Effect of Urinary Total Arsenic Level and Estimated Glomerular Filtration Rate on the Risk of Renal Cell Carcinoma in a Low Arsenic Exposure Area

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Abbreviations and Acronyms

As^{III} = arsenite

As^{V} = arsenate

DMA^{V} = dimethylarsinic acid

eGFR = estimated glomerular filtration rate

MMA^{V} = monomethylarsonic acid

RCC = renal cell carcinoma

UC = urothelial carcinoma

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For another article on a related topic see page 2353.

Purpose: We explored the relationship between urinary total arsenic and risk of renal cell carcinoma, and investigated whether having hypertension or a low estimated glomerular filtration rate would modify the risk of renal cell carcinoma.

Materials and Methods: The case-control study was conducted between November 2006 and May 2009 with 132 patients with renal cell carcinoma, and 260 sex and age matched controls from a hospital based pool. Pathological verification of renal cell carcinoma was completed by image guided biopsy or surgical resection of renal tumors. Urinary arsenic species, including inorganic arsenic, monomethylarsonic acid and dimethylarsinic acid, were determined with a high performance liquid chromatography linked hydride generator and atomic absorption spectrometry. Estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease Study equation.

Results: Urinary total arsenic was significantly associated with renal cell carcinoma risk in a dose-response relationship after multivariate adjustment. Low estimated glomerular filtration rate or hypertension was significantly related to renal cell carcinoma risk. Estimated glomerular filtration rate was significantly negatively related with urinary total arsenic. A significant interaction was seen between the urinary total arsenic and hypertension on renal cell carcinoma risk. The greatest odds ratio (6.01) was seen in the subjects with hypertension, low estimated glomerular filtration rate and high urinary total arsenic. A trend test indicated that the risk of renal cell carcinoma increased along with the accumulating number of these 3 risk factors ($p < 0.0001$).

Conclusions: Higher urinary total arsenic level was a strong predictor of renal cell carcinoma, and estimated glomerular filtration rate or hypertension interacts with urinary total arsenic in modifying the risk of renal cell carcinoma.

Key Words: arsenic; methylation; kidney; glomerular filtration rate; carcinoma, renal cell

RENAL cell carcinoma accounts for 85% of renal cancers and for 3% of all adult malignant neoplasms with a male-to-female ratio of 3:2.[1] In Taiwan 435 males and 230 females had RCC in 2006, which accounted for 0.9% of all patients with cancer.[2] Multiple potential etiologic factors con-

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tribute to the development of RCC including obesity, cigarette smoking, and exposure to trichloroethylene, asbestos and arsenic.[3] In addition, hypertension is a known risk factor for RCC,[4] while moderate alcohol consumption, coffee drinking and tea drinking are associated with a decreased risk of RCC.[5]

In a study on arsenic, one of the most significant hazards in the environment, Chen et al reported higher age standardized mortality in kidney cancer in the blackfoot disease endemic area.[6] Tan et al did not observe an association of high arsenic levels in drinking water with RCC, and concluded that the carcinogenicity of arsenic may be cell type specific for UC but not for RCC.[7] Based on these findings, the role of arsenic exposure in RCC needs to be clarified.

Huang et al conducted a 12-year followup study in the blackfoot disease endemic area and found that participants with high chronic arsenic exposure and with inefficient arsenic methylation had a higher risk of UC.[8] Pu et al reported that subjects with an unfavorable urinary arsenic profile have an increased UC risk even at low arsenic exposure levels.[9] These studies demonstrated that UC risk was influenced by arsenic methylation profiles in high and low arsenic exposure areas. Our recent study demonstrated that urinary total arsenic levels were significantly associated with chronic kidney disease, which is defined as an eGFR less than 60 ml/minute/1.73 m^2 in a dose-response relationship.[10] Renal tumors are common in the pre-transplant end stage renal disease population.[11] However, to our knowledge until now there was no epidemiological study to verify the relationship between individualized arsenic methylation capacity and risk of RCC. We clarified the association between arsenic methylation capacity and RCC risk, and explored whether low eGFR or hypertension modifies arsenic induced RCC risk even in a low arsenic exposure area.

