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Analysis of the association between mixed exposure to multiple metals and comorbidity of osteopenia or osteoporosis: baseline data from the Chinese Multi-Ethnic Cohort study (CMEC)

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Abstract

Both osteoporosis and metal exposure are well-recognized public health concerns globally, particularly in the aging population. However, studies investigating the relationship between metal exposure and bone health conditions such as osteopenia and osteoporosis have either produced inconsistent results or are scarce, especially among the ethnic minorities in China. Herein, we correlated single-metal and metal mixture exposure with osteopenia and osteoporosis using a log-binomial regression model and quantile g-computation. In total, 9,206 ethnic Chinese individuals (Dong and Miao) aged 30–79 years were investigated in this study utilizing the baseline data from the Chinese multi-ethnic cohort study. In the single-metal exposure model, urinary concentrations of arsenic (As), cadmium (Cd), chromium (Cr), iron(Fe), mercury(Hg), and manganese (Mn) were positively associated with of osteopenia, whereas those of cobalt(Co) and zinc(Zn) concentrations were negatively associated. Additionally, urinary As, Cd, Cr, and Mn concentrations were positively associated with osteoporosis, whereas that of vanadium(V) was negatively associated. Furthermore, Quantile g-computation results indicated that metal mixture exposure was positively associated with both osteopenia and osteoporosis. Altogether, these findings suggest that simultaneous exposure to multiple metals can affect bone health, providing a theoretical basis for further studies on underlying complex mechanisms.

Keywords Osteoporosis, Osteopenia, Co-exposure, Quantile g-comptation

Introduction

Characterized by lower-than-normal bone mineral density (BMD) and toughness, osteopenia serves as a precursor to osteoporosis (OP), a systemic bone disease characterized by decreased bone mass and damage to the microstructure of bone tissue, which increases fragility and susceptibility to fractures [1]. The accelerated aging of the global population has led to increased incidences of OP and bone loss, with varying prevalences among different countries [2–4], resulting in notable public health concerns. In a study, OP prevalence in people aged ≥ 50 years in various industrialized countries was reported to be as follows: 26.3% in Japan, 21.0% in the United States, 14.3% in Germany, 9.9% in France, 9.7% in Italy, 7.8% in the United Kingdom, 6.3% in Spain, 2.6% in Canada, and 2.0% in Australia [3]. A cross-sectional study of the

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Chinese population showed that the prevalence of OP among adults aged ≥ 40 years was 5.0% in men and 20.6% in women [5]. Furthermore, the standardized prevalence of OP in China has been predicted to range from 5.04% (2.12–11.34%) to 7.46% (3.13–16.32%) and 26.28% (15.38–40.40%) to 39.19% (25.74–53.95%) in men and women aged ≥ 50 years, respectively, by 2050, imposing a substantial economic burden [6]. BMD is usually measured by quantitative ultrasound (QUS), dual-energy X-ray absorptiometry (DXA), and quantitative computed tomography (QCT). Compared with DXA and QCT, QUS is preferred for on-site testing because it is mobile, cost-effective, time-efficient, and radiation-free. QUS has been reported as effective as DXA in measuring bone density [7]. Additionally, QUS results of the radius and Achilles tendon have presented reliable predictive ability for hip fractures [8].

In addition to the conventional risk factors for abnormal bone mass, including gender [9], age [10], smoking [11], drinking [12], and dietary factors [13], metal exposure has been notably associated with abnormal bone mass [14–16]. A 10-year cohort study in Korea suggested the increased risk of OP due to cadmium (Cd), even in lower doses, resulting in reduced BMD, particularly in women [17]. Lu et al. reported that both blood lead (Pb) and Cd levels in 20–35-year-old people in the United States were negatively associated with lumbar spine bone density in young women but not in young men [18]. Similarly, Wang et al. reported markedly lower BMD in women with higher serum Cd and Pb levels than those with lower levels. A study in the Chinese adult population suggested serum Pb as an independent risk factor for OP in men [19]. Sudjaroen et al. positively correlated serum Zn levels with BMD, indicating its protective effects against OP in postmenopausal women in Thailand aged 55–65 years, suggesting the association between OP and micronutrient depletion-induced antioxidant reduction [20]. These findings align with the results of Rondanelli et al. [21]. Additionally, iron (Fe) levels have been reported to show a U-shaped exposure–response relationship with the risk of OP, where both Fe deficiency and overload negatively affected bone health [22]. Present studies are limited to exploring the association between single-metal exposure and BMD, lacking comprehensive insights into the effects of metal mixture exposure. However, simultaneous exposure to multiple metals is more common than single-metal exposure, and it has been related to obesity [23], hypertension [24], and diabetes [25] in adults. This highlights the need for research on the effects of mixed exposure to multiple metals on bone density.

Most studies on metal exposure and bone density have focused on individuals from the Chinese Han population, resulting in a notable dearth of information concerning ethnic minorities, such as the Dong and Miao ethnic groups, who boast sizable minority populations in the Guizhou Province, China. These communities are characterized by relatively closed populations, robust genetic homogeneity, and stable dietary habits. Recently, quantile g-computation (Qgcomp) has been widely used in epidemiology-related investigations to elucidate the holistic effects of multiple interrelated exposures. Therefore, this study aimed to correlate single-metal and metal mixture exposure with osteopenia and osteoporosis using a log-binomial regression model and Qgcomp. Herein, we utilized the baseline survey data from the Chinese multi-ethnic cohort (CMEC) study [26]. QUS and inductively-coupled plasma mass spectrometry (ICP-MS) were performed to measure the heel bone BMD and urinary concentrations of 11 metals, respectively, in the Dong and Miao populations of Guizhou Province, China. The following 11 metals were investigated in this study: arsenic (As), cadmium(Cd), cobalt (Co), chromium (Cr), copper (Cu),iron (Fe), mercury (Hg), manganese (Mn), lead(Pb), vanadium (V), and zinc (Zn). The application of Qgcomp allowed the in-depth analysis of the overall relationship between metal mixture exposure and osteopenia and OP, thus, providing input for subsequent preventive measures.

Materials and methods

Study population

Based on the baseline data obtained from the CMEC Study, a baseline survey, including electronic questionnaires, face-to-face interviews, physical examinations, and clinical laboratory tests, was conducted from July 2018 to August 2019 among 12,766 Dong and Miao permanent residents aged 30 to 79 years using a multi-stage stratified whole cluster sampling method. The inclusion criteria were as follows: 1) individuals of Dong and Miao descent aged 30–79 years, who resided for a minimum of three generations, were in a relatively closed group with good genetic homology, and followed the unique dietary habits; 2) individuals who willingly participated, signed the informed consent form, and consented to biological sample collection; and 3) individuals without mental illness or other related diseases, presenting normal expression and understanding abilities. The exclusion criteria were as follows: 1) participants with missing bone density ultrasound testing results (n = 1,361); 2) participants lacking urine metal element data (n = 1,816); 3) individuals with current malignant tumors or those with family or personal history of related disease (n = 71); 4) participants with a history of organ resection (n = 216); and 5) participants with thyroid disease, congenital kidney disease, and/or kidney disease (n = 96). Finally,

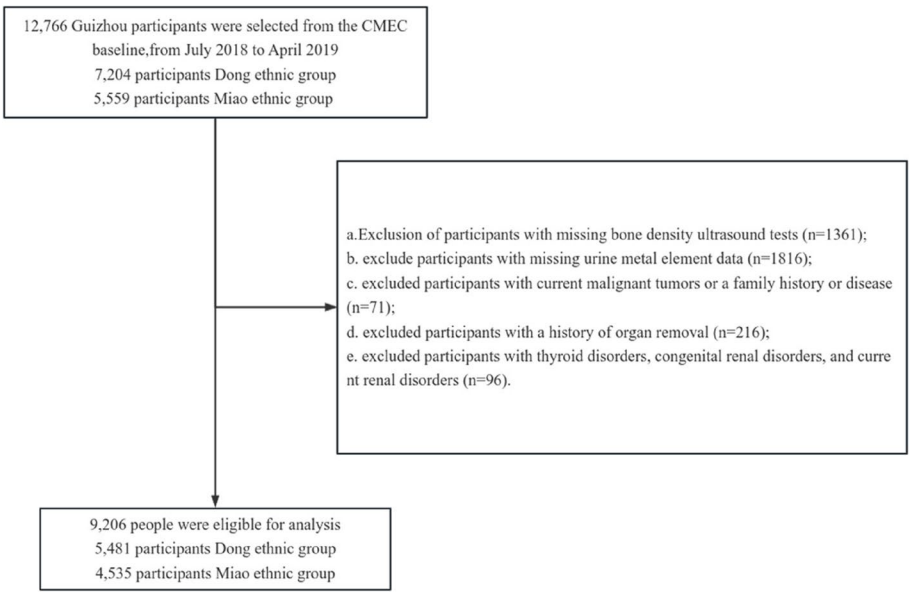

Fig. 1 Flowchart of the study. CMEC, Chinese multi-ethnic cohort

9,206 participants were included (Fig. 1). This study was approved by the Medical Ethics Committees of the West China Hospital of Sichuan University (K2016038) and the Affiliated Hospital of Guizhou Medical University (2018[094]). All research subjects participated voluntarily and signed informed consent forms.

