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Risk of Erectile Dysfunction Induced by Arsenic Exposure through Well Water Consumption in Taiwan

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Key words: arsenic exposure, calculated free testosterone, erectile dysfunction, sex hormone, total testosterone

Abbreviations:

ANOVA	Analysis of variance
cFT	Calculated free testosterone
CI	Confidence interval
CVD	Cardiovascular disease
DHT	Dihydrotestosterone
DM	Diabetes mellitus
ED	Erectile dysfunction
IIEF	International Index of Erectile Function
ISSAM	International Society for the Study of the Aging Male
MMAS	Massachusetts Male Aging Study
NO	Nitric oxide
NOS	Nitric oxide synthase
OR	Odds ratio
SHBG	Sex hormone binding globulin
TT	Total testosterone
U.S. EPA	U.S. Environmental Protection Agency

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Abstract

Background: Erectile dysfunction (ED) has a profound impact on life quality of many men. Many risk factors are associated with ED, such as ageing, sex hormone levels, hypertension, cardiovascular diseases and diabetes mellitus. Arsenic exposure could damage peripheral vessels and increase the risk of cardiovascular disease. However, the relationship between arsenic exposure and ED was seldom evaluated.

Objectives: This study aims to investigate whether exposure to arsenic enhances the risk of ED.

Methods: One hundred and seventy-seven males aged more than 50 were recruited through health examination conducted in three hospitals in Taiwan. A questionnaire of International Index of Erectile Function-5 was used to measure the level of erectile function. Sex hormones including total testosterone and SHBG were determined by radioimmunoassay. Another standardized questionnaire was also used to collect the background information and behaviors on cigarette smoking, alcohol, tea or coffee drinking and physical activity.

Results: The prevalence of ED was greater in the arsenic endemic area (83.3%) than in the non-arsenic endemic area (66.7%). Subjects with arsenic exposure more than 50 ppb had a significantly higher risk of developing ED than those with less than 50 ppb after this risk was adjusted for age, cigarette smoking, diabetes mellitus, hypertension and cardiovascular disease (OR=3.4). It also showed that the risk of developing severe ED was drastically enhanced due to arsenic exposure (OR=7.5) after adjusted for free testosterone and traditional risk factors of ED.

Conclusions: A negative impact of chronic arsenic exposure on erectile function was suggested.

Introduction

According to the definitions of the First International Consultation on erectile dysfunction (ED) co-sponsored by World Health Organization, ED is considered as 'the consistent or recurrent inability to attain and/or to maintain a penile erection sufficient for sexual performance' (Jardin et al. 2000). Although ED is a benign disorder, it is related to physical and psychological health, and has a notable impact on the life quality of both sufferers and their families (Wespes et al. 2002).

Aging is the most common risk factor associated with ED. Therefore, age-related factors including hormonal derangement, diabetes mellitus (DM), neural damage from surgery, side effects of drug, radiation therapy, and psychogenic factors are the most frequently cited causes of ED (Araujo et al. 1998; Wein and van Arsdalen 1988). Of the sex hormone levels, the changes in free testosterone correlate most closely with aging and have the closest correlation with sexual activity (Ahn et al. 2002). Androgens have been suggested to be essential in the maintenance of libido and important in regulating penile smooth muscle function in men (Salonia et al. 2003). In animal models, it was found that androgens could regulate the expression and activity of nitric oxide synthase (NOS) isoforms in the corpus cavernosum (Marin et al. 1999; Park et al. 1999; Shen et al. 2000). Nitric oxide (NO) was considered to mediate relaxation of the vascular smooth muscle of the resistance arteries of the corpus cavernosum and the trabeculae to facilitate penile erection. In castrated animals, testosterone and 5 α -dihydrotestosterone (DHT) administration restored the erectile response and NOS expression in the penis (Armagan et al. 2006; Baba et al. 2000a, 2000b). Within the follow-up data of Massachusetts Male Aging Study (MMAS), total testosterone declined 0.8% per year (Feldman et al. 2002), whereas both free and albumin-bound testosterone declined about 1.4%-2% per year (Feldman et al. 2002; Vermeulen et al. 1991). In our previous study, a significant association was found

between low levels of serum calculated free testosterone, bioavailable testosterone, and severity of ED in middle-aged and aged males in Taiwan (Hwang et al. 2007).