MATERIALS AND METHODS

Study Subjects and Questionnaire Interview

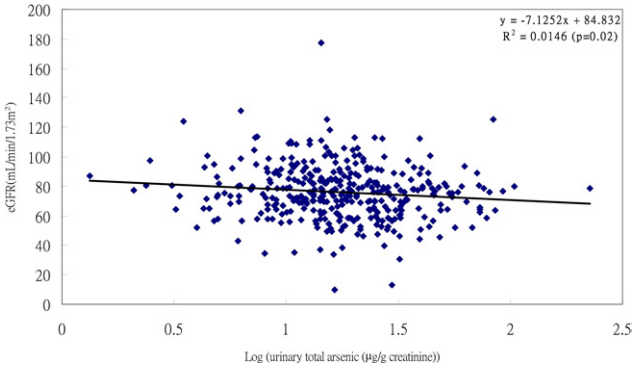
A total of 132 patients with pathologically proven RCC were diagnosed and recruited from the Department of Urology, National Taiwan University Hospital, between November 2006 and May 2009. Pathological verification of RCC was completed by image guided biopsy or surgical resection of renal tumors. A total of 260 age and sex matched control subjects with no evidence of RCC or any other malignancy were collected from those undergoing senior citizen health examinations at Taipei Medical University Hospital and those undergoing adult health examinations at Taipei Municipal Wan Fang Hospital. All 3 hospitals are located in Taipei, approximately 200 to 300 km away from the arsenic contaminated areas in Taiwan. None of the RCC cases or controls came from these areas. The majority of study participants (greater than 80%) lived in Taipei City and drank tap water from the Taipei Water Department of the Taipei City Government. The average arsenic concentration of tap water in Taipei City is 0.7 μg/l but concentrations range from nondetectable to 4.0 μg/l.

Well trained personnel performed standardized personal interviews based on a structured questionnaire. Information collected included demographic and socioeconomic characteristics, general potential risk factors for malignancies such as lifestyle, consumption of alcohol, tea and coffee, and cigarette smoking in quantified detail, as well as histories of hypertension and diabetes. Frequent alcohol, tea and coffee drinkers referred to those who consumed the respective drinks 2 or more days per week for at least 6 months. Those who consumed less than this level were classified as occasional drinkers. The Research Ethics Committee of National Taiwan University Hospital approved the study. All patients signed informed consent forms before sample and data collection.

Daytime midstream urine samples and serum samples were collected. Serum creatinine was measured after patient diagnosis but before surgery or treatment. Urine was stored at −20C until further use for urinary arsenic speciation. Urinary creatinine is almost universally used to adjust concentrations of urinary analytes for variations in hydration status. Therefore, we adjusted urinary total arsenic concentration with urinary creatinine (mg/dl). According to a report comparing the concentration of arsenic in individual urine voids with arsenic in a 24-hour urine collection, the concentration of arsenic in urine is stable throughout the day.[12] Therefore, the use of spot urine collection to reflect 24-hour excretions for arsenic may be reliable. The TNM classification of the American Joint Committee on Cancer was used for pathological staging.[13] Tumor grading was based on the Fuhrman grading system.[14] eGFR is traditionally considered the best overall index of renal function in health and disease. We used the abbreviated equation from the Modification of Diet in Renal Disease Study[15] to estimate eGFR as $186.3 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ for female})$.

Determination of Urinary Arsenic Species

Frozen urine samples were thawed at room temperature, dispersed by ultrasonic wave and filtered through a Sep-Pak® C18 column. A urine aliquot of 200 μl was used for the determination of arsenic species by high performance liquid chromatography (Waters 501, Waters Associates, Milford, Massachusetts) with columns obtained from Phenomenex (Nucleosil, Torrance, California). The contents of As^{III}, As^{V}, MMA^{V} and DMA^{V} were quantified by hydride generator atomic absorption spectrometry.[16] The method of arsenic speciation is not influenced by dietary factors such as the ingestion of shellfish, fish or other seafood.[17] Recovery rates of the 4 arsenic species were calculated by [(sample spiked standard solution concentration) - sample concentration]/standard solution concentration × 100. Recovery rates for As^{III}, As^{V}, MMA^{V} and DMA^{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 named total arsenic. The standard reference material, SRM 2670, contained 480 ± 100 μg/l inorganic



Association between log transformed urinary total arsenic and eGFR.

arsenic (National Institute of Standards and Technology, Gaithersburg, Maryland), and was used as a quality standard and analyzed along with urine samples. The mean concentration of SRM 2670 determined by our system was 507 ± 17 μg/l (n = 4).