Bone density measurement

The bone density was measured using the QSTEOK-J3OOO ultrasonic bone density meter (Nanjing Kejin Industrial Co., Ltd.). Before measurements, an appropriate coupling agent was applied to the heel (left/right) of all participants. Calibration of the ultrasonic bone densitometers was performed using a standard module before each use to ensure the accuracy of the measurements. All bone density measurements were performed individually by professionally trained personnel, with three readings recorded per participant to calculate the average as the final outcome. Subsequently, the measurement data were transferred to a database and cross-referenced with the personal code of respondents for accurate comparison. The original definition established by the World Health Organization in 1994, based on BMD, categorizes the T-score into the following three groups: normal bone: T-score > −1SD; osteopenia: −2.5SD < T-score ≤ −1SD; and OP: T-score ≤ −2.5SD [27].

Determination of urinary metal concentrations

The morning urine samples (5 mL) were collected and stored at −20 °C. They were allowed to reach room temperature before examination. From the original sample, 1 mL of the supernatant was mixed with 9 mL of 5% nitric acid solution (GR, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) in a 10-mL polyethylene centrifuge tube. After thorough mixing, the solution was passed through a 0.45-μm membrane filter. The urinary concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, V, and Zn were analyzed through ICP-MS (NexION 2000, PerkinElmer, Waltham, MA, USA). Internal online standardization was performed using an internal standard solution (10 μg/L; PerkinElmer), maintaining the regression coefficient of the standard curve above 0.999. To verify the accuracy, the Seronorm™ Trace Element Urine L-2 RUO (Sero, Billingstad, Norway) was used, ensuring an 80–120% recovery rate. Additionally, sample concentrations below the limit of detection were reported as half the limit of quantitation. The final concentration was calibrated by determining the specific gravity of urine [28], expressed in μg/L, using the following formula:

$$C = C_1 \frac{1.020 - 1.000}{d - 1.000}$$

where C and C_1 are the urinary metal concentration after and before correction, respectively; d is the specific gravity of urine.

Covariates

Face-to-face interviews with participants were conducted by trained investigators using a specialized electronic questionnaire prepared by the CMEC study project team. The questionnaire encompassed inquiries regarding the demographics, lifestyle, and medical history. The

variables were defined as follows: (1) ethnicity: Dong and Miao; (2) marital status: married/cohabiting, divorced/separated, widowed, and never married; (3) education level: junior high school and lower than junior high school diploma, high school diploma, college degree, and undergraduate degree or above; (4) annual income of the family(RMB): < 12,000, 12,000–19,999, 20,000–60,000, and > 60,000; (5) smoking: current smokers (participants who smoke ≥ 100 cigarettes), former smokers (those who quit smoking for > 6 months a year), and non-smokers; (6) drinking: regular drinkers (participants who consume alcohol every week for a continuous period of ≥ 1 year), occasional drinkers (those only drinking on special occasions or less than once a week), non-drinkers; (7) tea consumption: tea drinkers (participants who drink tea ≥ once a week) and non-tea drinkers (those who drink tea less frequently); (8) physical activity: the physical strength of participants was assessed by calculating the metabolic equivalents of occupation, transportation, housework, and leisure activities; (9) Body-Mass Index (BMI): In accordance with the "Guidelines for Prevention and Control of Overweight and Obesity in China", individuals can be classified as follows: underweight (BMI < 18.5 kg/m^2), normal weight (with 18.5 $kg/m^2 \leq$ BMI < 24 kg/m^2), overweight (where 24 $kg/m^2 \leq$ BMI < 28 kg/m^2), and obese (when BMI ≥ 28 kg/m^2) [29]; and (10) self-report: for conditions such as hypertension or diabetes, and if the participant have ever had a fracture.

Statistical analyses

Measurement data are expressed as medians (P25, P75), and intergroup comparisons were performed using the Kruskal–Wallis *H* test. Enumeration data are expressed as frequencies (percentages), and intergroup comparisons were performed using the chi-square test. The Statistical Package for the Social Sciences 25.0 (IBM, Armonk, NY, USA), R 4.3.1 software and SAS 9.4 software were used for statistical analyses of the data. Statistical significance was set at $P < 0.05$ based on two-sided probability.

Correlation between single-metal exposure and risk of osteopenia and OP

The urinary concentrations of 11 metals (arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, lead, vanadium, and zinc) were analyzed using Spearman's rank correlation to examine inter-metal correlations. Prevalence ratios (PRs) and 95% confidence intervals (CIs) for osteoporosis and osteopenia (OP) were calculated through a log-binomial regression model implemented in the PROC GENMOD macro of SAS 9.4. Individual urinary metal concentrations were divided into quartiles based on their distribution, with the lowest quartile designated as the reference group. The statistical significance of trends across quartiles was assessed by treating the median value of each quartile as a continuous variable in the regression model. Two models were constructed: Model I (unadjusted) and Model II (adjusted for age, gender, race, education, marital status, annual household income, smoking status, alcohol and tea consumption, hypertension, diabetes mellitus, body mass index, and physical activity). Urinary metal concentrations were log-transformed using the rcsci package in R (version 4.3.1). Additionally, restricted cubic spline (RCS) analysis was employed to evaluate the dose-response relationships between urinary metal concentrations and the prevalence of osteoporosis and OP in the Dong and Miao populations.

Association between metal mixtures and the risk of osteopenia and OP

Quantile g-computation was used to examine the correlation of metal mixture exposure with osteopenia and OP. Quantile g-computation, a statistical method rooted in parametric, generalized linear models, treated all metals as a mixture to examine the effect of each quartile increase on the risk of disease. The weighted indices in the model signify the relative contribution of the corresponding individual components of the mixture to the combined effect of the exposure. For heterogeneous effects, the weighting index is defined as the proportion of each component of the mixture effect to the outcome in each direction. These index weights for each exposure were constrained to be between 0 and 1, ensuring the sum of the weights for each metalloid in the positive and negative directions remains at 2.

Research results

Basic characteristics of research participants

In total, 9,206 participants were included in this study, among whom 347 (3.79%) were diagnosed with OP and 3,472 (37.71%) presented osteopenia. Both the osteopenia and OP groups exhibited increased detection in populations of higher age than in the normal group. The detection rates of OP and osteopenia were significantly higher in men than those in women. Compared with the normal group, the osteopenia and OP groups showed significant differences in the T score, marital status, annual family income, smoking, drinking, tea consumption, hypertension, diabetes, fracture history, BMI, and physical activity; however, those in ethnicity and education level were not statistically significant (Table 1). All *P*-value < 0.05.

Urinary metal concentrations

The median and interquartile range of the urinary metal concentrations (μg/L) in the total study population and different bone mass status groups are presented in Table 2.

Table 1 Basic characteristics of the study population (n [%] or median (25^{th}, 75^{th}])

Characteristics[a]	Normal	Osteopenia	Osteoporosis	χ^2/Z	*P*-Value[b]
Total	5387(58.50)	3472(37.71)	347(3.79)	/	/
Age	49.50(42.82,55.93)	56.03(48.03,65.78)	57.82(48.76,68.21)	701.047	< 0.001
T score	−0.10(−0.50,0.50)	−1.50(−1.80,−1.20)	−2.80(−3.30,−2.60)	6784.342	< 0.001
Sex				559.378	< 0.001
Male	1530(28.40)	1690(48.68)	253(72.91)		
Female	3857(71.60)	1782(51.32)	94(27.09)		
Ethnicity				5.268	0.072
Dong	3260(60.52)	2022(58.24)	199(57.35)		
Miao	2127(39.48)	1450(41.76)	148(42.65)		
Education				8.143	0.086
< high school	4298(79.78)	2717(78.25)	262(75.50)		
High school or vocational school graduate	462(8.58)	347(10.00)	39(11.24)		
≥ College graduate	627(11.64)	408(11.75)	46(13.26)		
Marital status				100.526	< 0.001
Married	4853(90.09)	2899(83.50)	301(86.74)		
Divorced	151(2.80)	112(3.23)	7(2.02)		
Widowed	350(6.50)	424(12.21)	33(9.51)		
Never married	33(0.61)	37(1.06)	6(1.73)		
Annual household income (RMB)				12.643	0.049
< 12,000	1506(27.96)	1006(28.98)	98(28.24)		
12,000–19999	1052(19.53)	647(18.63)	76(21.90)		
20,000–60000	1632(30.29)	983(28.31)	85(24.50)		
> 60,000	1197(22.22)	836(24.08)	88(25.36)		
Smoking				332.917	< 0.001
No	4524(83.98)	2467(71.05)	185(53.31)		
Current smoker	702(13.03)	821(23.65)	130(37.46)		
Former smoker	161(2.99)	184(5.30)	32(9.22)		
Drinking				72.765	< 0.001
No	2795(51.88)	1791(51.58)	155(44.67)		
Occasional drinkers	2061(38.26)	1186(34.16)	119(34.29)		
Regular drinkers	531(9.86)	495(14.26)	73(21.04)		
Tea consumption				59.368	< 0.001
No	4767(88.49)	2894(83.35)	277(79.83)		
Yes	620(11.51)	578(16.65)	70(20.17)		
Hypertension				57.674	< 0.001
No	4552(84.50)	2746(79.09)	257(74.06)		
Yes	835(15.50)	726(20.91)	90(25.94)		
Diabetes				15.325	< 0.001
No	5204(96.60)	3301(95.07)	327(94.24)		
Yes	183(3.40)	171(4.93)	20(5.76)		
Fracture history				53.400	< 0.001
No	5170(95.97)	3212(92.51)	319(91.93)		
Yes	217(4.03)	260(7.49)	28(8.07)		
BMI				86.938	< 0.001
< 18.5	102(1.89)	94(2.71)	8(2.30)		
18.5–24	2374(44.07)	1649(47.50)	124(35.73)		
24–28	2146(39.84)	1215(34.99)	112(32.28)		
≥ 28	765(14.20)	514(14.80)	103(29.69)		
Physical activity				220.533	< 0.001
Low	1231(22.98)	1210(35.16)	152(44.31)		
Medium	1353(25.36)	855(24.85)	81(23.62)		
High	2773(51.76)	1376(39.99)	110(32.07)		

