




Analysis of Threshold Effect of Urinary Heavy Metal Elements on the High Prevalence of Nephrolithiasis in Men

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Abstract

Exposure to heavy metals in the environment exerts serious effects on kidney health. However, the effects of joint exposure on the kidneys have been rarely studied, particularly in non-occupational exposure high-risk populations. This study provided a reference threshold range of heavy metals in urine and explored the effect of joint exposure on nephrolithiasis in men. The data were obtained from the China Multi-Ethnic Cohort database, and 1502 men were included in the study. A two-piece-wise regression model was used to assess the dose–response relationship between heavy metal exposure and nephrolithiasis. The least absolute shrinkage and selection operator regression model was used to calculate the score of joint exposure to heavy metals. The threshold effect analysis revealed a linear relationship between the concentration of arsenic (As) in the urine and the prevalence of nephrolithiasis, whereas a nonlinear relationship was observed with cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb). In addition, As, Cd, Cr, Hg, and Pb may significantly affect the joint exposure effect. Moreover, the final risk of nephrolithiasis increased by 123% (P for trend < 0.001). This study found a threshold relationship between heavy metals (Cd, Cr, Hg, Pb) in male urine and the occurrence of nephrolithiasis. Joint exposure to heavy metals in urine caused a high-risk effect on nephrolithiasis. The study provided a reference threshold value of related studies and indicated that environmental pollution caused by heavy metals should be reduced.

Keywords Heavy metals · Nephrolithiasis · Threshold effect · Joint exposure · Urine

Introduction

Heavy metals are natural metals with an atomic number greater than 20 and element density greater than 5 g/cm³ [1]. With the rapid development of the economy and society, heavy metal pollution has become one of the widespread environmental problems worldwide, with adverse effects on the aquatic, terrestrial, and atmospheric environments [2]. Among them, toxic heavy metals, including arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb),

are of utmost concern [3]. According to the International Agency for Research on Cancer, these metals were classified as human carcinogens (known or probable) [4]. Their toxicity depends on the dose, route of exposure, and chemical species, as well as the age, sex, genetics, and nutrition of the individual exposed. They affect multiple organs of the human body and were associated with several diseases [5]. Kidneys are particularly sensitive to contamination because filtrates can be concentrated (> threefold) in the proximal tubule during their transportation along with the renal tubules [6]. Heavy metals affect the kidneys through increased excretion of low-molecular-weight proteins in the urine due to damage to proximal tubular cells [7]. Oxidative stress generating reactive oxygen species (ROS) is the major mechanism underlying heavy metal-induced nephrotoxicity [8]. For example, hydronephrosis occurs when arsenic oxides deposited in the kidney are converted to methylated metabolites by As³⁺ methyltransferase [9]. The high levels of exposure to Cd cause increased lipid peroxidation and decreased antioxidant supply, resulting in its accumulation in the proximal tubules of kidneys and impairing tubular

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function and protein reabsorption [10, 11]. The analysis of the Cr metabolism pathway revealed that Cr and Cr compounds induced the oxidation and functional degradation of the kidneys, thereby damaging blood cells [12]. Hg²⁺ has long been known to cause necrosis of the renal unit [13]. Several functional dysregulations have been linked to Pb exposure, such as enzymuria, proteinuria, impaired anion, and glucose transportation, eventually leading to apoptosis and damage to kidney structure and function [14, 15]. In addition, the exposure to even low levels of these metals can cause them to slowly accumulate and result in chronic kidney disease and proteinuria [16].

Most of the effects of heavy metals on the kidneys are associated with nephrolithiasis, a common kidney disease with a prevalence rate of 15 to 20% worldwide [17]. It is caused by dehydration, decreased urine output or fluid flow rate, or increased mineral excretion [18]. It occurs because of the joint effect of environment and genes. The occurrence of the disease is higher in men than in women [19], because estrogen induces proteomic alterations in renal tubular cells, which decrease the expression of calcium oxalate crystal receptors and thus prevent nephrolithiasis [20]. Therefore, the analysis of heavy metal exposure in men's urine may contribute to explore the reasons for the consistently higher prevalence of nephrolithiasis in men than in women.

Although several studies have focused on heavy metals in the blood, studies on urine heavy metals are relatively rare. However, to evaluate renal diseases, the most useful information was acquired by measuring glomerular filtration rate and examining urine sediment [21]. Metal content in the urine is frequently used as a biomarker in human studies, especially in large group studies [22]. The 24-h urine test is widely considered as the gold standard to assess the excretion of various analytes over time [23]. Urinary heavy metal concentrations can reflect the excretion of heavy metals in the body and indirectly reflect the amount accumulated in the organism [24]. Therefore, urine element concentration forms an important basis for clinical monitoring and evaluation of heavy metal poisoning. A few studies exist on the normal dosage range of heavy metals in urine after being metabolized by the human body. Moreover, studies on nephrolithiasis are often restricted to single or interactive effects, with few reports on joint urinary metal exposure.

