Association of Urinary Arsenic with Serum 25(OH)D in Adults Living in an Area of Water-Borne Arsenicosis in Shanxi, China

Kunyu Zhang , Yunyi Yin , Man Lv , Xin Zhang , Meichen Zhang , Jia Cui , Ziqiao Guan , Xiaona Liu , Yang Liu , Yanhui Gao * , Yanmei Yang *

Posted Date: 20 October 2023

doi: 10.20944/preprints202310.1308.v1

Keywords: arsenic; serum 25(OH)D; methylation capacity; skin hyperkeratosis

Article

Association of Urinary Arsenic with Serum 25(OH)D in Adults Living in an Area of Water-Borne Arsenicosis in Shanxi, China

Kunyu Zhang [1,2], Yunyi Yin [1,2], Man Lv [1,2], Xin Zhang [1,2], Meichen Zhang [1,2], Jia Cui [1,2], Ziqiao Guan [1,2], Xiaona Liu [1,2], Yang Liu [1,2], Yanhui Gao [1,2,*] and Yanmei Yang [1,2,*]

[1] Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, Harbin 150081, Heilongjiang Province, China.
[2] Key Lab of Etiology and Epidemiology, Education Bureau of Heilongjiang Province & Ministry of Health of P. R. China, Harbin Medical University, Harbin 150081, Heilongjiang Province, China.
* Correspondence: gaoyanhui@hrbmu.edu.cn (Y. Gao), yangyanmei@hrbmu.edu.cn (Y. Yang).

Abstract: Limited studies have shown that exposure to arsenic is associated with serum vitamin D levels, but the results are still inconsistent. A cross-sectional study of 762 participants was conducted in Wenshui Country, Shanxi Province, which was identified as an area of water-borne arsenicosis. The results showed positive relationship between urinary arsenic species (iAs, MMA, DMA) and serum 25(OH)D. Binary logistic regression analysis showed that the significant increases of 0.4% and 0.6% in the risk of vitamin D excess for every 1-unit increment in the Box-Cox transformed urinary tAs and DMA, respectively. After stratifying populations based on arsenic methylation metabolic capacity, each one-unit increase in the Box-Cox transformed tAs level was associated with increases of 0.064 (95%CI: 0.032 to 0.096) in serum 25(OH)D in the populations with iAs% above the median. In the populations with skin hyperkeratosis, urinary iAs was positively associated with serum 25(OH)D (β=0.592, 95%CI: 0.041 to 1.143). Overall, our findings support the positive relationship between urinary arsenic and serum 25(OH)D.

Keywords: arsenic; serum 25(OH)D; methylation capacity; skin hyperkeratosis

1. Introduction

Vitamin D is known for its role in supporting bone growth, a few studies have also shown beneficial links between vitamin D and other non-skeletal health outcomes, including immune function, cardiovascular health and cancer[1]. Vitamin D functions in the body through both an endocrine mechanism (regulation of calcium absorption) and an autocrine mechanism (facilitation of gene expression)[2]. However, both vitamin D deficiency and excess can lead to serious health problems. Vitamin D deficiency is the most common nutritional deficiency worldwide. Insufficient vitamin D has been implicated in a range of disorders such as metabolic, autoimmune, psychiatric, and cardiovascular diseases, cancers (especially colon, prostate, and breast cancer), and chronic pain diseases [3-6]. Vitamin D as a fat-soluble steroid hormone raised concerns about the toxicity of over-supplementation. Early symptoms of vitamin D toxicity include drowsiness, continuous headaches, irregular heartbeat, and loss of appetite[2, 7].

The synthesis, absorption and metabolic processes of vitamin D can be influenced by a lot of factors, including environmental factors such as sun exposure, latitude, and season and individual factors such as adiposity, genetics, skin complexion, and age [8, 9]. Environmental pollutants are also suggested to be able to interfere with human vitamin D[10]. Epidemiologic studies suggest an association between air pollution and vitamin D deficiency. In cities with high levels of air pollutants (ozone, particulate matter, and sulfur dioxide), UV light is efficiently absorbed by these pollutants, thus reducing the skin synthesis of pro-vitamin D3[11]. In addition, due to toxic metals are widely distributed in the environment and their potential role in endocrine disruption, it is necessary to

 doi:10.20944/preprints202310.1308.v1

investigate the influences of exposure to toxic metals on human vitamin D status[10, 12]. A few population-based studies have been demonstrated that cadmium (Cd) and lead (Pb) are capable of interfering human vitamin D status. Several studies conducted in cadmium-contaminated areas of Japan found that exposure to environmental cadmium resulted in vitamin D deficiency[13, 14]. As an endocrine modulator in human populations, lead is also considered as one of the risk factors of vitamin D deficiency. One cohort study of pregnant women shown that vitamin D intake is negatively correlated with maternal blood lead and cord blood lead[15]. Another cross-sectional study demonstrated a significant negative correlation between blood lead level and serum 1,25(OH)2D in the children exposure to lead[16].