RMB Renminbi, *BMI* Body–mass index

[a] Data are presented as means ± standard deviation, median (25th, 75th), or n (%)

[b] *P-Value* was derived from the Kruskal–Wallis H test for continuous variables according to the data distribution and the Chi-square test for the category variables

Table 2 Concentrations of metals (μg/L) among the study population

Metal (μg/L)	LOD[a]	≥LOD	Median (25th,75th)				*P*
			Total	Normal	Osteopenia	Osteoporosis	
As	0.0104	9204(99.98%)	69.73(43.35,102.80)	67.76(40.84,101.03)	72.86(46.25,104.03)	74.26(49.14,107.81)	<0.001
Cd	0.0111	9069(98.51%)	1.90(1.12,3.08)	1.81(1.05,2.96)	2.00(1.21,3.23)	2.22(1.32,3.29)	<0.001
Co	0.0001	8380(91.03%)	0.40(0.20,0.71)	0.40(0.19,0.71)	0.39(0.20,0.68)	0.42(0.22,0.72)	0.214
Cr	0.0021	8929(96.88%)	41.72(25.55,64.48)	40.04(24.37,62.76)	43.97(26.85,66.76)	46.85(29.35,67.97)	<0.001
Cu	0.0016	8358(90.79%)	87.74(29.67,221.44)	87.33(28.93,223.52)	88.77(30.91,216.78)	78.44(25.96,220.62)	0.645
Fe	0.0201	9086(98.70%)	394.96(191.72,695.38)	391.18(193.48,682.37)	403.46(187.44,711.13)	376.45(185.53,719.42)	0.534
Hg	0.0168	7562(82.14%)	0.50(0.09,1.40)	0.48(0.06,1.35)	0.53(0.13,1.48)	0.56(0.15,1.37)	0.009
Mn	0.0002	7595(82.50%)	4.70(1.36,10.32)	4.57(1.26,10.14)	4.74(1.44,10.32)	6.32(2.13,12.83)	0.001
Pb	0.0001	7940(86.25%)	3.31(1.38,6.63)	3.22(1.29,6.49)	3.44(1.49,6.85)	3.58(1.65,6.72)	0.015
V	0.0166	9201(99.95%)	37.53(19.29,60.48)	37.52(19.06,61.06)	37.78(20.19,59.35)	33.87(16.53,60.81)	0.397
Zn	0.0180	9184(99.76%)	660.34(422.37,968.48)	640.86(406.42,938.72)	681.42(443.72,1003.60)	780.10(534.76,1153.91)	<0.001

Urinary metal concentrations were corrected for urine-specific gravity

As Arsenic, *Cd* Cadmium, *Co* Cobalt, *Cr* Chromium, *Cu* Copper, *Fe* Iron, *Hg* Mercury, *Mn* Manganese, *Pb* Lead, *V* vanadium, *Zn* zinc

[a] Limit of detection (LOD)

Notably, the detection rates for the 11 metals exceeded 80%, with statistically significant differences observed for 7 metals (namely As, Cd, Cr, Hg, Mn, Pb, and Zn) among different bone mass status groups ($P<0.05$). The correlation between urinary metal concentrations (Fig. 2) revealed a positive correlation between most metals and a negative correlation between Mn and Cu, with correlation coefficients ranging from −0.16 to 0.71 ($P<0.05$).

Association between urinary metal concentrations and osteopenia

The correlation between urinary metal concentrations and osteopenia in ethnic minorities is presented in Table 3. Urinary concentrations of Cd, Cr, Fe, and Hg were positively correlated with osteopenia in Model I, whereas those of Co and V exhibited negative correlations. The remaining urinary metals showed no correlation with osteopenia. In contrast, urinary concentrations of Cd, Cr, Fe and Hg were positively associated with osteopenia in Model II, whereas those of V, Co and Zn were negatively associated. The dose–response relationship between urinary concentrations of Cr and Fe, as well as the prevalence of osteopenia, exhibited a nonlinear trend (*P-overall < 0.05, P-nonlinear < 0.05*) (Fig. 3). In contrast, a linear association was observed between urinary concentrations of Cd, along with the prevalence of osteopenia (*P-overall < 0.05, P-nonlinear > 0.05*). Notably, no significant dose–response relationship was found between urinary concentrations of Co, in relation to the prevalence of osteopenia (*P-overall > 0.05*).

Association between urinary metals and OP

The correlation between urinary metal concentrations and the risk of OP is presented in Table 4. Urinary concentrations of As, Cd, Cr, and Mn were positively correlated with the risk of OP in Model I, whereas that of V exhibited a negative correlation with the risk of OP. Other elements did not exhibit any correlation with the risk of OP. In Model II, urinary concentrations of As, Cd, Cr, and Mn were positively correlated with the risk of OP, and the urinary concentration of V exhibited a significantly negative correlation with OP. The dose–response relationship between concentrations of urinary Cr and Mn and the prevalence of OP presented a nonlinear trend (P-overall < 0.05, P-nonlinear < 0.05), and that between urinary As and Cd concentrations and the prevalence of OP showed a linear trend (P-overall < 0.05, P-nonlinear > 0.05) (Fig. 4).

Quantile g-computation

The association between metal mixture exposure and the risk of osteopenia and OP was investigated via Qgcomp. Notably, a one-quartile increase in the mixture of 11 metals increased the risk of osteopenia by 1.59 times (OR: 1.59, 95% CI: 1.28–1.98, $P<0.001$). Co and Zn were the most substantial weighted indices in the negative association with osteopenia, whereas Cr and Cd exhibited the most significant positive association. Similarly, a one-quartile increase in the mixture of 11 metals increased the risk of OP by 1.98 times (OR: 1.98, 95% CI: 1.21–3.25, $P=0.006$) (Table 5). Regarding association with OP, V was the most weighted index in the negative association with OP, whereas Mn, As, and Cd showed a positive correlation with OP (Fig. 5).

Subgroup analysis of the association between metal mixture exposure in urine and osteopenia and OP

The data showing the correlation of urinary metal concentrations with the risk of osteopenia and OP in male ethnic minorities (Supplementary Tables 1 and

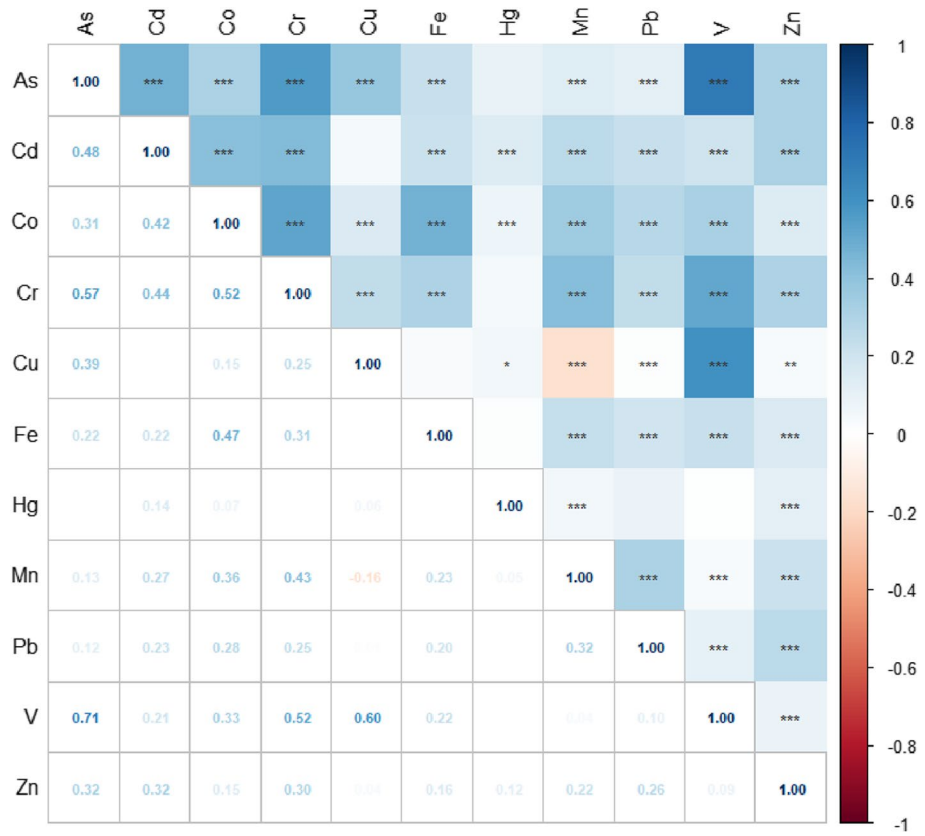

Fig. 2 Correlation of metal concentrations in urine. Urinary metal concentrations were corrected for urine-specific gravity. ***$P < 0.001$; **$0.001 < P \leq 0.01$; *$P \leq 0.05$. As, arsenic; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Hg, mercury; Mn, manganese; Pb, lead; V, vanadium; Zn, zinc

