**行政院國家科學委員會專題研究計畫成果報告**

計畫名稱：台灣烏腳病地區居民對無機砷甲基化能力與週邊血管疾病相關性的研究

計畫編號：93-2320-B-002-071

執行期限：93 年 08 月 01 日至 94 年 07 月 31 日

主持人：曾慶孝 台大醫院內科部

**Abstract**

This study examined the interaction between arsenic exposure and urinary arsenic speciation on the risk of peripheral vascular disease (PVD) in blackfoot disease (BFD)-hyperendemic area in Taiwan. A total of 479 (220 men and 259 women) adults residing in the BFD-hyperendemic area were studied. Doppler ultrasound was used to diagnose PVD. Arsenic exposure was estimated by an index of cumulative arsenic exposure (CAE). Urinary levels of total arsenic, inorganic arsenite ($As^{III}$) and arsenate ($As^{V}$), monomethylarsonic acid ($MMA^{V}$) and dimethylarsinic acid ($DMA^{V}$) were determined. Primary methylation index [$PMI=MMA^{V}/(As^{III}+As^{V})$] and secondary methylation index ($SMI=DMA^{V}/MMA^{V}$) were calculated. The association between PVD and urinary arsenic parameters was evaluated with consideration of the interaction with CAE and the confounding effects of age, sex, body mass index, total cholesterol, triglycerides, cigarette smoking and alcohol consumption. Results showed that PVD risk increased with a higher CAE and a lower capacity to methylate arsenic to $DMA^{V}$. The multivariate-adjusted odds ratios for CAE of 0, 0.1-15.4 and >15.4 mg/L×year were 1.00, 3.41 (0.74-15.78) and 4.62 (0.96-22.21), respectively ($p<0.05$, trend test); and for PMI≦1.77 and SMI>6.93, PMI>1.77 and SMI>6.93, PMI>1.77 and SMI≦6.93, and PMI≦1.77 and SMI≦6.93 were 1.00, 2.93 (0.90-9.52), 2.85 (1.05-7.73) and 3.60 (1.12-11.56), respectively ($p<0.05$, trend test). It was concluded that individuals with a higher arsenic exposure and a lower capacity to methylate inorganic arsenic to $DMA^{V}$ have a higher risk of developing PVD in BFD-hyperendemic area in Taiwan.

**Keywords**: peripheral vascular disease, environmental pollutants, arsenic methylation, urinary arsenic speciation

**Introduction**

The metabolism of inorganic arsenic involves 2 steps of chemical reactions: reduction and oxidative methylation [1]. Pentavalent arsenate is reduced to trivalent arsenite before it can be further metabolized. Arsenite is then oxidatively methylated to monomethylarsonic acid ($MMA^{V}$) and dimethylarsinic acid ($DMA^{V}$). Previously, methylation of inorganic arsenic has always been considered as a detoxification mechanism because $MMA^{V}$ and $DMA^{V}$ have relatively low toxicity and are rapidly excreted in the urine. However, recent studies have confirmed the existence of trivalent intermediates and products of monomethylarsonous acid ($MMA^{III}$) and dimethylarsinous acid ($DMA^{III}$), which are more toxic than inorganic arsenite. The capacity to metabolize inorganic arsenic differs among individuals; and its biologic effects on various organ systems depend not only on the ingested dosage, but also on the capacity of the individuals to metabolize and detoxify the related compounds. To achieve a more accurate assessment of arsenic methylation capacity, it is necessary to determine the specific arsenic species derived from inorganic arsenic, which are excreted in the urine. Studies evaluating the association between various urinary arsenic metabolites and clinical outcomes are still rare. Some recent studies have documented that subjects with higher cumulative arsenic exposure (CAE) and higher urinary $MMA^{V}$ percentage or lower urinary $DMA^{V}$ percentage suffered from a higher risk of skin cancer [2] and bladder cancer [3] among residents of the BFD areas. Our previous studies evaluating the association between inorganic arsenic and PVD have focused on the estimated ingested

dosage of total arsenic from drinking water [4,5].Whether the metabolism of arsenic could have an effect on the risk of developing PVD is an interesting issue that has not been studied before. Therefore, the present study aimed at evaluating the impact of the interaction between arsenic exposure dosage and urinary arsenic species on the development of PVD among residents in the BFD-hyperendemic area.

## Material and Methods

### Study subjects

The study subjects were recruited from residents in three BFD-hyperendemic villages in Putai Township of Chiayi County located along the southwest coast of Taiwan. The population with an age of 30 years or older in the studied villages as registered in the household registration office was 2258. Among them, 1571 (70%) were eligible and lived in the study villages 5 or more days a week. From September to December 1988, a total of 1081 (69%) of the eligible subjects were interviewed. All of the 1081 subjects were invited to participate in the first health examination during January and February 1989 and 941 (87%) subjects actually participated. Bi-annual health examinations were then carried out. The urinary samples used for the assay of arsenic metabolites in the present study were collected during the first health examination. The Doppler ultrasound examination for diagnosis of PVD was performed and blood samples were collected during the third health examination in February 1993. A total of 479 subjects having both urinary samples and receiving Doppler ultrasound examination were recruited for the present study.

### Questionnaire interview and blood sample collection

Information obtained included history of consuming high-arsenic artesian well water, residential history, and lifestyle variables including alcohol drinking and cigarette smoking. CAE (in mg/L×year) was derived from the arsenic concentration in artesian well water (mg/L) and the duration of consuming the artesian well water (year). Fasting blood samples were also collected for the measurement of serum total cholesterol and triglycerides in the third bi-annual health examination in 1993.