Arsenic, a well-recognized environmental contaminant, is listed by the Agency for Toxic Substances and Disease Registry (ATSDR) as the first on the list of hazardous substances[17]. Arsenic is widely distributed in environmental media such as air, water, and soil[18]. It is worth noting that water-borne arsenic contamination has led to the greatest concern for human health on a global scale[19]. Long term drinking water with excessive arsenic can induce liver and kidney damage, cardiovascular disease, diabetes and cancer[18]. Similar to heavy metals such as cadmium and lead, arsenic may affect vitamin D status. It was found that As_2O_3 increased the transcriptional activity of paricalcitol, a noncalcemic vitamin D analogue, probably by decreasing the function of the mitochondrial enzyme 24-hydroxylase, which functions to metabolize the active vitamin D in acute myeloid leukemia (AML) cells[20]. A cross-sectional study conducted near the lead smelter in city of Torreón in Mexico found positive associations between urinary arsenic and serum $1,25(OH)_2D$, but no association between urinary arsenic and serum 25(OH)D[21]. Another hospital-based prospective cohort study focused on the associations of urine metals during pregnancy with cord serum vitamin D levels and found that urinary arsenic was positively associated with serum 25(OH)D[22]. However, no studies on the correlation between urinary arsenic and serum vitamin D have been conducted in areas of water-borne arsenicosis.

The key factor in an individual's susceptibility to arsenic is the ability to metabolize and efficiently excrete arsenic[23]. Inorganic arsenic (iAs), namely the sum of [As (III)+As (V)], accepts a methyl group from S-adenosylmethionine and methylated into monomethylarsonic acid (MMA) or dimethylarsonic acid (DMA) after ingested by humans [19, 24]. In humans, efficient methylation from iAs to DMA is associated with reduced responsiveness and increased urinary arsenic excretion[25]. The extent of DMA methylation is different within and between individuals and populations, suggesting a genetic component to arsenic metabolism[26]. In general, high fractions of MMA and residual non-methylated inorganic arsenic in urine are associated with a higher risk of arsenic-related adverse health effects, suggesting that more efficient arsenic metabolism capacity (a high proportion of DMA) protects the body against arsenic toxicity [27, 28]. However, it is still not known whether the arsenic metabolism capacity can affect the association of arsenic and vitamin D levels.

As we know, skin is the only organ that has the capacity to produce vitamin D [29]. Most vitamin D is obtained from ultraviolet B (UVB) exposure of the skin, which stimulates the conversion of 7-dehydrocholesterol into vitamin D_3 (cholecalciferol)[30, 31]. The skin is also a toxic target organ for arsenic, and skin hyperpigmentation and hyperkeratosis have long been known to be the hallmark signs of chronic arsenic exposure. Therefore, the association of arsenic with vitamin d in a population with skin lesions deserves further study.

Serum 25(OH)D is considered as a reliable marker of vitamin D sufficiency because it has the highest abundance in all vitamin D metabolites in serum and its level remains stable for almost two weeks[32, 33]. In the present study, we analyzed the association of urinary arsenic species and arsenic metabolism capacity to serum 25(OH)D. We further assessed the association between urinary arsenic species and serum 25(OH)D according to the stratification of skin keratinization due to arsenic exposure.

 doi:10.20944/preprints202310.1308.v1

2. Materials and Methods

2.1. Study site and population

Data for this study was derived from a cross-sectional study of the association between arsenic exposure and diabetes conducted in 2019 in Wenshui County, Shanxi Province, People's Republic of China, which was identified as an area of water-borne arsenicosis according to long-term monitoring data provided by the Shanxi Institute of Endemic Disease Prevention and Control. All the participants were given face-to-face questionnaires and a general physical examination by well-trained staff. General demographic data including general demographic characteristics (age, gender, height, and weight), socioeconomic status (education and occupation), and milk consumption (≤1 glass/week and >1glass/week), because milk is fortified with vitamin D. The body mass index (BMI) of the participants was further calculated with height and weight. Skin hyperkeratosis (a hyperkeratotic thickening of the skin typically on the palms and soles) was ascertained by diagnosis of endemic arsenism (WS/T 211-2015) by study physicians. 762 participants were included for the final analysis after excluding participants with incomplete basic information and abnormal liver and kidney function. This study was approved by the Ethics Committee of Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, the number of ethical review is HRBMUECDC20210410.

2.2. Sample collection

Blood and urine samples of the participants were collected by professional nurses from the Affiliated Hospital of Shanxi Institute of Endemic Disease Prevention and Control. The venous blood of the cubital fossa was collected through blood collection vessels and the serum and blood cells were centrifuged on the site. The instant urine was collected and divided into 1.5 mL EP tubes after centrifugation. The samples were frozen and stored in the -80°C refrigerator until analysis.