2) indicates that urinary concentrations of Ca, Hg, and Pb were positively correlated with an increased risk of osteopenia. Furthermore, urinary concentrations of As, Ca, Cr, Fe, Mn, and Zn exhibited a positive association with the risk of OP. Additionally, Urinary concentrations of As, Ca, Cr, Hg, and Mn were associated with an increased risk of osteopenia in female ethnic minorities, and those of Ca, Cr, and Mn correlated positively with the risk of OP. Notably, V concentrations exhibited a negative correlation with the risk of OP.

The association of metal mixture exposure with abnormal bone mass was investigated through Qgcomp, stratified by sex, under the metal mixture exposure model. The findings indicated that in males, exposure to metal mixtures was associated with both reduced bone mass and an increased risk of OP. Specifically, each quartile increase in metal mixture exposure corresponded to a 1.51-fold (95% CI: 1.08–2.11) and 2.75-fold (95% CI: 1.49–5.07) increased risk of developing reduced osteopenia and OP, respectively. In females, exposure to metal mixtures was only associated with an increased risk of osteopenia but not with OP, with a 1.70-fold (95% CI: 1.33–2.17) increased risk for each quartile increase in metal mixture exposure (Supplementary Table 3). Furthermore, subgroup analysis revealed that Co, Cu, and Zn exhibited the most significant negative association with bone abnormalities among the metals, whereas Cd, Cr, Mn, and Hg exhibited notably positive association (Supplementary Fig. 1).

Sensitivity analyses

The results of sensitivity analyses indicated that excluding current and former smokers, the estimates of urinary metal concentrations contributing to osteopenia and OP were consistent with previous trends (Tables 6 and 7).

Although metal mixture exposure did not exhibit any notable association with OP, it presented a significantly positive association with osteopenia (Table 8, Fig. 6).

Discussion

The relationship between exposure to 11 metals and the risk of OP and osteopenia was investigated, along with the examination of the effects of concurrent exposure to

Table 3 Association between urinary metal concentrations and osteopenia

Metals	Grouping	*PR(95%CI)*			
		Model I	*P-value*	Model II	*P-value*
As(μg/L)	Q1(≤43.35)	1.00(Ref)		1.00(Ref)	
	Q2(43.35-69.73)	1.07(1.00-1.15)	0.053	1.09(0.98-1.22)	0.113
	Q3(69.73-102.80)	1.00(0.94-1.07)	0.895	1.09(0.96-1.25)	0.173
	Q4(>102.80)	0.99(0.94-1.04)	0.592	1.09(0.94-1.26)	0.253
P for trend			0.061		0.383
Cd(μg/L)	Q1(≤1.12)	1.00(Ref)		1.00(Ref)	
	Q2(1.12-1.90)	1.11(1.05-1.18)	<0.001	1.11(1.00-1.23)	0.047
	Q3(1.90-3.08)	1.04(0.99-1.10)	0.144	1.13(1.01-1.26)	0.028
	Q4(>3.08)	1.03(0.98-1.08)	0.289	1.21(1.08-1.37)	0.001
P for trend			<0.001		0.002
Co(μg/L)	Q1(≤0.20)	1.00(Ref)		1.00(Ref)	
	Q2(0.20-0.40)	0.85(0.80-0.90)	<0.001	0.97(0.88-1.08)	0.613
	Q3(0.40-0.71)	0.84(0.80-0.89)	<0.001	0.90(0.81-1.01)	0.064
	Q4(>0.71)	0.90(0.86-0.95)	<0.001	0.82(0.72-0.93)	0.003
P for trend			<0.001		0.002
Cr(μg/L)	Q1(≤25.55)	1.00(Ref)		1.00(Ref)	
	Q2(25.55-41.72)	1.05(0.99-1.10)	0.099	1.05(0.95-1.17)	0.354
	Q3(41.72-64.48)	1.14(1.07-1.22)	<0.001	1.11(0.99-1.25)	0.075
	Q4(>64.48)	1.10(1.04-1.16)	0.002	1.17(1.03-1.34)	0.019
P for trend			<0.001		0.014
Cu(μg/L)	Q1(≤29.68)	1.00(Ref)		1.00(Ref)	
	Q2(29.68-87.74)	1.02(0.96-1.08)	0.555	1.02(0.92-1.13)	0.766
	Q3(87.74-221.14)	0.99(0.94-1.04)	0.740	1.01(0.91-1.14)	0.802
	Q4(>221.14)	0.98(0.94-1.03)	0.516	1.03(0.91-1.16)	0.637
P for trend			0.989		0.671
Fe(μg/L)	Q1(≤191.74)	1.00(Ref)		1.00(Ref)	
	Q2(191.74-394.96)	1.03(0.98-1.09)	0.288	0.98(0.89-1.08)	0.709
	Q3(394.96-695.34)	1.07(1.02-1.13)	0.007	1.07(0.96-1.18)	0.218
	Q4(>695.34)	1.05(1.00-1.10)	0.048	1.12(1.01-1.25)	0.036
P for trend			0.042		0.015
Hg(μg/L)	Q1(≤0.09)	1.00(Ref)		1.00(Ref)	
	Q2(0.09-0.50)	1.06(1.01-1.11)	0.016	1.10(0.99-1.21)	0.064
	Q3(0.50-1.40)	1.01(0.96-1.06)	0.706	1.06(0.96-1.17)	0.269
	Q4(>1.40)	1.02(0.97-1.07)	0.375	1.12(1.01-1.23)	0.028
P for trend			0.052		0.092
Mn(μg/L)	Q1(≤1.36)	1.00(Ref)		1.00(Ref)	
	Q2(1.36-4.70)	0.99(0.94-1.05)	0.832	1.02(0.92-1.12)	0.733
	Q3(4.70-10.32)	0.99(0.93-1.04)	0.647	1.03(0.93-1.14)	0.620
	Q4(>10.32)	1.01(0.96-1.06)	0.812	1.06(0.95-1.19)	0.308
P for trend			0.420		0.397
Pb(μg/L)	Q1(≤1.38)	1.00(Ref)		1.00(Ref)	
	Q2(1.39-3.31)	1.05(1.00-1.10)	0.078	1.02(0.92-1.12)	0.766
	Q3(3.31-6.63)	1.02(0.97-1.07)	0.415	1.03(0.93-1.13)	0.619
	Q4(>6.63)	1.02(0.97-1.07)	0.390	1.04(0.93-1.15)	0.511
P for trend			0.172		0.598
V(μg/L)	Q1(≤19.29)	1.00(Ref)		1.00(Ref)	
	Q2(19.29-37.53)	0.90(0.84-0.97)	0.006	0.98(0.88-1.10)	0.729
	Q3(37.53-60.48)	0.91(0.86-0.97)	0.004	0.99(0.87-1.12)	0.830

Table 3 (continued)

Metals	Grouping	*PR(95%CI)*			
		Model I	*P-value*	Model II	*P-value*
	Q4(>60.48)	0.94(0.90-0.99)	0.027	0.93(0.80-1.08)	0.364
P for trend			0.007		0.426
Zn(μg/L)	Q1(≤422.40)	1.00(Ref)		1.00(Ref)	
	Q2(422.40-660.34)	1.03(0.98-1.09)	0.221	0.96(0.87-1.06)	0.426
	Q3(660.34-968.39)	1.02(0.97-1.08)	0.404	0.89(0.80-0.99)	0.035
	Q4(>968.39)	1.04(0.99-1.09)	0.152	0.90(0.81-1.01)	0.065
P for trend			0.150		0.128

P for trend across quartiles of metals was obtained by including the median of each quartile as a continuous variable in the regression models

Model I: non-adjusted model; Model II: model adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity

Ref reference, *PR* Prevalence ratio, *CI* Confidence interval, *As* Arsenic, *Cd* Cadmium, *Co* Cobalt, *Cr* Chromium, *Cu* Copper, *Fe* Iron, *Hg* Mercury, *Mn* Manganese, *Pb* Lead, *V* Vanadium, *Zn* Zinc

these trace elements on bone health. The findings indicated that single-metal exposure with increased urinary concentrations of As, Cd, Cr, Fe, Hg, and Mn and was positively associated with osteopenia, whereas increased concentrations of Co and Zn were negatively associated. Additionally, higher urinary concentrations of As, Cd, Cr, and Mn were positively associated with OP, whereas that of V exhibited a negative association with osteopenia. Notably, coexposure of multiple metals increased the risks of both osteopenia and OP.