Statistical Analysis

Student's t test was used to compare differences in the urinary arsenic profiles between case subjects and controls. ANOVA and Scheffe's multiple comparison were applied to compare urinary arsenic profiles between varied exposure strata. Multiple logistic regression models were used to estimate the multivariate adjusted odds ratios and 95% CIs. Cutoff points for continuous variables were the respective tertiles of distribution in controls. Significance tests for linear trends among ORs across exposure strata were calculated by categorizing exposure variables and treating scored variables as continuous. For the joint effect analysis the cutoff point for the urinary total arsenic or percentage of arsenic species were the respective medians of the controls. The joint effects of urinary total arsenic and hypertension on RCC risk were evaluated on multiplicative and additive scales. The binary interaction terms were calculated by multiplying the indicators for 2 explored risk factors and were added to the main effect models. Their significance was then tested by the likelihood ratio statistic based on a multiplicative model. The OR values and their variance covariance matrix were then used to calculate values for synergy index and 95% CIs.[18] SAS® version 8.2 was used for all statistical analyses.

RESULTS

The pathology characteristics revealed a distribution of the types of RCC cases as clear cell type 109, papillary type 9, chromophobe 10 and other types 4, similar to the previous study.[19] Participants with a lower eGFR had a significantly higher risk of RCC than those with a higher eGFR in a dose-response relationship after multivariate adjustment. Occasional alcohol, tea and coffee drinkers had a significantly lower RCC risk than nondrinkers. In contrast, subjects who were former cigarette smokers had a significantly higher OR of RCC than those who had never been smokers after multivariate adjustment. Hypertension was significantly related to RCC after adjustment for other risk factors.

Urinary total arsenic was marginally higher in cases than in controls (25.16 ± 2.22 vs 21.15 ± 1.02 μg/l, p = 0.09). Urinary total arsenic level was significantly associated with risk of RCC in a dose-response relationship after multivariate adjustment. Arsenic methylation indices did not show any relationship with RCC risk.

The log-transformed urinary total arsenic level was significantly negatively associated with eGFR after adjusting for multiple covariates (see figure). Hypertension or eGFR tended to interact multiplicatively with total urinary arsenic level in modifying RCC risk. The additive interactions were statistically insignificant.

The risk of RCC was estimated for each combination of hypertension, eGFR and urinary total arsenic using nonhypertension, eGFR 60 ml/minute/1.73 m^2 or greater and urinary total arsenic 15.95 μg/g creatinine or less as the reference group (see table). The greatest odds ratio (6.01) was seen in the subjects with hypertension, eGFR less than 60 ml/minute/1.73 m^2 and urinary total arsenic greater than 15.95 μg/g creatinine. A trend test indicated that the risk of RCC increased along with the accumulating number of these 3 risk factors (p <0.0001).

DISCUSSION

This study demonstrated that urinary total arsenic levels were associated with RCC risk in a dose-response relationship after adjustment for multiple risk factors. Participants with hypertension or with a low eGFR had a significantly increased risk of RCC compared to those with nonhypertension or a high eGFR after adjusting for multiple risk factors.

Adjusted odds ratios of RCC by hypertension, eGFR and urinary total arsenic

Risk Factors*	No. Cases/Controls	Age-Gender Adjusted OR (95% CI)	Multivariate Adjusted OR (95% CI)†
None	22/91	1.00	1.00
1	59/106	2.63 (1.46–6.45)‡	2.60 (1.37–4.94)‡
2	34/45	3.87 (1.95–7.67)§	4.36 (2.01–9.46)§
3	17/17	5.70 (2.36–16.04)§	6.01 (2.26–16.04)§

* Hypertension status, eGFR less than 60 ml/minute/1.73 m^2 and urinary total arsenic greater than 15.95 μg/g creatinine.

† Adjusted for age, gender, diabetes, body mass index, tea or coffee drinking and cumulative cigarette smoking.

‡ p <0.01.

§ p <0.001.

