have examined arsenic metabolism on decreased kidney function in humans. One study reported that the main detectable species were the relatively nontoxic compounds arsenobetaine and DMA, whereas levels of such toxic inorganic arsenic compounds as arsenite and arsenate were less than the detection limit in serum.[37] In the present study, we found that patients with CKD had significantly greater urinary total arsenic levels, greater MMA^V percentages, and lower DMA^V percentages than controls. Of these variables, only total arsenic level

Plasma lycopene	Total As	OR (95% CI)	Synergy index	*P*
High	Low	1.00	2.34 (0.68 - 8.09)	<0.001
	High	1.85 (0.66 - 5.16)		
Low	Low	2.04 (0.74 - 5.63)		
	High	5.43 (2.19 - 13.46)***		

Plasma lycopene	% inorganic As	OR (95% CI)	Synergy index	*P*
High	Low	1.00	1.16 (0.48 - 2.83)	0.5
	High	1.84 (0.65 - 5.19)		
Low	Low	3.52 (1.40 - 8.86)**		
	High	4.91 (1.88 - 12.84)**		

Plasma lycopene	% MMA	OR (95% CI)	Synergy index	*P*
High	Low	1.00	0.99 (0.25 - 4.01)	0.4
	High	0.76 (0.28 - 2.08)		
Low	Low	2.58 (1.10 - 6.04)*		
	High	2.33 (0.98 - 5.54)+		

Plasma lycopene	% DMA	OR (95% CI)	Synergy index	*P*
High	Low	1.00	0.76 (0.30 - 1.89)	<0.001
	High	1.62 (0.59 - 4.41)		
Low	Low	3.78 (1.56 - 9.05)**		
	High	3.57 (1.47 - 8.68)**		

Plasma lycopene	PMI	OR (95% CI)	Synergy index	*P*
High	Low	1.00	0.35 (0.12 - 1.04)	0.9
	High	1.22 (0.45 - 3.30)		
Low	Low	4.37 (1.85 - 10.30)***		
	High	2.27 (0.96 - 5.38)+		

Plasma lycopene	SMI	OR (95% CI)	Synergy index	*P*
High	High	1.00	1.15 (0.33 - 4.0)	0.2
	Low	1.03 (0.38 - 2.74)		
Low	High	2.70 (1.09 - 6.69)*		
	Low	2.99 (1.29 - 6.93)*		