There is also accumulated evidence supporting the association between ED and cardiovascular risk factors, such as hypertension and hyperlipidemia, as well as cardiovascular disease (CVD) (Billups 2005). Arsenic exposure was reported to damage peripheral vessels and cause black foot disease (Ch'i and Blackwell 1968). It has also been found that arsenic exposure was correlated with CVD such as atherosclerosis (Wang C et al. 2007; Wang Y et al. 2007; Wu et al. 2006). One of the possible explanations for the correlation between arsenic exposure and CVD was that arsenic exposure was associated with reduction of NOS activity (Zarazua et al. 2006), which was important in mediating relaxation of the vascular smooth muscle. As we mentioned before, NO was also play a critical role in penile erection. However, few studies discuss the relationship between arsenic exposure and ED. Therefore, our specific aim was to evaluate whether arsenic exposure would increase the risk of ED in males after adjusting conventional risk factors. Questionnaires of International Index of Erectile Function (IIEF-5) (Rosen et al. 1997) were used to measure the level of erectile function. To our knowledge, this is the first study to investigate the association between ED and arsenic exposure in humans.

Materials and Methods

Study subjects

A total of 8102 residents from 18 villages in four townships were interviewed and recruited as the study cohort from 1991 to 1994. In 1996, invitation letters were sent to 5146 subjects, who were still alive and had complete information of contact address and arsenic levels in well water, from the original cohort for attending a health examination held in 1997-1998. Finally, 1318 cohort members participated the health examination. In this study, due to a limited budget, the invitation letters were sent to a random sample of 300 males aged ≥ 50 years from the population of 1318 residents for attending a health screening held in Lotung Poh-Ai hospital in 2003. Eventually, a total of 66 males aged ≥ 50 years and live in a confirmed arsenic-endemic area of Lanyang Basin in Taiwan (Chiou et al. 1997) were recruited through health screening in Lotung Poh-Ai Hospital as an arsenic-endemic area group. The details of arsenic exposure for each study subjects were from our previous study (Chiou et al. 1997). 111 males aged ≥ 50 years were recruited through health examination in Taipei Wan-Fang Hospital and Taipei Medical University Hospital in 2003 as a non arsenic-endemic area group. Participants who failed to answer the question on ED or took hormone medicine (supplementation or deprivation) were excluded in this study. All study subjects were determined as ED cases or controls by IIEF-5 score. After screening by IIEF, there were 129 ED cases and 48 controls recruited in this study. This study was approved by the Institutional Review Board for human subjects of Taipei Medical University, Taipei, Taiwan and each subject provided a written informed consent prior to the study.

Data collection

The information was collected through a standardized self-completed questionnaire including demographic characteristics (age, marital status, occupation and education),

life style factors (cigarette smoking, alcohol drinking, tea, coffee and physical activity), and disease record. IIEF-5 was used to measure the level of erectile function.

Definition of erectile function

The total score of IIEF-5 items is 25. According to the ED assessment, healthy without ED is defined as score more than 21, score between 12 and 21 as mild ED, from 8 to 11 as moderate ED, and less than 7 is categorized as severe ED.

Hormones measurement

8 ml of non-fasting blood sample was drawn by venipuncture from each study subjects with a signed inform consent. All blood samples were collected at 8:00-12:00 in the morning and then centrifuged at 3000 rpm. Serum was stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Hormones including total testosterone (TT) and sex hormone binding globulin (SHBG) were determined by radioimmunoassay. Calculated free testosterone (cFT) was determined by total testosterone, albumin and SHBG using the method from the website of International Society for the Study of the Aging Male (ISSAM 2007). The serum level of TT more than 11 nmol/L and cFT more than 0.23 nmol/L were recognized as normal (Morales et al. 2004).