OP and osteopenia considerably affect the quality of life of patients, along with imposing a significant burden on society and economy. Compared with OP, the population affected by osteopenia is considerably larger; therefore, the effects of its prevalence on the economy should not be overlooked. Studies on the population of the Hubei Province, China, have reported the prevalence of OP in the region to be 12.19%, with 3.69% in men and 18.94% in women. Furthermore, the prevalence of osteopenia has been reported to reach 56.6%, with 44.96% in men and 65.84% in women [30]. These findings further underscore the widespread prevalence and severity of osteopenia. Proteomics analysis has revealed that both osteopenia and OP are associated with dysregulation of inflammatory signaling pathways; however, despite their overlapping physiological mechanisms, both conditions present notable differences in terms of biomarkers [31]. These differences in biomarkers provide an important basis for the clinical diagnosis and treatment of these conditions, along with providing future research directions.

Gender represents a significant factor exerting an impact on abnormal bone mass. The majority of studies have consistently revealed that the prevalence of osteoporosis is more pronounced in women compared to men. This disparity is principally attributed to sex hormones [32], especially estrogen, which can directly target osteoclasts via the RANKL—RANK—OPG system or induce anti—apoptotic effects on osteoclasts by activating ERK [33, 34]. Additionally, estrogen can restrain osteoclasts through modulating the activity of serum Calcitonin (CT) and Parathyroid Hormone (PTH) levels [35]. However, in the current study, an unexpected finding emerged: the detection rate of bone mass abnormality was higher in men within the Dong, Miao, and Buyi ethnic groups. This phenomenon might potentially be associated with the dietary preferences in ethnic minority regions. Specifically, most men in these areas exhibit a stronger inclination towards consuming cured fish/meat, and oil tea in contrast to women. Conversely, women tend to consume more sour soup. Moreover, a higher proportion of men engage in smoking and alcohol consumption, and all areas experience relatively higher stress levels. Nevertheless, the precise underlying reasons warrant further in-depth investigation.

Previous epidemiologic studies suggest the association of heavy metal exposure with an increased risk of developing OP, which is consistent with our findings. Herein, in the Dong and Miao populations of the Guizhou Province, the risks of osteopenia and OP were markedly associated with Cd and Mn exposures; this finding is consistent with the results of single-metal exposure and Qgcomp. Cd, a toxic heavy metal, is commonly found in nature in a chemosynthetic state and primarily released into the environment via waste gas, wastewater, and waste residues, leading to environmental pollution [36]. The biological half-life of Cd can range from 10 to 30 years, and it tends to accumulate in the body following exposure [37], with bone being among the primary target organs [38]. In a Chinese cohort study, urinary Cd levels were identified as an independent risk factor for OP in women aged 50–79 years, and the risk of OP in the high urinary cadmium group (> 10 μg/gcr) was 2.24 times

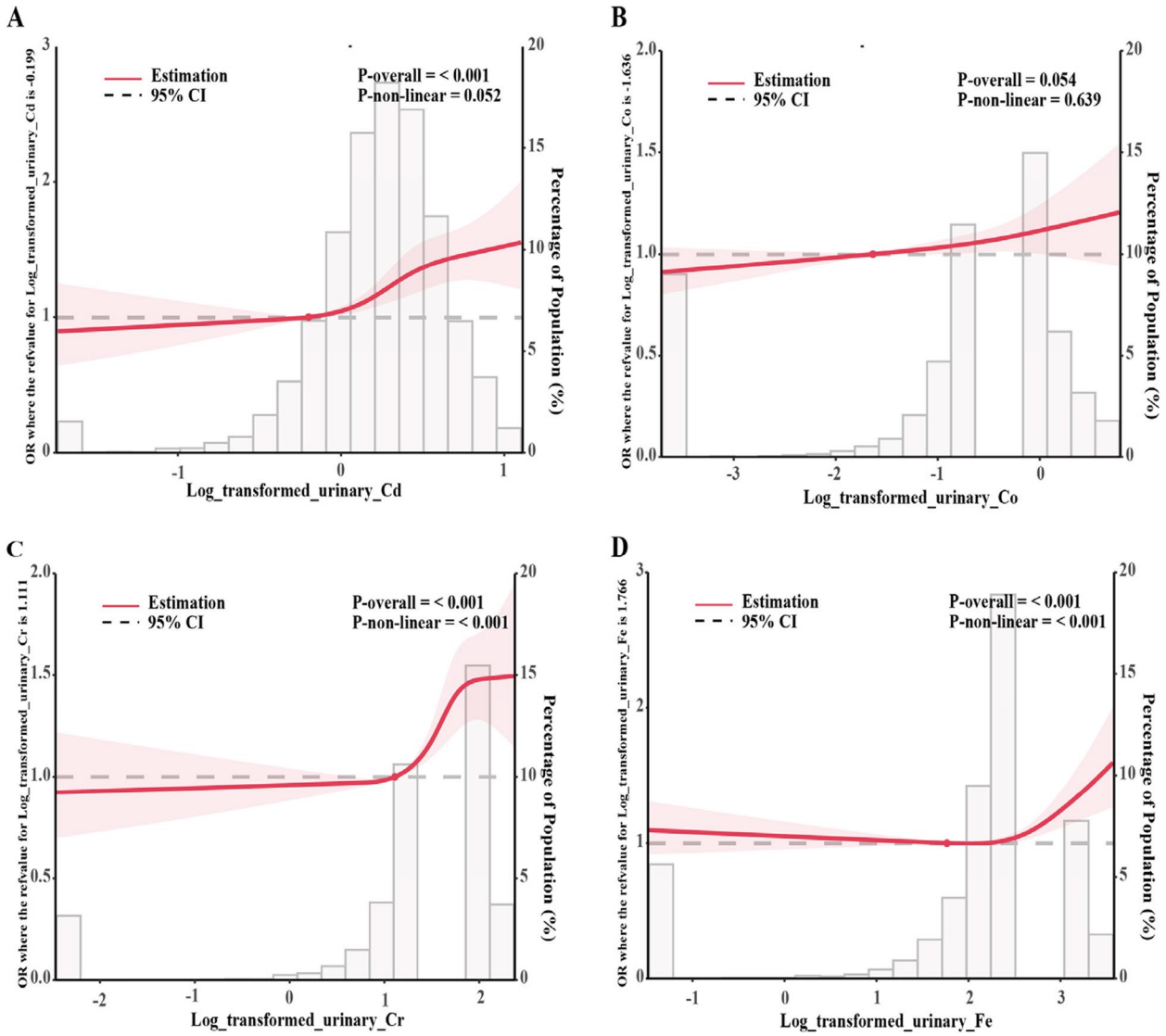

Fig. 3 Restricted cubic spline plots of urinary metals and the risk of osteopenia. Log-transformed urinary metal concentrations; adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity. Cd, cadmium; Co, cobalt; Cr, chromium; Fe, iron

higher than that in the low UCd group (< 5 μg/gcr) [39]. Another meta-analysis suggested UCd as an independent risk factor for OP and osteopenia [40]. Cd can induce abnormalities in calcium (Ca) and phosphorus metabolism within the bone tissue through mechanisms such as renal damage, particularly tubular damage, impaired Ca reabsorption, reduced vitamin D synthesis, and subsequent disturbances in Ca–phosphorus metabolism, resulting in the loss of Ca and phosphorus in the bone tissue, and ultimately resulting in abnormal bone mass [41]. Cd affects bone marrow mesenchymal stem cells by hindering their differentiation into osteoblasts and directly triggering apoptosis [42]. Moreover, it has been reported to directly affect osteoclast activation, fostering bone resorption, and causing damage to osteoblasts along with oxidative stress. This cascade leads to DNA damage, mitochondrial dysfunction, and endoplasmic reticulum stress, ultimately resulting in the activation of apoptosis [43].