2.3. Determination of urinary arsenic concentrations

Urinary arsenic species were determined using Liquid Chromatography Atomic Fluorescence Spectrometer (LC-AFS6500, Beijing Haiguang Instrument Co., Ltd., China). Chromatographic separation of the arsenic compounds was carried out in accordance with a previously validated method[34]. The limit of detection (LOD) of As^{3+}, As^{5+}, DMA, and MMA were 0.36μg/L, 0.42μg/L, 0.45μg/L and 0.81μg/L, respectively. When the arsenic content in the urine form of the sample is lower than LOD, the value is LOD $\sqrt{2}$.

2.4. Determination of serum 25(OH)D concentrations

Serum 25(OH)D was detected by biochemical analyzer 3100 (Hitachi High-Technologies Corporation, Japan). The vitamin D status of the participants was categorized according to the definition of the kit specification, which defines 25(OH)D <20 ng/mL as deficient, 21-29 ng/mL as inadequate, 30–100 ng/mL as adequate and >100 ng/mL as excessive. More information about reagents is available at https://www. nbmedicalsystem.com/.

2.5. Determination of blood glucose concentrations

Participants were asked to fast overnight. Fasting blood glucose was measured on the day of blood collection using the glucose tester (Acon Biotechnology co., Hangzhou, China).

2.6. Quality assurance and quality control

Training manual and work manual were developed to train the investigators and standardize the survey methods according to the relevant contents of the questionnaire. Before the test, the instruments and equipment used in the laboratory were corrected in time to reduce the measurement error. Frozen human serum samples were equilibrated at room temperature for 30 minutes prior to

 doi:10.20944/preprints202310.1308.v1

use, followed by centrifugation at 12000g for 5 minutes to filter out the precipitate. 1 mL urine stored at −80 °C was thawed at room temperature and centrifuged for 5 min at 13000 rpm. Filtered urine with MCE membrane was been analyzed after centrifugation.

2.7. Statistical analysis

Continuous variables were expressed as mean (± standard deviation) or median (P25-P75). Continuous variables with normal distribution were compared by student t-test, while continuous variables with non-normal distribution were compared by Mann-Whitney U test. Categorical variables were compared by the chi-square test. The concentration of different forms of arsenic (As^{3+}, As^{5+}, MMA, and DMA) was calculated by standard curve. iAs and tAs are calculated by the following formula: iAs = As^{3+}+As^{5+}; tAs= iAs + MMA + DMA. All concentrations of urinary arsenic species were transformed using an extension of the Box-Cox transformation to approach normality (tAs: $\lambda = 0.298$, iAs: $\lambda = 0.145$, MMA: $\lambda = 0.021$, DMA: $\lambda = 0.330$). Multivariable linear regressions were used to assess changes in serum 25(OH)D for each one-unit increment in the Box-Cox transformed urinary arsenic species. The baseline information and levels of these covariates were adjusted in these equations.

Model 1: Y= β (tAs or iAs or MMA or DMA) + ε

Model 2: Y= β (tAs or iAs or MMA or DMA) + β1 (age) + β2 (gender) + β3 (BMI) + ε

Model 3: Y= β (tAs or iAs or MMA or DMA) + β1 (age) + β2 (gender) + β3 (BMI) + β4 (occupation) + β5 (education) + β6 (skin hyperkeratosis) + β7 (milk consumption) + β8 (blood glucose) + ε

Binary logistic regression models were used to further evaluate the relationship between urinary arsenic species and vitamin D status, in which vitamin D status was determined with serum 25(OH)D and divided into two categories: vitamin D excess (>100 ng/mL) and normal (30-100 ng/mL) according to the instructions. 6 participants with serum 25(OH)D levels below 30ng/mL were excluded because the sample size was too small for follow-up analysis. The effect estimates were presented as β or odds ratios (ORs) with their 95% confidence intervals (95% CIs). The baseline information and levels of these covariates were adjusted in these equations.

Model 1: Y= β (tAs or iAs or MMA or DMA) + ε

Model 2: Y= β (tAs or iAs or MMA or DMA) + β1 (gender) + β2 (skin hyperkeratosis) + β3 (milk consumption) + ε

Stratification of arsenic methylation metabolic capacity by median iAs%, MMA%, and DMA%. All statistical analyses were performed in SPSS 26.0 software and p value < 0.05 (two sides) was considered significant.

3. Results

3.1. Basic characteristics of the study participants

There was a total of 762 participants and 86.1% of participants were farmers. Of these, 256 participants (33.7%) were male and 506 participants (66.3%) were female. The mean (± SD) age of enrolled participants was 57.92 ± 10.80 years and the mean (± SD) body mass index was 25.77 ± 4.01 kg/m^2. The median concentrations of urinary tAs, iAs, MMA, and DMA were 69.81, 3.46, 4.78, and 51.15 μg/L, respectively. The median concentration of blood glucose was 5.70 mmol/L. The mean serum 25(OH)D concentration was 74.03 ± 22.67 ng/mL. These results are presented in Table 1.

Table 1. Basic characteristics of study subjects.