Determination of arsenic in the well water and arsenic exposure

Well water samples were collected during the home interview and immediately acidified with hydrochloric acid. Then these samples were stored at $-20\text{ }^{\circ}\text{C}$ until the subsequent assay. Hydride generation combined with flame atomic absorption spectrometry was used to determine the arsenic concentration in these samples. It was found that the arsenic concentration in well water ranged from undetectable ($<0.15\text{ ppb}$) to $3.59 \times 10^3\text{ ppb}$. The details were described in our previous study (Chiou et al. 1997, 2001).

Statistical analyses

All data were analyzed by the SAS package (version 8.1, SAS Institute, Cary, NC), and were considered statistically significant at $p < 0.05$. The statistical significance was assessed by the chi-square test for categorical variables (Table 1) and by analysis of variance (ANOVA) for continuous variables (Table 2). A Duncan's post hoc test was used to determine which groups differ from each other after an ANOVA was completed and an effect (rejected the null) in the ANOVA was found. Multi-variable Logistic regression analyses were employed to estimate odds ratio (OR) and its 95% confidence interval (CI). In Table 3, models I to III were used to study the relationships between ED and arsenic exposure, testosterone and free testosterone respectively, after adjusted for age, smoking, diabetes mellitus, hypertension and cardiovascular disease. Model IV was employed to investigate the relationship between ED and arsenic exposure after adjusted for age, free testosterone, smoking, diabetes mellitus, hypertension and cardiovascular disease. The same methodology was applied in Table 4 to explore the relationship between severe ED and arsenic exposure.

Results

The characteristics of study subjects from arsenic endemic and non-arsenic endemic areas were shown in Table 1. There was no significant difference in the distributions of age, hypertension, DM, and cardiovascular disease between study subjects from these two areas. The average age was 67.8 and 67.4 in arsenic endemic and non-arsenic endemic areas, respectively. The percentage of cigarette smoking was significantly higher in subjects from arsenic endemic area than those from non-arsenic endemic area. Interestingly, the prevalence of ED was greater in arsenic endemic area (83.3%) than in non-arsenic endemic area (66.7%).

Though the safety level for arsenic in drinking water set by the U.S. Environmental Protection Agency was 10 ppb which was based on estimation of cancer risk (U.S. EPA 2007), a recent report from Wang Y et al. (2007) showed that a marked age- and gender-adjusted odds ratio of 3.3 for the development of carotid atherosclerosis was only observed among the high-arsenic exposure group who drank well water containing the arsenic level greater than 50 ppb. The risk of carotid atherosclerosis was not significant among the group with arsenic exposure between 10 ppb and 50 ppb. Therefore, subjects were classified into two groups based on the level of 50 ppb—one with arsenic exposure greater than 50 ppb and the other the rest. Each group was further divided into three sub-groups according to their IIEF-5 score (i.e. greater than 21, between 8 and 21, and less than 8) in order to investigate the relationship between sex hormone, arsenic exposure and ED. As shown in Table 2, significantly lower levels of testosterone or free testosterone were observed in severe ED cases compared with healthy controls. Moreover, both the arsenic exposure groups (≤ 50 ppb and > 50 ppb) showed marked liner trends of free testosterone across different severity of ED. However, in the item of testosterone, the observation

was only seen in the group with arsenic exposure ≤ 50 ppb. Without considering ED, the lower average levels of testosterone and free testosterone were found in subjects with arsenic exposure more than 50 ppb. However, the average level of SHBG was not significantly different between subjects with or without arsenic exposure. Only a significant linear trend of SHBG across different severity of ED was observed in the subjects with arsenic exposure more than 50 ppb.

In order to further clarify the relationships between ED and testosterone, free testosterone, and arsenic exposure respectively, traditional risk factors (e.g. age, smoking, DM, hypertension and CVD) were adjusted in models I to III as shown in Table 3. After that, the results showed that only subjects with arsenic exposure more than 50 ppb (model I) or free testosterone less than 0.23 nmol/l (model III) possessed significant risk of ED. The OR of arsenic exposure more than 50 ppb in model I and that of free testosterone less than 0.23 nmol/l in model III were 3.4 (95%CI=1.1-10.3) and 4.8 (95%CI=1.3-18.0) respectively. There was no significant risk of ED among subjects with testosterone less than 11 nmol/l in model II. Because free testosterone less than 0.23 nmol/l was found as an important risk factor of ED in model III, free testosterone was adjusted in model IV to derive a more apparent relationship between arsenic exposure and ED. After adjusted for serum free testosterone and traditional risk factors of ED, it still could be seen that arsenic exposure enhanced the risk of developing ED (OR=3.0, 95%CI=1.0-9.2) in model IV. A significant positive trend between age and ED was observed in all models. Moreover, study subjects older than 70 years old had the highest risk of developing ED (OR=6.0-7.0). As shown in Table 4, the same methodology was also applied to obtain the ORs of severe ED among subjects with arsenic exposure more than 50 ppb. In model IV from Table 4, a drastically enhanced OR (OR=7.5, 95%CI=1.8-30.9) for severe ED were observed in