Most studies in the cross-sectional direction of heavy metals have been conducted on occupationally exposed populations, with insufficient studies on populations at high risk of non-occupational exposure [16, 25–27]. In addition, studies on nephrolithiasis are usually limited to single or interactive effects, with a few reports on joint urinary metal exposure [28–30]. Although the levels of heavy metals in the urine vary from region to region [31], significant exposure can adversely affect kidney function [32, 33]. Thus, studying the relationship between exposure and nephrolithiasis in

non-occupational, high-risk populations is of high relevance. Although an increasing number of study groups are working on nephrolithiasis and heavy metals, a few have indicated their threshold ranges. This study investigated the threshold effect of heavy metals in the urine of Chinese Dong men under a stable genetic background. In addition, the effect of joint exposure to heavy metal elements in the environment on the prevalence of nephrolithiasis in men was investigated to understand the reasons for the differences in the occurrence rate between men and women with nephrolithiasis.

Materials and Methods

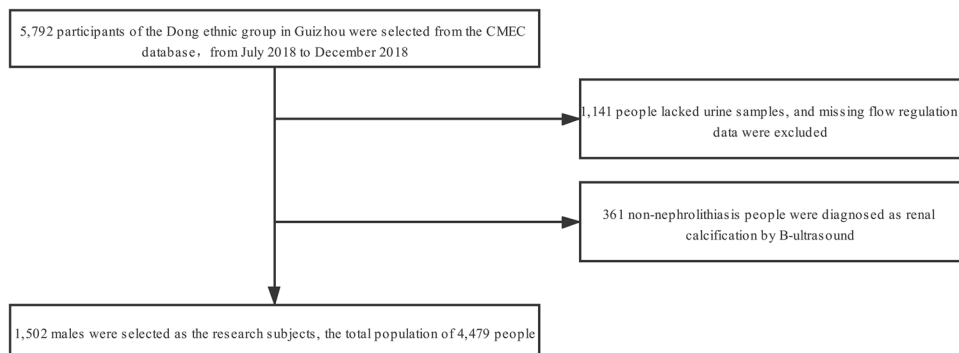
Research Subjects

From July to December 2018 in the Qiandongnan Prefecture, China (Kaili City, Liping County, Defeng Town, Yandong Town, Zhaoxing Town, and Zhongchao Town), the Dong people in the Guizhou Province of China aged 30 to 79 years who had been living for three generations were included in the study. A cross-sectional study was conducted using the multistage stratified cluster sampling method. The data of Dong population were obtained from the China Multi-Ethnic Cohort (CMEC) Study database. A total of 5792 people from the Dong ethnic group were selected from the database, and non-nephrolithiasis who lacked urine samples and whose abdominal B-ultrasound examination showed renal calcification were excluded. The selected nephrolithiasis-free population had no history of nephrolithiasis, no kidney disease conditions such as kidney calcification, with an intact urine sample. The final study population consisted of 4479 individuals, including 1502 men (Fig. 1). All participants signed an informed consent form before the investigation. This study was approved by the Sichuan University Medical Ethical Review Board (K2016038) and the Medical Ethics Committee of the Affiliated Hospital of Guizhou Medical University (2018[094]).

Basic Data Collection

We used a tablet computer with a self-developed application (CMEC application) to collect questionnaire information. It was collected through face-to-face interviews conducted by well-trained interviewers, who typically had medical backgrounds and were local college students [34]. Baseline information included gender, age, smoking status, alcohol intake, and physical examination. The physical examination was performed by a professional doctor; it included height, weight, blood pressure, chest X-ray, and abdominal ultrasound. The participant's body mass index (BMI) was calculated using weight (kg) and height (m²). The electronic sphygmomanometer measured blood pressure thrice in total,

Fig. 1 Flowchart showing the process of participants' selection



with an interval of 1 min between each measurement. The average value was considered the final systolic (SBP) and diastolic blood pressure (DBP). Glomerular filtration rate (GFR) was calculated based on gender, age, and serum creatinine (CR) according to the Chronic Kidney Disease Epidemiology Collaborative Group method [35, 36].

All participants fasted for more than 8 h, and blood samples were collected on the spot in the morning. Venous blood was collected, and one tube of serum and two tubes of ethylenediaminetetraacetic acid anticoagulant were prepared. The blood samples were transported under cold chain logistics ($-80\text{ }^{\circ}\text{C}$) to the Golden Field Medical Inspection Center on the same day for testing. Roche Modular P800 automatic biochemical analyzer (Roche, Basel, Kanton Basel, Switzerland) was used to measure CR, urea (UREA), and uric acid (UA). Britest 200B (URIT, Guilin, Guangxi, China) was used to measure urine protein (UPRO) and urine-specific gravity.

Assessment of Heavy Metal Elements in Urine

A 5 mL sample of urine was collected in the middle of the morning and stored at $-20\text{ }^{\circ}\text{C}$. The sample was placed at room temperature before use. Next, 1 mL of the original urine supernatant sample was diluted with 9 mL of nitric acid solution (5%) (GR, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) in a 10 mL polyethylene centrifuge tube, which was well-mixed and filtered through a $0.45\text{ }\mu\text{m}$ membrane. The diluted sample was measured within 24 h. Using an inductively coupled plasma mass spectrometer (NexION 2000, PerkinElmer, Waltham, MA, USA), the concentrations of heavy metal elements, including As, Cd, Cr, Hg, and Pb, were determined. Internal standard solutions of Bi, Ge, In, 6Li, Sc, Tb, and Y at a concentration of $10\text{ }\mu\text{g/L}$ provided by PerkinElmer were used for internal online standardization, and the regression coefficient of the standard curve was maintained above 0.999. SeronormTM Trace Elements Urine L-2 RUO (Sero, Billingstad, Norway) was used for checking the accuracy. Percent recovery was between 80 and 120% (Table 2). The detection limit falls

within 0.0001 and $0.0168\text{ }\mu\text{g/L}$ for the metals tested, and the concentration of the sample below the detection limit was expressed as half of the limit of quantification. The final concentration was corrected by urine-specific gravity and multiplied by 10 [37].