Characteristics	Value
Age, years, mean ± SD	57.92 ± 10.80
BMI, kg/m2, mean ± SD	25.77 ± 4.01
Gender, n (%)	
Man	256 (33.7)
Woman	506 (66.3)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 October 2023 doi:10.20944/preprints202310.1308.v1

Skin hyperkeratosis	
No	495 (64.9)
Yes	267 (35.1)
Occupations, n (%)	
Farmer	657 (86.1)
Others	105 (13.9)
Education, n (%)	
Primary and below	269 (35.4)
Junior high school	409 (53.6)
Senior high school and above	84 (11)
Milk consumption, n (%)	
≤1/week	471 (61.8)
>1/week	291 (38.2)
Urinary tAs, μg/L, median (P25-P75)	69.81 (27.77-137.51)
Urinary iAs, μg/L, median (P25-P75)	3.46 (0.83-14.67)
Urinary MMA, μg/L, median (P25-P75)	4.78 (0.32-16.14)
Urinary DMA, μg/L, median (P25-P75)	51.15 (19.00-102.93)
iAs%, median (P25-P75)	9.94 (2.39-18.58)
MMA%, median (P25-P75)	9.41 (1.60-16.88)
DMA%, median (P25-P75)	78.48 (66.53-90.18)
Blood glucose, mmol/L, median (P25-P75)	5.70 (5.10-6.70)
25(OH)D, ng/mL, mean ± SD	74.03 ± 22.67

3.2. Multivariate linear regression analysis between urinary arsenic species and serum 25(OH)D

Table 2 reflects the association between urinary arsenic species and serum 25(OH)D. For the urinary tAs, multivariate linear regression analysis found that the Box-Cox transformed urinary tAs was positively associated with serum 25(OH)D in the non-adjusted model (β=0.046, 95%CI: 0.020 to 0.071, $p < 0.01$). And this positive relationship still existed after adjusted other potential confounders (Model 2: β=0.044, 95%CI: 0.021 to 0.067, $p < 0.01$; Model 3: β=0.044, 95%CI: 0.020 to 0.069, $p < 0.01$).

Table 2. Association between urinary arsenic species and serum 25(OH)D.

Exposure	**Box-Cox transformed β (95%CI)**	**p-Value**
tAs		
Model 1	0.046 (0.020, 0.071)	< 0.01
Model 2	0.044 (0.021, 0.067)	< 0.01
Model 3	0.044 (0.020, 0.069)	< 0.01
iAs		
Model 1	0.330 (0.035, 0.624)	0.028
Model 2	0.155 (-0.115, 0.425)	0.260
Model 3	0.100 (-0.178, 0.377)	0.482
MMA		
Model 1	0.447 (0.145, 0.748)	< 0.01
Model 2	0.276 (0.001, 0.551)	0.049

 doi:10.20944/preprints202310.1308.v1

Model 3	0.272 (-0.013, 0.556)	0.061
DMA		
Model 1	0.057 (0.024, 0.089)	< 0.01
Model 2	0.060 (0.031, 0.091)	< 0.01
Model 3	0.062 (0.030, 0.094)	< 0.01

Model 1: non-adjusted. Model 2: adjusted for age, gender, and BMI. Model 3: adjusted for age, gender, BMI, occupation, education, skin hyperkeratosis, milk consumption and blood glucose.

For the urinary iAs, we found a positively associated between Box-Cox transformed urinary iAs and serum 25(OH)D in the non-adjusted model (β=0.330, 95%CI: 0.035 to 0.624, $p < 0.01$). However, when we adjusted other potential confounders, the association between urinary iAs and serum 25(OH)D became null in model 2 (β=0.155, 95%CI: -0.115 to 0.425, $p = 0.260$) and model 3 (β=0.100, 95%CI: -0.178 to 0.377, $p = 0.482$).

For the urinary MMA, our results demonstrated that the Box-Cox transformed urinary MMA was positively associated with serum 25(OH)D in the non-adjusted model (β=0.447, 95%CI: 0.145 to 0.748, $p < 0.01$). And this positive relationship still existed after we adjusted for age, gender, and BMI (β=0.276, 95%CI: 0.001 to 0.551, $p = 0.049$). However, when we added potential confounders including occupation, education, skin lesions, and milk consumption, this positive relationship was not existed (β=0.272, 95%CI: -0.013 to 0.556).

For the urinary DMA, our multivariate linear regression analysis found that the Box-Cox transformed urinary DMA was positively associated with serum 25(OH)D in the non-adjusted model (β=0.057, 95%CI: 0.024 to 0.089, $p < 0.01$). After we adjusted other potential confounders, this positive relationship still existed (Model 2: β=0.060, 95%CI: 0.031 to 0.091, $p < 0.01$; Model 3: β=0.062, 95%CI: 0.030 to 0.094, $p < 0.01$).