subjects with arsenic exposure greater than 50 ppb after adjusted for age, free testosterone, diabetes mellitus and cardiovascular disease. Besides arsenic exposure, the risk factors of severe ED in model IV included age more than 70 years (OR=30.9, CI=5.2-182.1), abnormal level of serum free testosterone (OR=4.7, CI=1.2-18.9), diabetes mellitus (OR=5.5, 95%CI=1.2-24.2) and cardiovascular disease (OR=1.0, 95%CI=1.0-26.8). We used the current WHO drinking water guideline of 10 ppb as cut-point to analyze the data again. The OR of abnormal erectile function among subjects with arsenic exposure more than 10 ppb was 1.9 (95%CI=0.7-5.3), not reach statistical significance, after adjustment of age, free testosterone, smoking, DM, hypertension and CVD (data not shown). This indicated that there was no significant risk of abnormal erectile function among subjects with arsenic exposure more than 10 ppb. However, a significant OR of 4.0 (95%CI=1.2-13.6) for severe ED was observed in subjects with arsenic exposure more than 10 ppb after adjustment of age, free testosterone, smoking, diabetes mellitus, hypertension and cardiovascular disease (data not shown). The results showed that employing arsenic exposure of 50 ppb as cut-point revealed a stronger effect of arsenic in ED than using 10 ppb as cut-point.

Discussion

In our study, the prevalence of ED in arsenic endemic and non-arsenic endemic areas was 83.3% and 66.7%, respectively (Table 1). Ansong et al. (2000) did a self-administered survey among 5198 randomly selected men 50-76 years old, living in four rural counties in Central New York State. They found that age-specific prevalence was 26.0%, 34.9%, 46.9%, 57.8% and 69.4% among men 50-54, 55-59, 60-64, 65-69 and 70-76 years old, respectively. Shiri et al. (2003) estimated the prevalence of ED in a population based sample of 50 to 75-year-old Finnish men. The overall prevalence of ED was 76.5%. The prevalence of ED increased from 67% for men 50 years old to 89% for those 75 years old. The observations from the above studies indicated that ED was a highly prevalent disorder among men older than 50 years old and the prevalence of ED increased with advancing age. In our study, age distribution was not significantly different between subjects from arsenic endemic area (67.77 ± 6.76) and non-arsenic endemic area (67.42 ± 8.70), but a greater prevalence of ED was observed in the arsenic endemic area than non-arsenic endemic area suggesting that arsenic may play a role of developing ED.

Lower prevalence of DM and hypertension was observed in study subjects from the arsenic endemic area than those from the non-arsenic endemic area (Table 1), but this did not reach statistical significance. Because the age distribution was similar in subjects from these two areas, different prevalence of DM and hypertension observed between these two areas may be due to the health examination bias. However, even under the health examination bias, the prevalence of ED was drastically higher in study subjects from the arsenic endemic area than those from the non-arsenic endemic area. This indicates that the health examination bias generated limited influence to the observation of high prevalence of ED in the arsenic endemic area.

A significantly association between concentration of serum testosterone, free testosterone and severity of ED was seen in the group with arsenic exposure less than 50 ppb (Table 2). Interestingly, the average levels of free testosterone and testosterone were significant less in the subjects with arsenic exposure more than 50 ppb compared with those less than 50 ppb. The data suggest that arsenic was strongly associated with lower proportion of free fraction, bioavailable testosterone or total testosterone in circulating blood. Several reports supported the theory that sex hormones especially the free testosterone was associated with sexual function (Kim 1999; Martínez-Jabaloyas et al. 2006). Therefore, arsenic probable increases the risk of ED through reducing the level of free testosterone. Data from a rat model indicated that arsenic has a suppressive influence on spermatogenesis, gonadotropin and testosterone released (Sarkar et al. 2003). However, the actual mechanism(s) by which arsenic impairs male reproductive function remains unclear.

