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

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Abstract

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

Keywords: Plasma folate level; Urinary arsenic species; Interaction; Urothelial carcinoma

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Study subjects

One hundred and seventy-one patients, age range 24–93 years, with pathologically proven UC were recruited from the Department of Urology, National Taiwan University Hospital, between September 2002 and May 2004. Pathological verification of UC was done by routine urological methods including endoscopic biopsy or surgical resection of urinary tract tumors followed by histopathological examination by board-certified pathologists. A total of 488 control subjects with no evidence of UC or any other malignancy were recruited from a hospital-based pool, including those receiving senior citizen health examinations at Taipei Medical University Hospital and those receiving health examinations at Taipei Municipal Wan Fang Hospital. These three hospitals are medical center and their clinical clients' bases are similar and located in Taipei approximately 200–300 km away from the arsenic-contaminated areas in Taiwan. In this study, no case subjects or controls have lived in the arsenic-contaminated areas in southwestern (Chen et al., 2003b) or northeastern Taiwan (Chiou et al., 2001). Although we only collected tap water from 37 UC cases and determined the total arsenic levels, the mean ± standard error was 17.14 ± 0.55 μg/L. However, urinary total arsenic levels in cases and controls were 24.47 ± 2.56 μg/L and 24.85 ± 1.06 μg/L, respectively (p-value is 0.89 for Student's t-test). These results may indicate no difference in arsenic exposure between cases and controls.

2.2. Questionnaire interview and specimens collection

Well-trained personnel carried out standardized personal interviews based on a structured questionnaire. Information collected included demographic and socioeconomic characteristics, general potential risk factors for malignancies such as lifestyle, quantified details of alcohol consumption, cigarette smoking, exposure to potential occupational and environmental carcinogens such as hair dyes and pesticides, chronic medication history, consumption of conventional and alternative medicines, and personal and family history of urological diseases. Regular alcohol drinkers referred to those who consumed alcohol three or more days per week, continuing for at least six months. The Research Ethics Committee of National Taiwan University Hospital, Taipei Medical University Hospital and Taipei Municipal Wan Fang Hospital approved the study. All subjects provided informed consent forms before specimen's collection and questionnaire interview. The study was consistent with the World Medical Association Declaration of Helsinki.

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

2.3. Plasma folate assays

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

2.4. Determination of urinary arsenic species

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