3.3. Associations between urinary arsenic species and vitamin D status

We excluded 6 participants with vitamin deficiency (serum 25(OH)D < 30ng/mL), and defined participants with serum 25(OH)D levels of 30-100ng/mL as a normal group and those with higher than 100ng/mL as a vitamin D excess group. Table S1 reveals the basic characteristics of the population between two groups. No significant difference was noted in age, BMI, education, occupations, urinary iAs, urinary MMA, and blood glucose between two groups (all $p > 0.05$). But we found the gender, skin hyperkeratosis, and milk consumption were varied between two groups (all $p < 0.05$). We noticed that the populations in the vitamin D excess group had higher urinary tAs and urinary DMA levels compared to the normal group (all $p < 0.05$).

Table 3 shows the relationship between urinary arsenic species and vitamin D status. For urinary tAs, our binary logical regression analysis showed that the significant increases of 0.4% in the risk of vitamin D excess for every 1-unit increment in the Box-Cox transformed urinary tAs in the non-adjusted model (OR=1.004, 95%CI: 1.001 to1.007, $p < 0.01$). After adjusting for gender, skin health and milk consumption, this positive relationship was still existed (OR=1.004, 95%CI: 1.001 to1.007, $p = 0.045$). For urinary DMA, we found that the significant increases of 0.6% in the risk of vitamin D excess for every 1-unit increment in the Box-Cox transformed urinary DMA in the non-adjusted model (OR=1.006, 95%CI: 1.001 to1.010, $p < 0.01$). After adjusting for above mentioned covariates, this positively relationship was still existed (OR=1.006, 95%CI: 1.001 to1.011, $p = 0.017$).

Table 3. Association between urinary tAs and vitamin D status.

Exposure	**OR (95% CI)**	**p-Value**
tAs		
Model 1	1.004 (1.001, 1.007)	< 0.01
Model 2	1.004 (1.001, 1.007)	0.045

 doi:10.20944/preprints202310.1308.v1

iAs		
Model 1	1.023 (0.986, 1.061)	0.222
Model 2	1.004 (0.966, 1.044)	0.838
MMA		
Model 1	1.026 (0.988, 1.065)	0.187
Model 2	1.007 (0.967, 1.049)	0.724
DMA		
Model 1	1.006 (1.001, 1.010)	0.010
Model 2	1.006 (1.001, 1.011)	0.017

Model 1: non-adjusted. Model 2: adjusted for gender, skin hyperkeratosis and milk consumption.

3.4. Association between arsenic metabolism efficiency and serum vitamin D

We performed the linear regression analyses to further identify the effect of arsenic metabolism efficiency on the association between urinary tAs and serum 25(OH)D in different populations (Table 4). Interestingly, no significant correlation was observed between urinary tAs and serum 25(OH)D (β=0.015, 95%CI: -0.027 to 0.057, p = 0.484) in the populations with iAs% below the median. However, in the populations with iAs% above the median, each one-unit increase in the Box-Cox transformed tAs level was associated with increases of 0.064 (95%CI: 0.032 to 0.096, p < 0.01) in serum 25(OH)D. This result suggests arsenic methylation capacity might affect the positive relationship between urinary tAs and serum 25(OH)D levels.

Table 4. Multivariable regression analyses of the associations between urinary tAs and serum 25(OH)D stratified for arsenic metabolism efficiency.

	Box-Cox transformed β (95%CI) [b]	**p-value** [b]
iAs% [a]		
< 10.19	0.015 (-0.027, 0.057)	0.484
≥ 10.19	0.064 (0.032, 0.096)	< 0.01
MMA% [a]		
< 9.54	0.053 (0.012, 0.094)	0.036
≥ 9.54	0.038 (0.006, 0.070)	0.044
DMA% [a]		
< 78.15	0.046 (0.013, 0.078)	< 0.01
≥ 78.15	0.047 (0.006, 0.087)	0.027

a Stratified for median of each metabolite. b The multiple regression without adjusting factors.

3.5. Association between urinary arsenic species and serum 25 (OH)D in subgroups stratified by skin hyperkeratosis

The skin is both an organ for vitamin D synthesis and a target organ for arsenic toxicity. Therefore, stratified analyses were performed to determine the relationship between urinary arsenic species and serum 25(OH)D in populations with normal and arsenic-induced hyperkeratotic skin. Table S2 shows the basic characteristics of the population in two groups. We found that the serum 25(OH)D in the skin hyperkeratosis group was higher than that in the normal group. Table 5 shows the associations between urinary arsenic species and serum 25(OH)D in two groups. In the normal group, the positive associations were found between tAs (β=0.041, 95%CI: 0.013 to 0.069, p < 0.01), MMA (β=0.425, 95%CI: 0.075 to 0.776, p < 0.01), and DMA (β=0.054, 95%CI: 0.017 to 0.090, p < 0.01) with serum 25(OH)D. However, we only found the positive association between urinary iAs (β=0.592,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 October 2023 doi:10.20944/preprints202310.1308.v1

95%CI: 0.041 to 1.143, p = 0.129) and serum 25(OH)D in the arsenic-induced hyperkeratotic skin group.