Assessment of Nephrolithiasis

Nephrolithiasis was diagnosed by a specialized physician at the Second Affiliated Hospital of Guizhou Medical University using ultrasonography (Apogee 1200, Shantou Ultrasound, Shanghai, China) that revealed strong urinary light spots or clusters with an echogenic diameter $\geq 4\text{ mm}$ or results of a chest X-ray (1250 Shimadzu 500 mA X-ray machine, Shimane Prefecture, Japan) were used [34, 38].

Statistical Analysis

We performed a statistical analysis of the final population data. Continuous variables are represented as mean \pm standard deviation. Categorical variables are expressed in proportion. Nonparametric methods were used to compare the difference between continuous variables in the nephrolithiasis and non-nephrolithiasis groups. After dividing the measured quartiles of five heavy metals into the groups, using the first group as a reference, multiple regression equations were used to adjust multiple variables (age, BMI, SBP, DBP, CR, GFR, UREA, UA, smoking status, and alcohol intake). The results showed the independent effect of each group on the prevalence of nephrolithiasis by odds ratio (OR), with a confidence interval of 95% (95% CI). A linear trend test was performed by entering the median of the concentration of each group of heavy metal elements in the model as a continuous variable. After logarithmic transformation of the concentration data of five heavy metals, smooth curve fitting was performed according to the generalized additive model.

The threshold effect analysis was used to describe the linear/nonlinear relationship between the concentration of heavy metal elements and the prevalence of nephrolithiasis. First, we determined whether the *P*-value of the

log-likelihood ratio test (LRT) was significant ($P < 0.05$). If model 1 was not significantly selected, model 2 was selected significantly. Model 1 was a univariate linear regression, and model 2 was a two-piece-wise regression model. Next, the bootstrap resampling method was used to determine the credible interval of the threshold [39, 40].

To solve the collinearity problem among heavy metal elements, we adopted the least absolute shrinkage and selection operator (Lasso) regression method. A formula was obtained for calculating the score value of five heavy metal elements and their regression coefficients. The score value was then subjected to smooth curve fitting and threshold effect analysis to obtain the final relationship between the five heavy metal elements in urine and nephrolithiasis.

All statistical analyses were performed by statistical packages R (The R Foundation; <http://www.r-project.org>; version 3.4.3.) and Empower (R) (www.empowerstats.com, X&Y solutions, Inc. Boston, MA, USA). A P value of < 0.05 was considered statistically significant.

Results

Participants' Characteristics

The characteristics of the total participants are given in Supplementary Table 1. A total of 4479 people were included (1502 men and 2977 women), and the total prevalence of nephrolithiasis was 13.89% (622/4479). The prevalence of nephrolithiasis was 23.77% (357/1502) in men, which was 2.67-fold higher than that of 8.90% in women (265/2977) and the overall prevalence of the high-risk group of nephrolithiasis. The age, BMI, SBP, DBP, CR, UREA, and UA values of men in the nephrolithiasis group were overall 1 to 7% higher to various degrees than those in the non-nephrolithiasis group. The GFR of men in the nephrolithiasis group was generally 6% lower than that of the non-nephrolithiasis group. Compared with the nonpatient group, the prevalence of nephrolithiasis was 10 to 20% higher in men with UPRO and smoking and drinking habits (Table 1).

Correlation Between Heavy Metal Elements and Nephrolithiasis

Compared with the non-nephrolithiasis group, the concentrations of As, Cd, and Hg in men in the nephrolithiasis group were about 8 to 16% lower than those in the non-nephrolithiasis group, whereas those of Cr and Pb were higher (Table 2). As and Cd may be significantly associated with the occurrence of nephrolithiasis ($P < 0.001$). Figure 2 showed a small difference between the unadjusted and adjusted OR values,

Table 1 Baseline characteristics of male participants ($N = 1502$)