Mn is an essential nutrient for intracellular activity, and it serves as a cofactor for various enzymes such as arginase, glutamine synthetase, pyruvate carboxylase, and Mn superoxide dismutase. Various physiological processes, including digestion, reproduction, antioxidant defense, energy production, immune response, and the regulation of neuronal activity, require the involvement of Mn

Table 4 Association between urinary metal concentrations and osteoporosis

Metal	Grouping	*PR*(*95%CI*)			
		Model I	*P-value*	Model II	*P-value*
As(μg/L)	Q1(≤43.35)	1.00(Ref)		1.00(Ref)	
	Q2(43.35-69.73)	1.16(0.91-1.25)	0.162	1.17(0.83-1.21)	0.366
	Q3(69.73-102.80)	1.04(1.01-1.13)	0.027	1.12(0.94-1.20)	0.120
	Q4(>102.80)	1.11(1.10-1.27)	0.005	1.13(1.00-1.20)	0.012
P for trend			0.004		0.043
Cd(μg/L)	Q1(≤1.12)	1.00(Ref)		1.00(Ref)	
	Q2(1.12-1.90)	1.18(1.13-1.22)	0.008	1.16(1.09-1.22)	0.014
	Q3(1.90-3.08)	1.17(1.12-1.31)	<0.001	1.12(1.06-1.27)	<0.001
	Q4(>3.08)	1.11(1.17-1.26)	<0.001	1.08(1.02-1.29)	<0.001
P for trend			<0.001		<0.001
Co(μg/L)	Q1(≤0.20)	1.00(Ref)		1.00(Ref)	
	Q2(0.20-0.40)	1.08(0.77-1.31)	0.654	1.07(0.85-1.22)	0.725
	Q3(0.40-0.71)	1.08(0.75-1.34)	0.465	1.08(0.84-1.28)	0.437
	Q4(>0.71)	0.97(0.86-1.25)	0.673	1.08(0.84-1.25)	0.255
P for trend			0.627		0.270
Cr(μg/L)	Q1(≤25.55)	1.00(Ref)		1.00(Ref)	
	Q2(25.55-41.72)	1.31(1.01-1.36)	0.042	1.07(0.88-1.14)	0.079
	Q3(41.72-64.48)	1.32(1.12-1.37)	0.003	1.13(1.06-1.24)	0.021
	Q4(>64.48)	1.29(1.04-1.31)	0.012	1.08(1.00-1.19)	0.045
P for trend			0.007		0.039
Cu(μg/L)	Q1(≤29.68)	1.00(Ref)		1.00(Ref)	
	Q2(29.68-87.74)	0.91(0.87-1.13)	0.252	0.81(0.76-1.06)	0.257
	Q3(87.74-221.14)	0.86(0.83-1.16)	0.435	0.79(0.74-1.02)	0.081
	Q4(>221.14)	0.81(0.86-1.20)	0.541	0.83(081-1.14)	0.549
P for trend			0.587		0.628
Fe(μg/L)	Q1(≤191.74)	1.00(Ref)		1.00(Ref)	
	Q2(191.74-394.96)	1.02(0.85-1.38)	0.716	1.11(0.81-1.22)	0.544
	Q3(394.96-695.34)	0.88(0.84-1.21)	0.436	1.05(0.83-1.20)	0.328
	Q4(>695.34)	1.05(0.87-1.42)	0.608	1.25(0.98-1.38)	0.068
P for trend			0.853		0.064
Hg(μg/L)	Q1(≤0.09)	1.00(Ref)		1.00(Ref)	
	Q2(0.09-0.50)	1.04(0.83-1.26)	0.427	1.08(0.88-1.21)	0.579
	Q3(0.50-1.40)	1.04(0.96-1.25)	0.057	1.15(0.99-1.26)	0.064
	Q4(>1.40)	1.15(0.93-1.37)	0.423	1.14(0.82-1.59)	0.671
P for trend			0.751		0.621
Mn(μg/L)	Q1(≤1.36)	1.00(Ref)		1.00(Ref)	
	Q2(1.36-4.70)	0.99(0.81-1.31)	0.479	1.02(0.72-1.25)	0.769
	Q3(4.70-10.32)	1.18(0.93-1.25)	0.257	1.23(1.02-1.29)	0.044
	Q4(>10.32)	1.21(1.17-1.33)	0.004	1.07(1.10-1.36)	0.006
P for trend			<0.001		<0.001
Pb(μg/L)	Q1(≤1.38)	1.00(Ref)		1.00(Ref)	
	Q2(1.39-3.31)	1.10(0.80-1.12)	0.574	0.94(0.87-1.12)	0.781
	Q3(3.31-6.63)	1.13(1.05-1.24)	0.031	1.11(0.96-1.22)	0.066
	Q4(>6.63)	1.14(0.90-1.20)	0.183	1.09(0.88-1.31)	0.613
P for trend			0.185		0.488
V(μg/L)	Q1(≤19.29)	1.00(Ref)		1.00(Ref)	
	Q2(19.29-37.53)	0.89(0.86-0.91)	0.014	0.94(0.85-0.98)	0.026
	Q3(37.53-60.48)	0.93(0.85-0.99)	0.069	0.91(0.87-0.99)	0.019

Table 4 (continued)

Metal	Grouping	*PR*(*95%CI*)			
		Model I	*P-value*	Model II	*P-value*
	Q4(>60.48)	0.91(0.82-0.99)	0.034	0.88(0.85-0.95)	0.047
P for trend			0.077		0.298
Zn(μg/L)	Q1(≤422.40)	1.00(Ref)		1.00(Ref)	
	Q2(422.40-660.34)	1.03(0.92-1.18)	0.582	1.00(0.99-1.06)	0.863
	Q3(660.34-968.39)	1.14(0.98-1.31)	0.339	0.96(0.92-1.16)	0.562
	Q4(>968.39)	1.19(0.99-1.37)	0.674	1.03(0.90-1.12)	0.901
P for trend			0.876		0.283

P for trend across quartiles of metals was obtained by including the median of each quartile as a continuous variable in the regression models

Model I: non-adjusted model; Model II: model adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity

Ref Reference, *PR* Prevalence ratio, *CI* Confidence interval, *As* Arsenic, *Cd* Cadmium, *Co* Cobalt, *Cr* Chromium, *Cu* Copper, *Fe* Iron, *Hg* Mercury, *Mn* Manganese, *Pb* Lead, *V* Vanadium, *Zn* Zinc

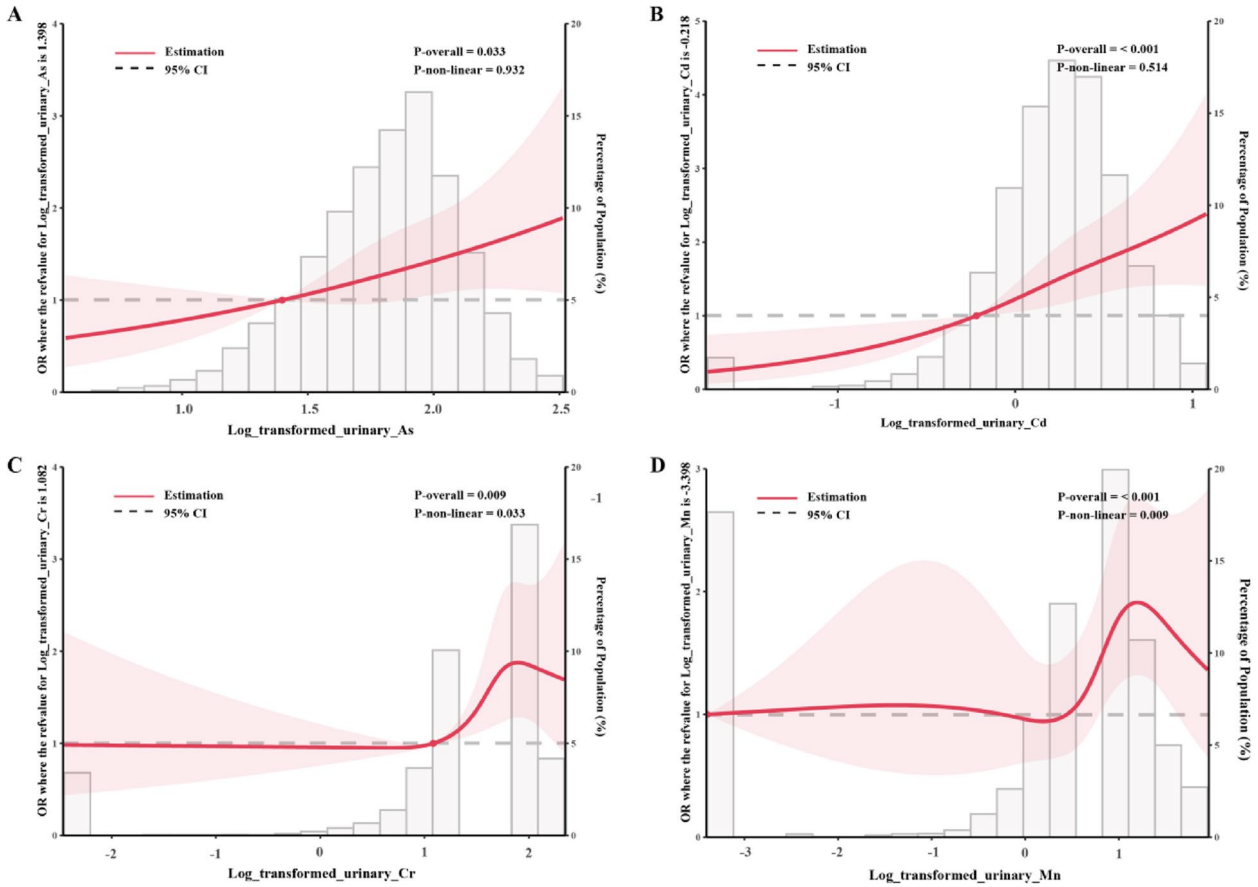

Fig. 4 Restricted cubic spline plots of urinary metals and the risk of osteoporosis. Log-transformed urinary metal concentrations; adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity. OR, odds ratio; CI, confidence interval; As, arsenic; Cd, cadmium; Cr, chromium; Mn, manganese

with metalloproteins [44]. Bone tissues are one of the major depositional sites for Mn [45]; however, the results across different studies are not consistent. Women with OP have been reported to exhibit lower serum Mn levels compared with those with normal bone density. Conversely, two studies based on the National Health and Nutrition Examination Survey (NHANES) data reported a negative correlation between blood Mn levels and