Table 5. Association between urinary arsenic species and serum 25(OH)D by skin hyperkeratosis stratified.

	Box-Cox transformed β (95%CI) [a]			
Exposure	**Normal**	**p-Value**	**Skin hyperkeratosis**	**p-Value**
tAs	0.041 (0.013, 0.069)	< 0.01	0.046 (-0.007, 0.099)	0.090
iAs	0.169 (-0.173, 0.511)	0.332	0.592 (0.041, 1.143)	0.035
MMA	0.425 (0.075, 0.776)	< 0.01	0.430 (-0.139, 0.999)	0.138
DMA	0.054 (0.017, 0.090)	< 0.01	0.052 (-0.015, 0.119)	0.129

[a] The multiple regression without adjusting factors.

4. Discussion

Vitamin D is a group of biologically active steroid compounds. It is found in nature as vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol)[35, 36]. Vitamin D2 is considered a plant form of vitamin D, because it produced by fungi and yeast by UVB-exposure of ergosterol (provitamin D2)[37]. For most vertebrate animals, 7-dehydrocholesterol is converted under ultraviolet B (UVB) rays to vitamin D3[38]. When vitamin D2 and vitamin D3 are ingested, they are both metabolized in the liver to form 25(OH)D. As the main storage form of vitamin D, total serum or plasma 25(OH)D concentrations reflect exposure to skin synthesis, dietary intake, and mobilization of tissue stores, therefore 25(OH)D is often considered as reliable biomarker of the nutritional status of the vitamin D endocrine system[39].

Studies on the association between urinary arsenic and serum vitamin D are scarce. A prospective cohort study focused on association of maternal metals exposure during pregnancy with newborns' vitamin D status in China found a positive correlation between urinary arsenic and serum 25(OH)D.[22]. In Torreón, a town in Mexico, no association of urinary arsenic with serum 25(OH)D was reported in a case-control study focused on adolescents[21]. In this study, we found that urinary iAs and MMA was positively associated with serum 25(OH)D in the incompletely adjusted models. However, the positive associations between urinary tAs and DMA with serum 25(OH)D were exist in all linear regression models. The results of binary logical regression analysis also demonstrated urinary tAs and DMA was positively associated with the risk of vitamin D excess. These findings suggest that arsenic metabolism efficiency may affect serum 25(OH)D levels.

Once inorganic arsenic (iAs) is ingested into the body, it will be metabolized in the liver. iAs then accepted a methyl group from S-adenosylmethionine and metabolized to monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) through a series of oxidation and methylation processes[19, 40]. These arsenic species (iAs, MMA, and DMA) are excreted from the urine and can be measured and expressed as a percentage of total urinary arsenic (iAs%, MMA%, and DMA%)[41]. Generally, low iAs% indicates enhanced methylation capacity, while high iAs% indicates decreased methylation capacity. It is hypothesized that increased methylation capacity may reduce susceptibility to arsenic-related toxicity[41-43]. The results in present study showed the significantly positively association between urinary tAs and serum 25(OH)D in the populations with iAs% above the median. However, no significant correlation was observed between urinary tAs and serum 25(OH)D in the populations with iAs% below the median. Therefore, our study support that the positive association between urinary arsenic and serum 25(OH)D exist in the incompletely methylated population and this correlation might be altered by efficiency of arsenic metabolism. However, further work is needed to expand sample size for relevant studies.

The maintenance of the superficial structure of the skin is dependent on the balance between the differentiation and proliferation of keratinocytes. Chronic exposure to inorganic arsenic dramatically disrupts this balance, ultimately leading to hyperkeratosis of the skin[44-46]. The epidermis is also

 doi:10.20944/preprints202310.1308.v1

the major source of vitamin D for the body, because the keratinocytes within the epidermis are capable of producing 25 (OH)D3[29, 47, 48]. The results of our stratified analyses showed the positive association between urinary iAs and serum 25(OH)D in the arsenic-induced hyperkeratotic skin group. Future work is required to explore the mechanisms.

One potential limitation of our study is its cross-sectional nature, which restricts inferences about causality, and more longitudinal studies are needed. Further, we didn't collect the information of time spent outside because the sun exposure is a potential confounder, but our study population was homogenous, consisting entirely of adult, 86.1% of whom were farmers and the lifestyle of them is similar. Finally, further in vitro experiments are recommended to verify whether arsenic leads to elevated vitamin D levels by promoting hyperproliferation of keratinocytes.

5. Conclusions

In summary, our findings support the positive association between urinary arsenic and serum 25(OH)D. The precise mechanism of arsenic in vitamin D remains to be elucidated.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Population's characteristics description of the normal group and vitamin D excess group; Table S2: Population's characteristics description of the normal group and skin hyperkeratosis group.