Age, DM, cigarette smoking, hypertension, sex hormone and cardiovascular disease have been reported to be associated with ED. In the present study, the two risk factors of ED, diabetes mellitus and cardiovascular disease, reached the statistical significance only in the subjects with severe ED. After adjusted for age, free testosterone, DM, cigarette smoking, hypertension and cardiovascular disease, the increased risk of ED was still found in the subjects with arsenic exposure more than 50 ppb (Tables 3 and 4). Therefore, arsenic may also influence erectile function via non-hormone dependent pathway. Oxidative stress has been suggested to be a major cause of male reproductive failure. Indeed, some studies suggested that the toxicity resulting from chronic arsenic exposure was due to oxidative stress (Liu et al. 2001; Maiti and Chatterjee 2001; Ramanathan et al. 2002; 2003; Santra et al. 2000). It was believed that alterations in neural and impaired penile vascular systems were mainly

responsible for ED rates approaching 75% in some reports (Benet and Melman 1995; Hakim and Goldstein 1996). Nitric oxide (NO), derived from vascular endothelial and neural sources, played a critical role in the early steps of the normal cascade of the penile vasculature and the relaxation of cavernous smooth muscle (Andersson and Wagner 1995; Burnett et al. 1992; Moreland et al. 2001). The presence of oxygen free radicals inactivated NO and reduced its physiologic impact. Direct inactivation of NO, largely by superoxide anions, may be involved in producing impaired cavernosal relaxation (Katusic 1996).

In conclusion, our study suggests that chronic arsenic exposure has a negative impact on erectile function. The potential pathways of arsenic exposure leading to ED may be that arsenic could either through inhibit the level of sex hormone or reduce NOS activity to impair the functions of smooth muscle and vessel in penile. Future work could further elucidate the interaction of arsenic exposure, sex hormone, and oxidative stress related factors on the risk of ED with a larger sample size.

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Table 1 : Characteristics of study subjects from arsenic endemic and non-arsenic endemic areas

		<u>arsenic endemic area^a non-arsenic endemic area</u>		P value ^b
		N (%)	N (%)	
Age (years)	<60	9 (13.6)	22 (19.8)	0.295
	≥ 60	57 (86.4)	89 (80.2)	
Cigarette smoking		47 (72.3)	55 (49.6)	0.003
Diabetes mellitus		4 (6.1)	17 (15.3)	0.066
Hypertension		15 (22.7)	38 (34.2)	0.106
Cardiovascular disease		6 (13.6)	16 (14.4)	0.900
ED		55 (83.3)	74 (66.7)	0.016

^a The average duration of arsenic exposure was 42 years.

^b χ^2 -test

Table 2: Sex hormone levels of study subjects categorized by erectile function and arsenic exposure

	Arsenic exposure (ppb)	IIEF (mean±SD)			P value ^a	Total (N=174)	P value ^b
		>21 (n=48) (normal ED)	8-21 (n=49) (moderate/mild ED)	≤ 7 (n=77) (severe ED)			
Testosterone (nmol/l)	≤ 50 ^c	19.28±7.21 ^d	17.50±4.28 ^{de}	16.03±5.28 ^e	0.01	17.55±5.85	
	>50	16.74±4.19	15.57±2.96	14.46±5.16	0.22	15.04±4.64	0.009
Free testosterone (nmol/l)	≤ 50 ^c	0.42±0.17 ^d	0.40±0.13 ^{de}	0.33±0.17 ^e	0.01	0.38±0.16	
	>50	0.39±0.11	0.34±0.09	0.28±0.14	0.03	0.31±0.13	0.003
SHBG (nmol/l)	≤ 50	33.53±15.74	31.25±14.21	37.31±17.03	0.26	34.20±15.86	
	>50	28.71±11.57	31.29±11.34	41.79±19.17	0.03	37.61±17.50	0.23

^a test for trend

^b test by different arsenic exposure

^c A Duncan's post hoc test was used to determine which groups differ from each other after an ANOVA was completed and an effect (rejected the null) in the ANOVA was found. Means with different letters showed significant difference between them (i.e. d vs. e represents significant difference; d vs. de or de vs. e represent no significant difference).