Characteristics	Non-nephrolithiasis ($N = 1145$)	Nephrolithiasis ($N = 357$)	P -value ^c
Age (years) ^a	55.07 ± 11.55	55.73 ± 10.28	0.347
BMI (kg/m ²) ^a	23.46 ± 3.36	24.03 ± 3.56	0.010
SBP (mmHg) ^a	128.43 ± 18.08	130.43 ± 18.24	0.058
DBP (mmHg) ^a	83.17 ± 11.37	85.38 ± 11.21	< 0.001
CR (μmol/L) ^a	82.31 ± 21.61	87.67 ± 27.82	< 0.001
GFR (mL/min/1.73 m ²) ^a	73.31 ± 16.64	68.97 ± 17.59	< 0.001
UREA (mmol/L) ^a	5.53 ± 1.69	5.68 ± 1.81	0.218
UA (μmol/L) ^a	398.93 ± 92.68	426.76 ± 112.46	< 0.001
UPRO ^b			0.175
No	1021 (89.17%)	309 (86.55%)	
Yes	124 (10.83%)	48 (13.45%)	
Smoking status ^b			0.682
No	454 (39.65%)	133 (37.25%)	
Yes	564 (49.26%)	185 (51.82%)	
Ever	127 (11.09%)	39 (10.92%)	
Alcohol intake ^b			0.079
No	822 (71.79%)	239 (66.95%)	
Yes	323 (28.21%)	118 (33.05%)	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CR, serum creatinine; GFR, glomerular filtration rate; UREA, serum urea; UA, uric acid; UPRO, urine protein

^aContinuous variables are represented by mean ± SD

^bCategorical variables are expressed as numbers (percentages)

^cKruskal–Wallis rank test for continuous variables, chi-square test for categorical variables. Fisher's exact test for categorical variables with expects < 10

indicating stable results. The relationship between Cr, Hg, and Pb and nephrolithiasis was not clear.

Threshold Effect Analysis of Heavy Metals in Urine and Nephrolithiasis

According to the smooth fitting curve between the urinary heavy metal concentration and the prevalence of nephrolithiasis as shown in Fig. 3, a linear relationship was observed between As and the prevalence of nephrolithiasis, whereas Cd, Cr, Hg, and Pb showed a nonlinear relationship. The dose–response relationship was consistent with the results of threshold effect analysis, and P was < 0.05 for the maximum likelihood ratio test (Table 3). When the concentration of Cd in urine was in the range of 0.20 to 0.34 μg/L, the risk of nephrolithiasis was significantly increased by 101% ($P < 0.05$). After reaching the key point (0.34 μg/L), for every 1 μg/L increase in urine Cd concentration, the risk of nephrolithiasis was significantly reduced by 61% ($P < 0.05$). When the Cr concentration in urine was in the range of 17.78

Table 2 Distribution of five heavy metal elements in male urine

Exposures	Percent recovery	LOD	Median (Q1–Q3)	Nephrolithiasis (N=1,502)		P-value
				No (N=1,145)	Yes (N=357)	
As (µg/L)	97.50%	0.0104	55.40 (32.77–85.68)	56.85 (34.13–87.98)	49.40 (28.52–76.32)	<0.001
Cd (µg/L)	119.94%	0.0111	1.42 (0.84–2.34)	1.47 (0.87–2.46)	1.23 (0.75–2.02)	<0.001
Cr (µg/L)	84.86%	0.0021	30.20 (16.57–45.12)	29.96 (16.03–45.16)	30.45 (18.24–44.41)	0.459
Hg (µg/L)	115.71%	0.0168	0.69 (0.15–1.84)	0.71 (0.15–1.86)	0.65 (0.18–1.77)	0.839
Pb (µg/L)	114.62%	0.0001	3.13 (1.43–5.14)	3.09 (1.38–5.09)	3.17 (1.56–5.25)	0.221

Non-normally distributed continuous variables are represented by median (quartile 1, quartile 3)

Kruskal–Wallis rank test for continuous variables. P-value in bold <0.05

LOD, limit of detection; Q1, quartile 1; Q3, quartile 3

to 25.12 µg/L, the risk of nephrolithiasis was significantly increased by 24% (*P* < 0.05). Similarly, when the Hg concentration in urine was in the range of 0.04 to 0.08 µg/L, the risk of nephrolithiasis disease increased by 3.00-fold higher (*P* < 0.05). When the Pb concentration in urine was in the range of 4.90 to 6.17 µg/L, the risk of nephrolithiasis was significantly reduced by 42% (*P* < 0.05). However, after reaching the key point (6.17 µg/L), for every 1 µg/L increase in urine Pb, the risk of nephrolithiasis disease increased by 3.53-fold higher (*P* < 0.05).

Joint Effects of Heavy Metal Elements on the Prevalence of Male Nephrolithiasis

We used the Lasso regression model to eliminate the possible collinearity problem between heavy metal elements. Furthermore, the effect of the joint exposure of five heavy metal elements on nephrolithiasis was evaluated. We calculated the score according to the model formula for subsequent analysis (Formula 1). When a threshold effect analysis was performed on the score, a dose–response relationship was consistent with that shown in Fig. 4a. When the score reached the key point, the