Author Contributions: Kunyu Zhang: Conceptualization, Methodology, Visualization, Formal analysis, Writing–original draft. Yunyi Yin: Methodology, Software, Formal analysis. Man LV: Formal analysis, Resources, Validation. Xin Zhang: Visualization, Investigation. Meichen Zhang: Methodology, Software, Formal analysis. Jia Cui: Validation. Ziqiao Guan: Validation. Xiaona Liu: Software, Validation, Investigation. Yang Liu: Software, Validation, Investigation. Yanmei Yang: Writing-review & editing, supervision, project administration. Yanhui Gao: Writing-review & editing, supervision, project administration, Funding acquisition.

Funding: This research was funded by the National Key R&D Program of China（2022YFC2503000）.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, the protocol code is HRBMUECDC20210410.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Acknowledgments: We sincerely thank the Institute of Endemic Disease Prevention and Control of Shanxi Province for their strong support in this cross-sectional survey.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Wilson, L.R., et al., *Vitamin D deficiency as a public health issue: using vitamin D2 or vitamin D3 in future fortification strategies.* Proc Nutr Soc, 2017. **76**(3): p. 392-399.
2. Heaney, R.P., *Vitamin D in health and disease.* Clin J Am Soc Nephrol, 2008. **3**(5): p. 1535-41.
3. Rosen, C.J. and C.L. Taylor, *Common misconceptions about vitamin D--implications for clinicians.* Nat Rev Endocrinol, 2013. **9**(7): p. 434-8.
4. Alshahrani, F. and N. Aljohani, *Vitamin D: deficiency, sufficiency and toxicity.* Nutrients, 2013. **5**(9): p. 3605-16.
5. Holick, M.F., *Vitamin D deficiency.* N Engl J Med, 2007. **357**(3): p. 266-81.
6. Nair, P., B. Venkatesh, and J.R. Center, *Vitamin D deficiency and supplementation in critical illness-the known knowns and known unknowns.* Crit Care, 2018. **22**(1): p. 276.
7. Marcinowska-Suchowierska, E., et al., *Vitamin D Toxicity-A Clinical Perspective.* Front Endocrinol (Lausanne), 2018. **9**: p. 550.
8. Tsiaras, W.G. and M.A. Weinstock, *Factors influencing vitamin D status.* Acta Derm Venereol, 2011. **91**(2): p. 115-24.

9. Meza-Meza, M.R., A.I. Ruiz-Ballesteros, and U. de la Cruz-Mosso, *Functional effects of vitamin D: From nutrient to immunomodulator.* Crit Rev Food Sci Nutr, 2022. **62**(11): p. 3042-3062.
10. Mousavi, S.E., et al., *Air pollution, environmental chemicals, and smoking may trigger vitamin D deficiency: Evidence and potential mechanisms.* Environ Int, 2019. **122**: p. 67-90.
11. Hoseinzadeh, E., et al., *The impact of air pollutants, UV exposure and geographic location on vitamin D deficiency.* Food Chem Toxicol, 2018. **113**: p. 241-254.
12. Bimonte, V.M., et al., *The endocrine disruptor cadmium: a new player in the pathophysiology of metabolic diseases.* J Endocrinol Invest, 2021. **44**(7): p. 1363-1377.
13. Nogawa, K., et al., *Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1 alpha, 25-dihydroxyvitamin D level.* Int Arch Occup Environ Health, 1987. **59**(1): p. 21-30.
14. Nogawa, K., et al., *Serum vitamin D metabolites in cadmium-exposed persons with renal damage.* Int Arch Occup Environ Health, 1990. **62**(3): p. 189-93.
15. Arbuckle, T.E., et al., *Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study.* Chemosphere, 2016. **163**: p. 270-282.
16. Rosen, J.F., et al., *Reduction in 1,25-dihydroxyvitamin D in children with increased lead absorption.* N Engl J Med, 1980. **302**(20): p. 1128-31.
17. Garbinski, L.D., B.P. Rosen, and J. Chen, *Pathways of arsenic uptake and efflux.* Environ Int, 2019. **126**: p. 585-597.
18. Zhao, J., et al., *The association of arsenic exposure with hypertension and blood pressure: A systematic review and dose-response meta-analysis.* Environ Pollut, 2021. **289**: p. 117914.
19. Oremland, R.S. and J.F. Stolz, *The ecology of arsenic.* Science, 2003. **300**(5621): p. 939-44.
20. Kumagai, T., et al., *19-Nor-1,25(OH)2D2 (a novel, noncalcemic vitamin D analogue), combined with arsenic trioxide, has potent antitumor activity against myeloid leukemia.* Cancer Res, 2005. **65**(6): p. 2488-97.
21. Zamoiski, R.D., et al., *Association of arsenic and metals with concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D among adolescents in Torreón, Mexico.* Environ Health Perspect, 2014. **122**(11): p. 1233-8.
22. Fang, X., et al., *Associations of urine metals and metal mixtures during pregnancy with cord serum vitamin D Levels: A prospective cohort study with repeated measurements of maternal urinary metal concentrations.* Environ Int, 2021. **155**: p. 106660.
23. Ameer, S.S., et al., *Exposure to Inorganic Arsenic Is Associated with Increased Mitochondrial DNA Copy Number and Longer Telomere Length in Peripheral Blood.* Front Cell Dev Biol, 2016. **4**: p. 87.
24. Ventura-Lima, J., M.R. Bogo, and J.M. Monserrat, *Arsenic toxicity in mammals and aquatic animals: a comparative biochemical approach.* Ecotoxicol Environ Saf, 2011. **74**(3): p. 211-8.
25. Gardner, R.M., et al., *Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status.* Reprod Toxicol, 2011. **31**(2): p. 210-8.
26. Zhang, M., et al., *Association between arsenic (+3 oxidation state) methyltransferase gene polymorphisms and arsenic methylation capacity in rural residents of northern China: a cross-sectional study.* Arch Toxicol, 2023. **97**(11): p. 2919-2928.
27. Vahter, M.E., *Interactions between arsenic-induced toxicity and nutrition in early life.* J Nutr, 2007. **137**(12): p. 2798-804.
28. De Loma, J., et al., *Elevated arsenic exposure and efficient arsenic metabolism in indigenous women around Lake Poopó, Bolivia.* Sci Total Environ, 2019. **657**: p. 179-186.
29. Bikle, D.D., *Vitamin D metabolism and function in the skin.* Mol Cell Endocrinol, 2011. **347**(1-2): p. 80-9.
30. DeLuca, H.F., *Overview of general physiologic features and functions of vitamin D.* Am J Clin Nutr, 2004. **80**(6 Suppl): p. 1689s-96s.
31. Chen, T.C., et al., *Factors that influence the cutaneous synthesis and dietary sources of vitamin D.* Arch Biochem Biophys, 2007. **460**(2): p. 213-7.
32. Holick, M.F., *The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention.* Rev Endocr Metab Disord, 2017. **18**(2): p. 153-165.
33. Kennel, K.A., M.T. Drake, and D.L. Hurley, *Vitamin D deficiency in adults: when to test and how to treat.* Mayo Clin Proc, 2010. **85**(8): p. 752-7; quiz 757-8.
34. Lou, Q., et al., *Arsenic exposure elevated ROS promotes energy metabolic reprogramming with enhanced AKT-dependent HK2 expression.* Sci Total Environ, 2022. **836**: p. 155691.
35. Wiciński, M., et al., *Impact of Vitamin D on Physical Efficiency and Exercise Performance-A Review.* Nutrients, 2019. **11**(11).