### Diagnosis of PVD

During the third health examination in February 1993, 582 subjects including 263 men and 319 women underwent Doppler ultrasound examination [4,5]. Systolic pressures on bilateral brachial, posterior tibial and dorsal pedal arteries were measured with Medacord PVL (Meda-Sonic Inc., Mountain View, CA) and the device automatically calculated the right and left ankle-brachial indices (ABI). Diagnosis of PVD was based on an ABI$<$0.90 on either side [4,5].

### Determination of urinary arsenic species

The frozen urinary samples were thawed at room temperature, dispersed by ultrasonic wave, filtered through Sep-Pak $C_{18}$ column (Mallinckrodt Baker In., NJ, USA) and tested for levels of $As^{III}$, $As^{V}$, $MMA^{V}$ and $DMA^{V}$. An aliquot of 200 μL was used for separation of arsenic species by HPLC (Waters 501, Waters Associates, Milford, MA, USA) with columns obtained from Phenomenex (Nucleosil 10SB, Torrance, CA, USA). The levels of various species of inorganic arsenic and their metabolites were quantified by HGAAS. Freeze-dried urine SRM 2670, which was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and contained 480±100 μg/L of arsenic, was analyzed together with urinary samples of the subjects to control for the quality.

### Data analyses and statistical methods

Arsenic methylation capacity was assessed by primary methylation index (PMI) defined as the ratio between $MMA^{V}$ and inorganic arsenic ($As^{III}$+$As^{V}$) level, and secondary methylation index (SMI) as the ratio between $DMA^{V}$ and $MMA^{V}$. Logistic regression models were used to estimate the multivariate-adjusted ORs and their 95% CIs for PVD, with regards to CAE and urinary

arsenic profile.

## Results

The multivariate-adjusted odds ratios for CAE of 0, 0.1-15.4 and >15.4 mg/L×year were 1.00, 3.41 (0.74-15.78) and 4.62 (0.96-22.21), respectively ($p$<0.05, trend test); and for PMI≦1.77 and SMI>6.93, PMI>1.77 and SMI>6.93, PMI>1.77 and SMI≦6.93, and PMI≦1.77 and SMI≦6.93 were 1.00, 2.93 (0.90-9.52), 2.85 (1.05-7.73) and 3.60 (1.12-11.56), respectively ($p$<0.05, trend test).

## Discussion

The present study confirmed the association between PVD and increased $MMA^V$ percentage and/or decreased $DMA^V$ percentage, taking into account the ingested arsenic dosage. Therefore, the results of this study suggested that PVD susceptibility is not only related to the exposure dosage of arsenic, the metabolism of arsenic has a significant and great impact on the susceptibility and development of PVD in subjects chronically exposed to arsenic: the more efficient to methylate to $DMA^V$, the lower the risk.

The importance of a complete second methylation is also implicated from the observation that human beings excrete significant amounts of $MMA^V$ in the urine and are more susceptible to arsenic-related health hazards while compared to animals like mice and hamsters that can methylate arsenic very efficiently, resulting in urinary excretion of $MMA^V$ in less than 10% of the urinary arsenic species [6]. The more efficient methylation of arsenic has also been suggested as the explanation for the lower sensitivity to arsenic-induced tumor in rodents [7].

In conclusion, PVD risk in BFD-hyperendemic area in Taiwan is associated with a higher exposure dosage of arsenic and a lower capacity to methylate inorganic arsenic to $DMA^V$. These observations could explain partly why some subjects with a high exposure dosage would not develop clinical PVD. However, the association between PVD and the undetected trivalent forms of methylated metabolites in this study awaits further clarification.

## References


1. Thomas, D.J., Waters, S.B., Styblo, M., 2004. Elucidating the pathway for arsenic methylation. Toxicol. Appl. Pharmacol. 198, 319-326.

2. Chen, Y.C., Guo, Y.L., Su, H.J., Hsueh, Y.M., Smith, T.J., Ryan, L.M., Lee, M.S., Chao, S.C., Lee, J.Y., Christiani, D.C., 2003. Arsenic methylation and skin cancer risk in southwestern Taiwan. J. Occup. Environ. Med. 45, 241-248

3. Chen, Y.C., Su, H.J., Guo, Y.L., Hsueh, Y.M., Smith, T.J., Ryan, L.M., Lee, M.S., Christiani, D.C., 2003. Arsenic methylation and bladder cancer risk in Taiwan. Cancer Causes Control 14, 303-310.

4. Tseng, C.H., Chong, C.K., Chen, C.J., Tai, T.Y., 1996. Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis 120, 125-133.
5. Tseng, C.H., Chong, C.K., Chen, C.J., Tai, T.Y., 1997. Lipid profile and peripheral vascular disease in arseniasis-hyperendemic villages in Taiwan. Angiology 48, 321-335.
6. Marafante, E., Vahter, M., Norin, H., Envall, J., Sandstrom, M., Christakopoulos, A., Ryhage, R., 1987. Biotransformation of dimethylarsinic acid in mouse, hamster and man. J. Appl. Toxicol. 7, 111-117.
7. WHO, 1980. IARC monographs on the evaluation of the carcinogenic risk of chemical to humans: some metal and metallic compounds. World Health Organization, Lyon, IARC.