Table 5 Quantile g-computation-based assessment of the association of metal mixture exposure with osteopenia and osteoporosis

	OR(95%CI)	*P*-value
Osteopenia	1.59(1.28,1.98)	< 0.001
Osteoporosis	1.98(1.21,3.25)	0.006

Adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity

OR Odds ratio, *CI* Confidence interval

BMD, both in adolescents and adults aged > 18 years [46, 47]. Similarly, another cross-sectional study on Mn exposure in occupational groups showed a negative correlation between blood Mn levels and bone density [48]. The results of this study indicate that Mn contributes to osteopenia and an increased risk of OP, with single-metal exposure outcomes aligning with those of metal mixture exposure.

Single-metal exposure of Fe and Hg was found to be positively correlated with the risk of osteopenia and OP. Hg, which exists in various forms such as metallic, inorganic compound, and organic compounds, exhibited a high affinity for the sulfhydryl groups of proteins, similar to Cd [49]. The results of the present research on Hg and OP are inconsistent. Hg compounds have been reported to interfere with enzymatic and hormonal reactions in the human body. Blood Hg levels have been significantly correlated with bone density in adolescents aged 12–19 years. Consistent with another study based on the United States NHANES database [50], an inverse correlation has been reported between blood Hg levels and spinal bone density in adults, with low blood Hg levels (< 3 μg/L), leading to an increased risk of OP [51]. Another meta-analysis found no association between Hg exposure and the risk of osteopenia or OP [52]. This is inconsistent with the results of the present study, possibly because of the differences in the selected biological samples and detection methods. Fe is one of the trace elements necessary for life activities and the maintenance of various physiological functions of the human body. Cellular Fe is mainly stored in cells in the form of Fe^{2+} (binds to proteins and participates in various physiological reactions) or Fe^{3+} (the main form of iron transport in the human body) [53]. Fe metabolism-associated disorders, including Fe deficiency and Fe overload, can lead to OP. Zhao et al. [54] reported that excess Fe inhibited the activity of osteoblasts, mild Fe deficiency promoted bone cell activity, and severe Fe deficiency decreased bone levels. Patients with a history of Fe deficiency and anemia have been reported to be at a higher risk of OP (approximately twice the risk) compared with patients without Fe deficiency and anemia [55]. Fe overload is one of the main characteristics of ferroptosis [56]. In 2012, Dixo et al. [57] first reported a type of Fe ion-dependent programmed cell death, which is morphologically different from apoptosis, autophagy, and necrosis. Presently, ferroptosis-related mechanisms and signaling pathways in OP are not fully understood. Fe overload has been shown to reduce cell viability, superoxide dismutase levels, and glutathione levels; increase reactive oxygen species production, lipid peroxidation, malondialdehyde levels, ferroptosis-related protein expression; and induce ultrastructural changes

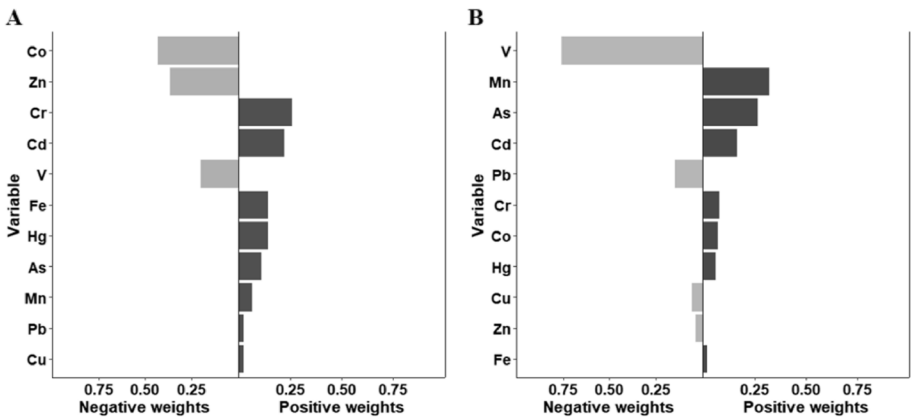

Fig. 5 Weights representing the proportion of the positive or negative partial effect for each metal in the quantile g-computation model with all metals. **A** The correlation of metal mixture exposure with osteopenia. **B** The correlation of metal mixture exposure with osteoporosis. Translogarithmic conversion was performed on urinary metal concentrations. Models were adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, the prevalence of hypertension, the prevalence of diabetes, history of fractures, body–mass index, and physical activity. As, arsenic; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Hg, mercury; Mn, manganese; Pb, lead; V, vanadium; Zn, zinc

Table 6 Sensitivity analysis of the association between urinary metal concentrations and osteopenia

Metals	Grouping	PR(95%CI)			
		Model I	*P*-value	Model II	*P*-value
As(μg/L)	Q1(≤ 43.03)	1.00(Ref)		1.00(Ref)	
	Q2(43.03–69.62)	0.88(0.81,0.99)	< 0.001	1.32(1.13,1.54)	0.006
	Q3(69.62–102.64)	0.91(0.83,1.16)	0.064	1.36(1.16,1.58)	< 0.001
	Q4(> 102.64)	1.03(0.87,1.18)	0.079	1.40(1.19,1.64)	< 0.001
P for trend			0.028		< 0.001
Cd(μg/L)	Q1(≤ 1.10)	1.00(Ref)		1.00(Ref)	
	Q2(1.10–1.86)	0.86(0.80,0.92)	0.033	1.19(1.00,1.30)	0.001
	Q3(1.86–3.00)	0.98(0.84,1.10)	0.738	1.24(1.15,1.29)	0.004
	Q4(> 3.00)	1.00(0.87,1.12)	0.953	1.26(1.08,1.34)	0.002
P for trend			0.041		< 0.001
Co(μg/L)	Q1(≤ 0.20)	1.00(Ref)		1.00(Ref)	
	Q2(0.20–0.41)	1.04(0.95,1.20)	0.411	1.01(0.96,1.18)	0.868
	Q3(0.41–0.72)	1.19(1.04,1.27)	0.039	0.96(0.92,1.03)	0.499
	Q4(> 0.72)	1.12(0.98,1.29)	0.113	0.95(0.90,1.04)	0.114
P for trend			0.187		0.129
Cr(μg/L)	Q1(≤ 25.68)	1.00(Ref)		1.00(Ref)	
	Q2(25.68–41.78)	0.96(0.94,0.99)	0.029	1.10(0.94,1.18)	0.241
	Q3(41.78–64.54)	0.92(0.91,0.96)	< 0.001	1.13(1.10,1.22)	0.042
	Q4(> 64.54)	0.95(0.94,0.99)	0.037	1.13(1.03,1.19)	0.023
P for trend			< 0.001		< 0.001
Cu(μg/L)	Q1(≤ 28.98)	1.00(Ref)		1.00(Ref)	
	Q2(28.98–86.62)	0.97(0.88,1.16)	0.274	1.06(0.92,1.24)	0.460
	Q3(86.62–219.63)	1.05(0.94,1.26)	0.440	1.09(0.95,1.24)	0.377
	Q4(> 219.63)	1.04(0.92,1.20)	0.196	1.09(0.94,1.28)	0.469
P for trend			0.738		0.322
Fe(μg/L)	Q1(≤ 201.47)	1.00(Ref)		1.00(Ref)	
	Q2(201.47–407.08)	0.94(0.84,1.02)	0.063	0.99(0.85,1.15)	0.675
	Q3(407.08–706.98)	0.86(0.81,0.96)	0.007	1.12(0.96,1.30)	0.151
	Q4(> 706.98)	0.89(0.87,1.03)	0.116	1.19(1.02,1.29)	0.017
P for trend			0.047		0.025
Hg(μg/L)	Q1(≤ 0.09)	1.00(Ref)		1.00(Ref)	
	Q2(0.09–0.50)	0.89(0.77,1.03)	0.110	1.03(1.00,1.14)	0.036
	Q3(0.50–1.40)	1.05(0.91,1.21)	0.510	1.14(0.88,1.23)	0.094
	Q4(> 1.40)	0.95(0.82,1.09)	0.438	1.12(1.05,1.42)	0.021
P for trend			0.341		0.322
Mn(μg/L)	Q1(≤ 1.39)	1.00(Ref)		1.00(Ref)	
	Q2(1.39–4.85)	1.01(0.95,1.19)	0.785	1.06(0.91,1.24)	0.448
	Q3(4.85–10.47)	1.02(0.87,1.20)	0.610	1.13(0.97,1.31)	0.412
	Q4(> 10.47)	0.99(0.82,1.15)	0.698	1.26(1.08,1.36)	0.003
P for trend			0.556		0.002
Pb(μg/L)	Q1(≤ 1.32)	1.00(Ref)		1.00(Ref)	
	Q2(1.32–3.28)	0.90(0.89,1.06)	0.478	0.97(0.83,1.13)	0.661
	Q3(3.28–6.60)	0.92(0.84,1.09)	0.652	1.03(0.89,1.20)	0.665
	Q4(> 6.60)	0.98(0.95,1.15)	0.820	1.04(0.89,1.21)	0.619
P for trend			0.561		0.459
V(μg/L)	Q1(≤ 19.54)	1.00(Ref)		1.00(Ref)	
	Q2(19.54–37.97)	0.96(0.83,1.10)	0.264	0.96(0.80,1.15)	0.470
	Q3(37.97–60.97)	1.11(0.95,1.28)	0.222	0.92(0.84,1.12)	0.292