36. Mazur, A., et al., *Vitamin D and Vitamin D3 Supplementation during Photodynamic Therapy: A Review.* Nutrients, 2022. **14**(18).
37. Jäpelt, R.B. and J. Jakobsen, *Vitamin D in plants: a review of occurrence, analysis, and biosynthesis.* Front Plant Sci, 2013. **4**: p. 136.
38. Cashman, K.D. and M. Kiely, *EURRECA-Estimating vitamin D requirements for deriving dietary reference values.* Crit Rev Food Sci Nutr, 2013. **53**(10): p. 1097-109.
39. Castano, L., et al., *25(OH)Vitamin D Deficiency and Calcifediol Treatment in Pediatrics.* Nutrients, 2022. **14**(9).
40. Chen, H., et al., *Prenatal arsenic exposure, arsenic metabolism and neurocognitive development of 2-year-old children in low-arsenic areas.* Environ Int, 2023. **174**: p. 107918.
41. Tseng, C.H., *A review on environmental factors regulating arsenic methylation in humans.* Toxicol Appl Pharmacol, 2009. **235**(3): p. 338-50.
42. Pierce, B.L., et al., *Arsenic metabolism efficiency has a causal role in arsenic toxicity: Mendelian randomization and gene-environment interaction.* Int J Epidemiol, 2013. **42**(6): p. 1862-71.
43. Valenzuela, O.L., et al., *Urinary trivalent methylated arsenic species in a population chronically exposed to inorganic arsenic.* Environ Health Perspect, 2005. **113**(3): p. 250-4.
44. Bangert, C., P.M. Brunner, and G. Stingl, *Immune functions of the skin.* Clinics in Dermatology, 2011. **29**(4): p. 360-376.
45. Yu, H.S., W.T. Liao, and C.Y. Chai, *Arsenic carcinogenesis in the skin.* J Biomed Sci, 2006. **13**(5): p. 657-66.
46. Wu, S., et al., *The potential of Diosgenin in treating psoriasis: Studies from HaCaT keratinocytes and imiquimod-induced murine model.* Life Sci, 2020. **241**: p. 117115.
47. Lehmann, B., et al., *UVB-induced conversion of 7-dehydrocholesterol to 1alpha,25-dihydroxyvitamin D3 in an in vitro human skin equivalent model.* J Invest Dermatol, 2001. **117**(5): p. 1179-85.
48. Bikle, D.D., et al., *1,25-Dihydroxyvitamin D3 production by human keratinocytes. Kinetics and regulation.* J Clin Invest, 1986. **78**(2): p. 557-66.