Table 3: Multivariate-adjusted ORs and 95% CIs in subjects with ED (IIEF \leq 21) comparing to those with normal erectile function

		Model I OR (95% CI)	Model II OR (95% CI)	Model III OR (95% CI)	Model IV OR (95% CI)
Age (years)	<60	1.0	1.0	1.0	1.0
	60-70	1.7 (0.6-4.7)	1.9 (0.7-5.0)	1.7 (0.6-4.6)	1.6 (0.6-4.4)
	\geq 70	7.0 (2.4-20.6) ***	7.0 (2.4-20.7) ***	6.0 (2.0-18.1) ***	6.1 (2.0-18.4) ***
		p for trend: <0.001	p for trend: <0.001	p for trend: <0.001	p for trend: <0.001
Testosterone < 11 (nmol/l)			1.5 (0.5-4.5)		
Free testosterone < 0.23 (nmol/l)			4.8 (1.3-18.0) **	4.3 (1.1-16.7) **	
Arsenic exposure (ppb)	\leq 50	1.0			1.0
	>50	3.4 (1.1-10.3) **			3.0 (1.0-9.2) *
Cigarette smoking		1.2 (0.5-2.5)	1.3 (0.6-2.8)	1.3 (0.6-2.9)	1.2 (0.6-2.7)
Diabetes mellitus		3.0 (0.9-10.6)	2.5 (0.7-8.5)	2.7 (0.8-9.3)	3.2 (0.9-11.0)
Hypertension		0.5 (0.2-1.3)	0.4 (0.2-1.1)	0.4 (0.2-1.0)	0.5 (0.2-1.2)
Cardiovascular disease		3.6 (0.9-14.0)	3.1 (0.8-11.6)	3.1 (0.8-12.2)	3.4 (0.8-13.4)

* p=0.05 ** p<0.05 *** p<0.005

Table 4: Multivariate-adjusted ORs and 95% CIs in subjects with severe ED (IIEF ≤ 7) comparing to those with normal erectile function

		Model I OR (95% CI)	Model II OR (95% CI)	Model III OR (95% CI)	Model IV OR (95% CI)
Age (years)	<60	1.0	1.0	1.0	1.0
	60-70	4.9 (1.0-24.0) *	4.5 (1.0-20.6) *	4.3 (0.9-20.7)	4.8 (0.9-25.4)
	≥ 70	35.9 (6.5-197.5) ***	27.1 (5.4-134.8) ***	22.1 (4.3-114.4) ***	30.9 (5.2-182.1) ***
		p for trend: <0.001	p for trend: <0.001	p for trend: <0.001	p for trend: <0.001
Testosterone <11 (nmol/l)			2.4 (0.7-7.8)		
Free testosterone <0.23 (nmol/l)				4.9 (1.3-18.9) **	4.7 (1.2-18.9) **
Arsenic exposure (ppb)	≤ 50	1.0			1.0
	>50	7.7 (2.0-30.0) ***			7.5 (1.8-30.9) **
Cigarette smoking		1.2 (0.4-3.0)	1.4 (0.6-3.7)	1.4 (0.5-3.5)	1.2 (0.4-3.2)
Diabetes mellitus		5.9 (1.3-27.22) **	3.8 (1.0-15.2)	3.7 (0.9-14.5)	5.5 (1.2-24.2) **
Hypertension		0.5 (0.2-1.5)	0.4 (0.1-1.1)	0.4 (0.1-1.2)	0.4 (0.1-1.4)
Cardiovascular disease		6.5 (1.3-32.1) **	4.4 (1.0-19.4) *	4.2 (0.9-19.3)	5.3 (1.0-26.8) **

* p=0.05 ** p<0.05 *** p<0.005