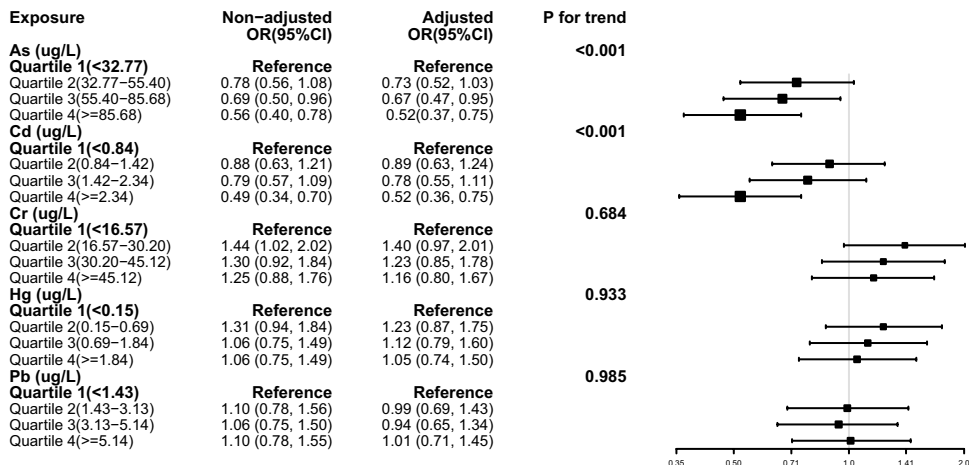
risk of nephrolithiasis significantly increased by 5.85-fold higher (Table 4). After performing multiple regression analysis using the score and after adjusting for confounding factors, the risk of nephrolithiasis increased by 123% in the high-risk group compared with the low-risk group (OR: 2.23, 95% CI: 1.57–3.17) (Fig. 4b).

$$\text{Score} = -0.0038 * \text{As} - 0.133 * \text{Cd} + 0.00538 * \text{Cr} - 0.00109 * \text{Hg} + 0.01818 * \text{Pb} \quad (1).$$

Discussion

This study revealed the relationship between heavy metal pollutants in the urine and nephrolithiasis in males. According to Fig. 3, it can be initially determined that the As curve is linear, whereas the Cd, Cr, Hg, and Pb curves are non-linear. The LRT shown in Table 3 indicated that when the *P*-value was <0.05, there was an inflection point; therefore, model 2, representing a nonlinear relationship, was selected. When the *P*-value was nonsignificant (>0.05), model 1 representing a linear relationship was selected. In this study, the *P*-value in the LRT of As was 0.111, indicating a linear relationship. The *P* values of Cd, Cr, Hg, and Pb were

Fig. 2 Forest chart of the prevalence of five heavy metal elements and male nephrolithiasis. The multiple regression model shows the independent relationship between metal elements and the prevalence of male nephrolithiasis, and the trend test of each metal element was performed. The quartile of heavy metal elements was based on the first group. Adjusted factors included age, BMI, SBP, DBP, CR, GFR, UREA, UA, UPRO, smoking status, and alcohol intake. OR, odds ratio; CI, confidence interval



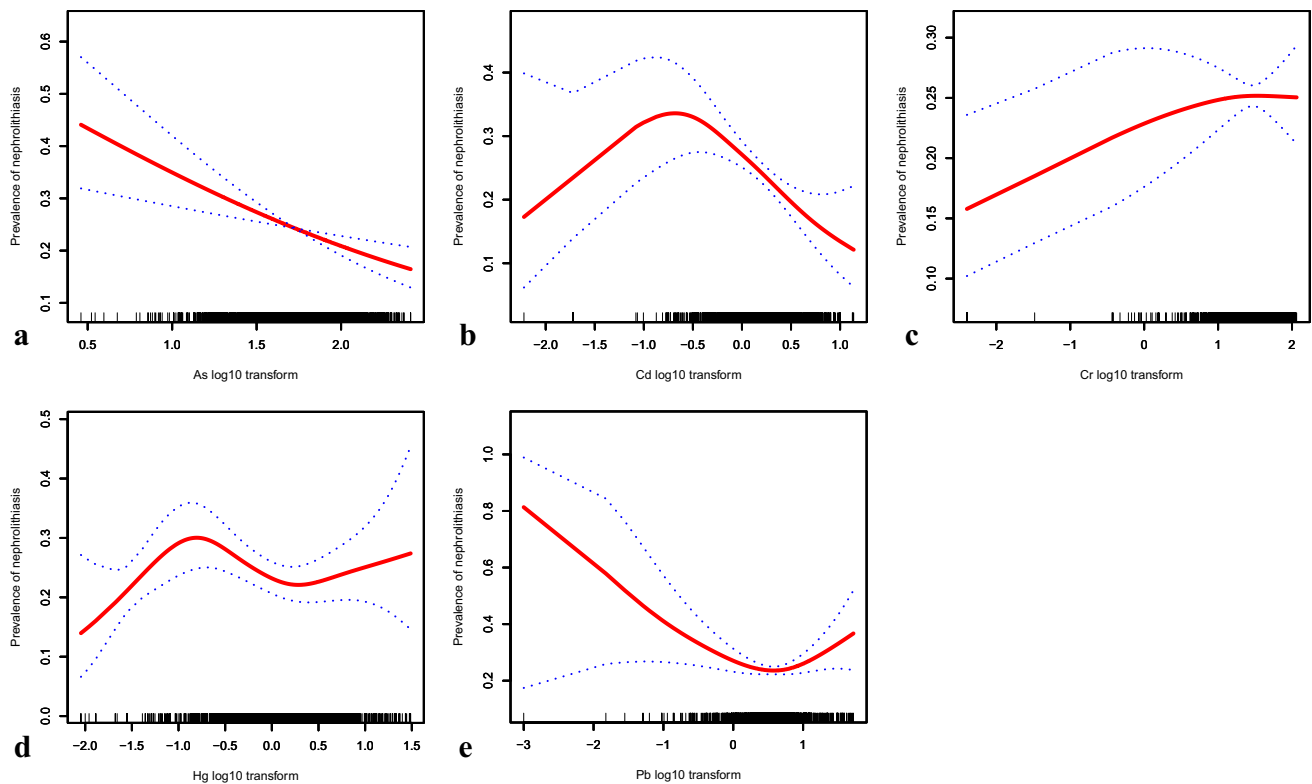


Fig. 3 The smooth fitting curve of five heavy metals in urine and the prevalence of nephrolithiasis. Curve-adjusted age, BMI, SBP, DBP, CR, GFR, UREA, UA, UPRO, smoking status, and alcohol intake.