Urinary total arsenic levels of participants in this study were significantly lower than those in our previous study, which included skin cancer cases and healthy controls from the arsenic contaminated area in southwestern Taiwan (104.1 ± 15.2 and 89.5 ± 7.2 μg/l, respectively).[16] Nevertheless, the total arsenic levels reported here (RCC cases 25.2 ± 2.2, controls 21.4 ± 1.0 μg/l) suggest that subjects in this study had been exposed to low levels of arsenic. We still observed an increased RCC risk in subjects with high urinary total arsenic, which is the same result seen in our previous studies.[9,10]

Absorbed arsenic undergoes complicated biomethylation in the liver to form MMA^V and DMA^V that are excreted by the kidneys into the urine.[20] This pathway is the major pathway for the metabolism of inorganic arsenic in humans, followed as $As^V \rightarrow As^{III} \rightarrow MMA^V \rightarrow MMA^{III} \rightarrow DMA^V$, and involves reduction and oxidative methylation. Reduction is catalyzed by purine nucleoside phosphorylase or glutathione s-methyltransferase omega, and oxidative methylation is catalyzed by arsenite/MMA^{III} methyltransferase.[21] MMA^{III} and DMA^{III} have been identified in human urine[22] and many studies have demonstrated that those arsenic species are more toxic than the inorganic compounds.[23] However, the trivalent methylated arsenic metabolites are not stable, and whether they can be detected depends on the conditions of sample storage and concentrations in the urine. We did not observe any trivalent methylated metabolites in this study because the analytical method used lacks the requisite specificity. In general, arsenic methylation is considered a detoxification process in which MMA^V and DMA^V are considered nontoxic. In the present study we only found that urinary total arsenic but not methylation capability was significantly associated with RCC risk, although subjects ingested low levels of arsenic in drinking water.

The inorganic arsenic percentage and DMA percentage was significantly different between stages I and II, and stages I and III, and there was no association between the other arsenic indices and tumor stage, grade, cell type and tumor size (data not shown). Therefore, these results may indicate that arsenic methylation capability may not affect RCC prognosis.

The major function of the kidneys is to regulate systemic water and electrolyte balance, and excrete waste products and foreign chemicals. Many studies have reported that the relative risk of RCC in patients with end stage renal failure is estimated to be 5 to 20-fold higher than that of the general population.[11] In this study hypertension was related to RCC, and the eGFR of patients with hypertension was significantly lower than in those without hypertension (data not shown). However, a lower eGFR was associated with a higher risk of RCC and eGFR was also significantly negatively related with log-transformed urinary total arsenic. These results may suggest that urinary total arsenic induced hypertension[24] or influenced the kidney function[10] to modify the risk of RCC, and this association needs further examination. The mechanism by which hypertension contributes to RCC is unclear, but it may be associated with related functional or metabolic changes such as decreased blood flow to the kidney and cell proliferation in the renal tubule that can subsequently induce hypoxia and promote angiogenesis.[25]

In this study urinary total arsenic was significantly related to the risk of RCC, possibly through mechanisms or modes of action for arsenic carcinogenesis, including oxidative stress. Inorganic arsenic induced oxidative damage results in chronic pathological states in the kidney that involve reactive oxygen species production.[26] 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 and lipid peroxidation end products, and reduced glutathione in a mouse model.[27] Our recent study showed that arsenic methylation species were associated with oxidative damage, which was assessed using urinary 8-hydroxydeoxyguanosine,[28] suggesting that arsenic metabolites are related to oxidative stress. On the other hand, the strong local electronic molecular interactions between arsenic ions and the membrane bound neural endopeptidase CD10[29] that might induce arsenic hydrate microcrystals might also be involved in arsenic induced carcinogenicity in terms of the development of renal malignancy.[30] These mechanisms merit further investigation.

Our study has some important limitations that need to be considered when interpreting the results. The accuracy of spot evaluation of urinary arsenic species may be in doubt. However, the values might be reliable because none of the subjects experienced a change in lifestyle and appeared to maintain their homeostatic metabolism. In addition, the information on some risk factors for RCC, including environmental exposures such as asbestos or advanced kidney disease,[3] was unavailable to control in our analyses. Because of the small sample size, statistical significance should be interpreted with caution. Finally, RCC prevalence cases were recruited in this study. However, we cannot exclude the fact that the association between urinary total arsenic levels and RCC in this study might be the result of and not the cause of RCC.

CONCLUSIONS

This is the first study to our knowledge showing that higher urinary total arsenic is significantly associated with an increased risk of RCC. Our data also provided evidence that subjects with high urinary total arsenic levels may experience an increased risk of RCC if they had hypertension and if they had an eGFR less than 60 ml/minute/1.73 m^2, even if they ingested low levels of arsenic in drinking water.

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