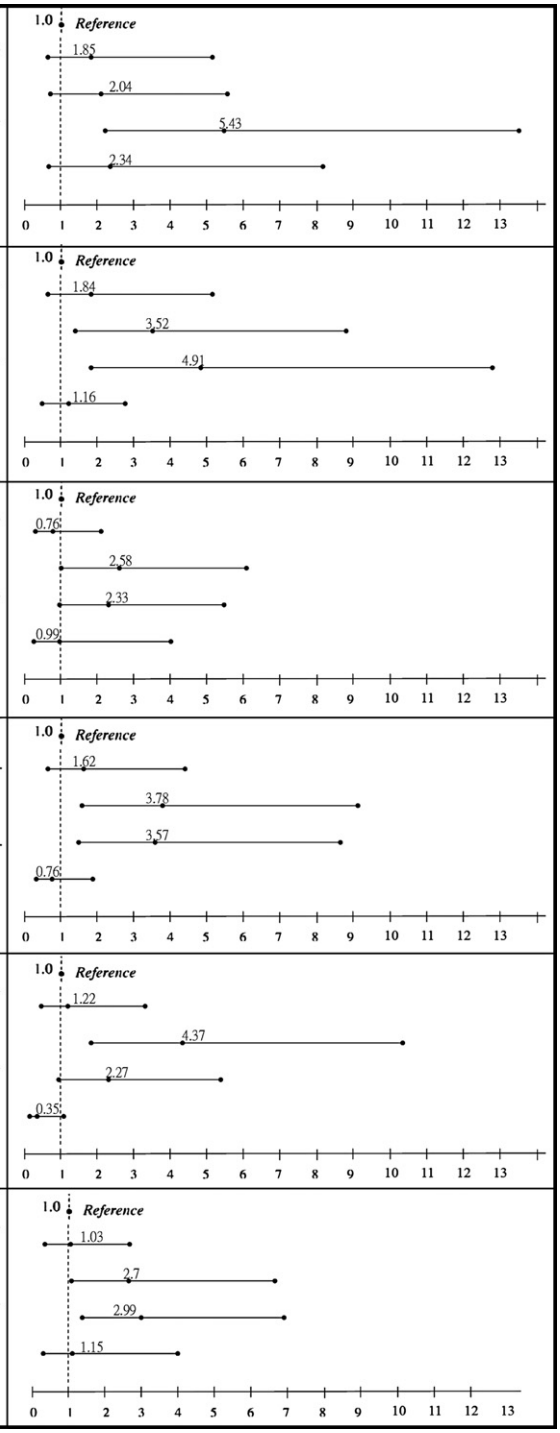

Figure 4. Multiple logistical regression analysis of the combination of urinary total arsenic, arsenic species percentage, and plasma lycopene on chronic kidney disease. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The unit of total arsenic is μg/g creatinine. The relative proportion of each arsenic species (% inorganic As, % MMA and % DMA) was calculated by dividing the levels of each species by the total arsenic level. Abbreviations: PMI, primary methylation index; SMI, secondary methylation index. High is defined as a value greater than the median; low, as a value equal to the median or less. Odds ratios (ORs) based on analyses adjusted for age, sex, educational level, paternal and maternal ethnicity, cigarette smoking, coffee drinking, hypertension, and diabetes history. *P* represents statistical interaction as a departure from joint multiplicative effects.

was associated significantly with CKD risk. Whether the capacity of arsenic methylation is related to patients with CKD when they ingest low arsenic levels in drinking water needs further investigation.

Inorganic arsenic-induced oxidative damage results in chronic kidney pathological states involving ROS production, reduction/oxidation-related gene expression, and cytotoxicity.[38] However, oxidative stress has been identified as an important mechanism in arsenic-induced decreased kidney function through accumulation of arsenic in kidney tissue; increased levels of serum urea nitrogen, creatinine, and lipid peroxidation end products; and reduced glutathione in a mouse model.[39] Our recent study showed that arsenic methylation species were associated with oxidative damage assessed by using urinary 8-hydroxy-2′-deoxyguanosine,[40] suggesting that arsenic metabolites are related to oxidative stress.

Antioxidants could be considered an alternative approach to mitigate arsenic-induced oxidative damage.[41] In our previous study, a significant inverse dose-response relationship was observed between arsenic-related ischemic heart disease and serum α- and β-carotene levels.[42] Our study also showed that serum β-carotene level was related negatively to arsenic-induced skin cancer.[43] In the present study, we found that participants with high plasma lycopene levels had a significantly decreased risk of CKD compared with patients with low plasma lycopene levels. Additionally, participants with low plasma lycopene levels were at greater risk of having CKD when they presented with at least 1 of high total arsenic level or low DMA^V percentage. Although these data suggest that participants with low antioxidant capacity may not easily mitigate oxidative stress produced by arsenic metabolites and therefore may be at risk of CKD, these findings need additional study.

Our study had some important limitations that need to be considered when interpreting results. First, there is the possibility of selection bias because cases and controls were recruited from 2 different hospitals; however, bias was minimized because these hospitals both belonged to medical centers and were located in Taipei. Furthermore, the majority of cases and controls lived in Taipei and were similar in age and sex distribution (Table 1) with respect to demographic characteristics. Possible selection bias may have occurred because the recruited CKD cases more often had an elementary school education than controls. However, in a large-scale screening program, it has been reported that participants with a high level of education had lower CKD risk than those with a low level of education in Taiwan.[2] Second, the accuracy of a single spot evaluation of plasma antioxidants and urinary arsenic species may be in doubt. However, the values might be reliable because all participants had no change in lifestyle and appeared to maintain their homeostatic metabolism. Third, because of the small sample size, statistical significance should be interpreted with caution. Fourth, CKD cases were recruited in this study; however, we cannot exclude that the findings of an association between lycopene or arsenic and its various metabolites and CKD might be the result and not the cause of CKD.

In conclusion, this is the first study showing that high urinary total arsenic levels or low plasma lycopene levels are associated positively with CKD. Similarly, our data suggest that the capacity for arsenic methylation may be associated with CKD in individuals who also had low plasma lycopene levels when they ingested low arsenic levels in drinking water.

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