The red curve in the middle represents the estimated value, and the blue curves on both sides represent the 95% confidence intervals

all < 0.05 , implying a nonlinear relationship, with the presence of inflection points and segments. The dose–response relationship between the levels of heavy metal elements, such as Cd, Cr, Hg, Pb, and nephrolithiasis in urine was two-piece-wise linear, with a threshold effect. The effect of individual elements on nephrolithiasis was not evident. However, when the five elements were evaluated together, the higher concentration of Cr and Pb, the lower concentration of As, Cd, and Hg, and all five heavy metal elements significantly increased the risk of nephrolithiasis. Therefore, the joint exposure of multiple heavy metal elements may play a crucial role in the occurrence of nephrolithiasis.

Exposure to heavy metals was associated with the occurrence of nephrolithiasis [41]. Studies have shown that it could be because heavy metals can induce renal cell apoptosis, leading to kidney damage [42, 43]. A high or small amount of them in the human body can affect health. Among them, As is an essential element for the human body. It exists in different forms in the environment. A higher or smaller dose of it can affect the health of the human body. A study on Taiwan people reported a J-shaped relationship between As intake and kidney damage. As the daily intake of inorganic substances increased, the damage intensified [44]. However, the dose–response of arsenous acid in non-Hispanic white

individuals was negatively associated with nephrolithiasis [28]. In our study, the concentration of As in the nephrolithiasis group was significantly lower than that in the non-nephrolithiasis group in this study, and the risk of nephrolithiasis was reduced as the urine concentration increased. A study of China’s Tibetans, Hans, and Huis showed that different individuals showed different urinary arsenic metabolism patterns, and the bioaccumulation of arsenic in the kidneys of different individuals might be different [5, 45]. In addition, the level of UPRO (renal biomarker) in the nephrolithiasis group was higher, indicating that the kidneys of the Dong male nephrolithiasis population might have been damaged, thereby reducing the excretion capacity of As [30, 46]. Urine As is an interactive term that regulates several associations between renal biomarkers and urine Pb, Cd, and Hg [47]. The As in the nephrolithiasis group might interact with other heavy metals in the body, resulting in a decrease in urine content.

Cd is an extremely toxic heavy metal even at low concentrations [48]. Cd accumulation in the human body can lead to kidney damage by potentially harming the proximal tubular epithelial cells [49]. Cd exposure was related to a significant decrease in the glomerular filtration rate [50]. Table 1 showed that the glomerular filtration rate of

Table 3 Threshold effect analysis of five heavy metal elements and prevalence of male nephrolithiasis using two-piece-wise regression model

Exposure	As log ₁₀ transform			Cd log ₁₀ transform			Cr log ₁₀ transform			Hg log ₁₀ transform			Pb log ₁₀ transform		
	OR (95% CI)	P ^d	OR (95% CI)	P ^d	OR (95% CI)	P ^d	OR (95% CI)	P ^d	OR (95% CI)	P ^d	OR (95% CI)	P ^d	OR (95% CI)	P ^d	
Model I ^a	0.49 (0.33, 0.74)	< 0.001	0.73 (0.57, 0.94)	0.015	1.15 (1.00, 1.31)	0.045	1.03 (0.89, 1.19)	0.700	0.88 (0.65, 1.18)	0.384	0.79 (0.69, 0.91)	0.018	0.58 (0.40, 0.83)	0.002	
Model II ^b (key point)	1.22 (1.19, 1.41)		-0.47 (-0.69, -0.37)		1.38 (1.25, 1.46)		-1.1 (-1.39, -0.66)		6.17 (4.90, 8.13)		3.53 (1.61, 7.75)		< 0.001		
Key point (μg/L)	16.60 (15.49, 25.70)		0.34 (0.20, 0.43)		25.12 (17.78, 28.84)		0.08 (0.04, 0.22)		0.098		0.098		0.098		
< K	3.05 (0.27, 34.02)	0.364	2.01 (1.08, 3.76)	0.029	1.24 (1.06, 1.45)	0.007	3.00 (1.21, 7.46)	0.018	0.58 (0.40, 0.83)	0.003	0.098		0.098		
≥ K	0.41 (0.25, 0.65)	< 0.001	0.39 (0.25, 0.59)	< 0.001	0.51 (0.23, 1.11)	0.091	0.81 (0.64, 1.04)	0.098	3.53 (1.61, 7.75)	0.002	0.098		0.098		
P for LRT ^c	0.111		< 0.001		0.037		0.018		< 0.001		< 0.001		< 0.001		

^aModel I: The linear relationship was described by a univariate linear regression model

^bModel II: Used two-piece-wise regression model to describe nonlinear relationship. The obtained log-likelihood ratio was less than 0.05 and model II was selected, otherwise, model I was selected. The result was the log-rotated value and the corresponding 95% confidence interval

^cLRT: log-likelihood ratio test

^dWald test for the difference between two regression coefficients. P-value in bold < 0.05

Adjusted variables: age, BMI, SBP, DBP, CR, GFR, UREA, UA, UPRO, smoking status, alcohol intake

OR, odds ratio; CI, confidence interval

the nephrolithiasis group was lower than that of the non-nephrolithiasis group. It could be inferred that when the Cd concentration was greater than 0.34 μg/L, renal function was significantly impaired. The concentration of cadmium in urine reflected the burden on the kidneys after long-term exposure to cadmium. Therefore, the threshold for nephrotoxicity observed in diseased kidneys might be lower than that observed in healthy kidneys [51]. In addition, the interaction of elements in the body would affect the excretion of urinary Cd. For example, due to the competition with metallothionein binding, an appropriate concentration of zinc exerts an antagonistic effect on the toxicity of cadmium [52].

We found that the dose–response relationship of Cr exposure was nonlinear, and the toxicity of < 25.12 μg/L Cr exposure increased the risk of nephrolithiasis by 24%. The literature pointed out that it was because Cr (+6) was reduced to Cr (+3) in the stomach and gastrointestinal tract and was excreted by the human body [53]. The concentration of Cr in urine was used as an internal Cr exposure biomarker [54], and it was affected by many factors. Studies had shown that metabolic disorders of obesity might affect changes in Cr in the body [55]. We found that the BMI of people with nephrolithiasis was 2% higher than that of people without nephrolithiasis, suggesting that increased Cr excretion in people with nephrolithiasis might partly lead to a decrease in body storage. Moreover, the co-exposure of Cr, Pb, and Cd might cause a further decrease in glomerular filtration rate [56], leading to an increased risk of nephrolithiasis.

Hg and Pb are heavy metal elements that are extremely harmful to the human body and can accumulate in human tissues, including the brain [57]. Most of the Hg in the environment that affects the human body is inorganic Hg [58]. The toxic effects of inorganic Hg were mainly seen in the kidneys of humans and animals, and experiments in rats had shown effects including increased kidney weight, tubular necrosis, UPRO, and hypoalbuminemia [53]. Our results also showed that UPRO was significantly higher in the nephrolithiasis group, suggesting that Hg might have started to impair kidney function, leading to excretory dysfunction with more Hg accumulation in the body and less in the urine. Pb causes toxicity in living cells by generating ions and oxidative stress [59]. This oxidative stress was manifested as an increase in the level of malondialdehyde in the kidneys, a decrease in the level of intracellular glutathione, and an increase in the infiltration of inflammatory cells into the organs [60]. Our results confirmed the damage of Pb to the kidneys. When the urine Pb concentration was ≥ 6.17 μg/L, the risk of nephrolithiasis disease increased by 3.53-fold higher. Therefore, the urine Pb concentration < 6.17 μg/L might be the result of normal kidney detoxification in Dong men.

The occurrence of stones may occur mainly because of environmental metal exposure [61]. However, a few studies have indicated the joint exposure effect between heavy

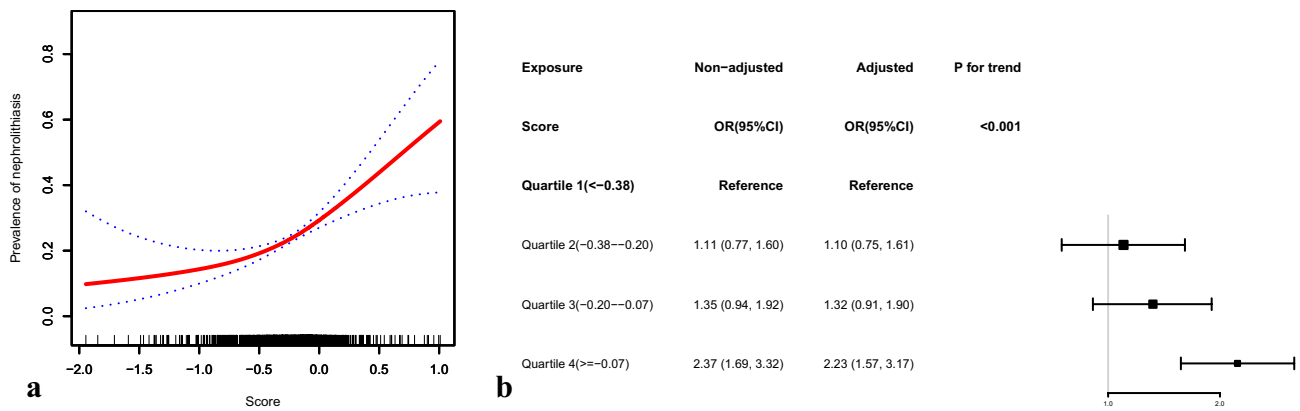


Fig. 4 The relationship between the score of metal combination and the risk of nephrolithiasis. **a** The smooth fitting curve of five environmental heavy metal elements score and the prevalence of nephrolithiasis. The red curve in the middle represents the estimated value, and the blue curves on both sides represent 95% confidence intervals. **b** The multiple regression model shows the independent relationship

between metal elements and the prevalence of male nephrolithiasis, and the trend test of each metal element was performed. The quartile of heavy metal elements was based on the first group. Adjusted factors included age, BMI, SBP, DBP, CR, GFR, UREA, UA, UPRO, smoking status, and alcohol intake

metals. In a 2015–2016 cross-sectional study on the blood levels of Co, Cr, Hg, and Pb and renal function, exposure to a mixture of heavy metals was associated with decreased renal function [30]. Low-dose metal mixtures (Pb, Hg, As, and Cd) interacted with toxic and essential metals, and the interactions between metal mixtures had a synergistic effect on kidney toxicity to a large extent [62]. Studies had found that long-term exposure to Cd would produce greater renal toxicity than As, and the combination of Cd and As could cause kidney damage even greater than the damage caused by using any chemical alone [63]. Rat studies had shown that the combination of Pb and Cd had an enhanced effect and produced renal subcellular changes [64]. The results of a large-scale prospective study of middle-aged and elderly people in China showed that exposure to multiple metals could cause a decline in kidney function in middle-aged and elderly people, and simultaneous exposure to multiple metals might have a synergistic effect on the decline in kidney function [65]. Our study found that the joint effect of heavy metals significantly increased the risk of nephrolithiasis by calculating the score using the Lasso regression model. In other words, the effect of individual heavy metals

on nephrolithiasis may not be clear. Using the prediction formula derived from the collinearity study, we found that the joint exposure of five heavy metals affected the risk of nephrolithiasis.

There are several limitations to this study. First, these results were based on a cross-sectional study and could not explain the cause and effect. Second, the influence of genetics, eating habits, and other urinary metal elements were not discussed. Finally, the difference in male and female participants also needs further investigation. Most of the baseline characteristics were significantly different ($P < 0.05$) in the analyzed group (Supplementary Table 1). In contrast to males, we did not observe a significant difference ($P > 0.05$) in metal exposure levels between non-nephrolithiasis ($N = 2,712$) and nephrolithiasis ($N = 265$) female participants (Supplementary Table 2). The relationship between the baseline markers and metal exposure level remains unclear. Therefore, dietary intake and genetics should be considered to improve the monitoring system in future studies.

This study also had several advantages. The effect of joint exposure to heavy metals in the research

Table 4 Threshold effect analysis of five heavy metal elements scores and prevalence of male nephrolithiasis using two-piece-wise regression model

Key point	Unadjusted			Adjusted		
	OR (95% CI)	P-value ^a	P for LRT ^b	OR (95% CI)	P-value ^a	P for LRT ^b
<-0.62	0.76 (0.21, 2.71)	0.669	0.039	0.69 (0.18, 2.57)	0.577	0.041
≥-0.62	4.26 (2.57, 7.08)	<0.001		5.85 (1.21, 28.30)	<0.001	

Two-piece-wise regression model was used to describe a nonlinear relationship

^aWald test for the difference between two regression coefficients

^bLRT: log-likelihood ratio test. P-value in bold <0.05

Adjusted variables: age, BMI, SBP, DBP, CR, GFR, UREA, UA, UPRO, smoking status, alcohol intake

environment on people with a high incidence of nephrolithiasis is rare. We included three generations of Dong people, with fixed diet and living habits, and high genetic stability. Their data were more valuable than those of other floating population studies. Moreover, the male population was selected, which reduced the influence of gender on the study. Although several studies have examined nephrolithiasis and heavy metals, only a few have indicated their threshold ranges. This study will serve as a reference for evaluating and monitoring potential heavy metals in different environmental areas and residential bioregions.

Conclusions

The potential association between urinary heavy metal concentrations and the prevalence of nephrolithiasis in men among the ethnic minority (Dong) population of China was demonstrated. Urinary As showed a linear relationship with the risk of nephrolithiasis. However, Cd, Cr, Hg, and Pb had a nonlinear relationship with the risk of nephrolithiasis. This result provided a reference for the studies of other high-risk groups. Moreover, a single heavy metal in the urine may not have a large effect on the risk of nephrolithiasis. However, the joint exposure of As, Cd, Cr, Hg, and Pb in urine may play a crucial role in the high incidence of nephrolithiasis in men. The results showed that exposure to heavy metals in the environment is of great significance to the human body. Therefore, we should reduce heavy metal pollution in the environment to prevent the occurrence of diseases.

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Author Contribution YL prepared the data, conducted the analysis, and drafted and revised the manuscript. CZ and QY conducted the experiments. ZQ, JL, XT, and QW provided technical support and contributed to the project. FH monitored all aspects of research implementation and contributed to the writing of the manuscript. All authors approved the final version.

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Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics Approval This study was approved by the Sichuan University Medical Ethical Review Board (K2016038) and the Medical Ethics Committee of the Affiliated Hospital of Guizhou Medical University (2018[094]).

Consent to Participate All participants provided the written informed consent for the current study.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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