Table 6 (continued)

Metals	Grouping	PR(95%CI)			
		Model I	*P*-value	Model II	*P*-value
	Q4(> 60.97)	1.13(0.97,1.25)	0.879	0.85(0.77,1.07)	0.873
P for trend			0.496		0.236
Zn(μg/L)	Q1(≤ 402.89)	1.00(Ref)		1.00(Ref)	
	Q2(402.89–636.52)	1.03(0.88,1.20)	0.566	0.81(0.89,0.95)	0.031
	Q3(636.52–945.42)	0.98(0.85,1.14)	0.383	0.85(0.81,0.89)	< 0.001
	Q4(> 945.42)	0.95(0.82,1.09)	0.458	0.81(0.80,0.94)	< 0.001
P for trend			0.667		0.001

P for trend across quartiles of metals was obtained by including the median of each quartile as a continuous variable in the regression models

Model I: non-adjusted model; Model II: model adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity

Ref Reference, *PR* Prevalence ratio, *CI* Confidence interval, *As* Arsenic, *Cd* Cadmium, *Co* Cobalt, *Cr* Chromium, *Cu* Copper, *Fe* Iron, *Hg* Mercury, *Mn* Manganese, *Pb* Lead, *V* Vanadium, *Zn* Zinc

in mitochondria [58]. Reportedly, Fe overload can cause osteoblast apoptosis [59].

As and Cr were positively correlated with the risk of osteopenia and OP on single-metal exposure and presented a positive weight in Qgcomp. Cr is a naturally occurring heavy metal present in the crust of the Earth and seawater and is widely used in industrial processes. It exhibits multiple oxidation states from −2 to +6, with Cr^{3+} and Cr^{6+} being the most stable and commonly occurring forms [60]. Cr^{6+} is a strong carcinogen and is involved in various diseases and pathologies [61]. Cr^{3+}, in trace amounts, is crucial for natural lipid and protein metabolisms, and it is an important component of the glucose tolerance factor [62] and enhances insulin activity [63]. Sankaramanivel et al. showed that in vivo accumulation of Cr^{6+} in the femur of rats led to a systemic decrease in alkaline phosphatase and tartrate-resistant acid phosphatase, affecting both bone formation and resorption [64]. In vitro studies have shown that osteoblasts absorb Cr^{6+} through membrane transporters, rapidly reducing them to Cr^{3+}, leading to increased reactive oxygen species, oxidative stress, and DNA damage [65, 66]. In an animal experiment, the effect of Cr^{3+} on bone density has been reported to be similar to that of low-dose estradiol; the effect of estradiol on bone density is dose-related and can be modified by Cr^{3+} [67]. Exposure to As can increase the risk of various bone diseases. Chronic exposure to low-level As can induce bone resorption by promoting osteoclast differentiation. Following low-level As exposure, osteoclast precursor cells generate hydrogen peroxide, leading to their differentiation into cells that disintegrate the bone matrix [68]. Moreover, As has been reported to induce apoptosis in osteoblast cell lines (including hFOB, MC3T3-E1, and MG-63) and mouse bone marrow stromal cells (M2-10B4), while triggering endoplasmic reticulum stress [69]. Akbal et al. associated As exposure with bone loss in men [70]. These results are consistent with the findings of this study, indicating that exposure to the abovementioned metals increases the risk of osteopenia and OP.

V, Zn, and Co were positively correlated with BMD, aligning with the findings of previous studies highlighting their correlative roles in bone formation. The element V belongs to group VB, and V-incorporating compounds can emulate the biological functions of insulin and growth factors, exhibiting various osteogenic effects related to extracellular matrix and collagen formation in bone cells, thus, promoting the osteogenic activity [71]. In an animal study, V exhibited hypoglycemic properties and improved the bone condition of patients with diabetes [72]. Zn is a trace element essential for the normal growth of human and animal bones. Zn concentrations have been strongly associated with OP in elderly people with proximal femur fractures, suggesting its key role in bone development and maintenance of bone mass [73]. It can stimulate runt-related transcription factor 2 and promote osteoblast differentiation [74]. In contrast, excess Zn can induce osteoclast apoptosis [75]. Herein, Zn played a protective role in osteopenia but could not exhibit a considerable protective effect against OP, possibly because of the differences in urinary Zn concentrations. Co is commonly used in bone and joint implants and may exert its potential mechanism on bone formation by inducing hypoxia-induced factor-1α [76]. However, it is noteworthy that in Qgcomp, Co exhibited different directional weights in association with osteopenia and OP. This suggests that metals may play distinct roles at different stages of the disease; further studies are warranted for verification.

In the metal mixture exposure model, a marked positive association between 11 metal mixtures and the risk of osteopenia and OP was found in the Dong and Miao populations. The association between metal mixtures and bone health has been reported previously. Exposure

Table 7 Sensitivity analysis of the association between urinary metal concentrations and osteoporosis

Metals	Grouping	PR(95%CI)			
		Model I	*P*-value	Model II	*P*-value
As(μg/L)	Q1(≤ 43.03)	1.00(Ref)		1.00(Ref)	
	Q2(43.03–69.62)	0.95(0.92,0.99)	0.032	1.04(0.94,1.17)	0.420
	Q3(69.62–102.64)	0.95(0.86,1.08)	0.349	1.06(0.99,1.19)	0.216
	Q4(> 102.64)	1.05(0.93,1.16)	0.561	1.18(0.99,1.21)	0.106
P for trend			0.060		0.079
Cd(μg/L)	Q1(≤ 1.10)	1.00(Ref)		1.00(Ref)	
	Q2(1.10–1.86)	0.99(0.97,1.23)	0.675	1.17(1.11,1.28)	0.023
	Q3(1.86–3.00)	1.03(0.97,1.20)	0.083	1.21(1.03,1.31)	< 0.001
	Q4(> 3.00)	1.03(0.98,1.19)	0.477	1.25(1.06,1.33)	0.014
P for trend			0.627		0.034
Co(μg/L)	Q1(≤ 0.20)	1.00(Ref)		1.00(Ref)	
	Q2(0.20–0.41)	1.03(0.99,1.12)	0.361	1.01(0.86,1.31)	0.517
	Q3(0.41–0.72)	1.06(0.98,1.17)	0.659	0.96(0.82,1.03)	0.276
	Q4(> 0.72)	1.08(1.00,1.21)	0.034	0.95(0.90,1.04)	0.331
P for trend			0.287		0.682
Cr(μg/L)	Q1(≤ 25.68)	1.00(Ref)		1.00(Ref)	
	Q2(25.68–41.78)	0.87(0.84,0.91)	0.019	1.12(0.95,1.25)	0.068
	Q3(41.78–64.54)	0.87(0.80,1.05)	0.674	1.14(1.09,1.24)	0.030
	Q4(> 64.54)	0.85(0.82,1.05)	0.806	1.12(1.08,1.27)	0.041
P for trend			0.036		0.012
Cu(μg/L)	Q1(≤ 28.98)	1.00(Ref)		1.00(Ref)	
	Q2(28.98–86.62)	0.88(0.85,1.06)	0.795	0.92(0.80,1.31)	0.666
	Q3(86.62–219.63)	0.90(0.85,1.07)	0.709	0.95(0.82,1.35)	0.921
	Q4(> 219.63)	1.02(0.92,1.05)	0.286	0.85(0.84,1.22)	0.478
P for trend			0.866		0.214
Fe(μg/L)	Q1(≤ 201.47)	1.00(Ref)		1.00(Ref)	
	Q2(201.47–407.08)	1.01(0.82,1.11)	0.582	1.04(0.99,1.17)	0.847
	Q3(407.08–706.98)	1.10(0.86,1.16)	0.438	0.95(0.91,1.17)	0.190
	Q4(> 706.98)	0.91(0.57,1.07)	0.889	1.00(0.94,1.15)	0.989
P for trend			0.107		0.460
Hg(μg/L)	Q1(≤ 0.09)	1.00(Ref)		1.00(Ref)	
	Q2(0.09–0.50)	1.01(0.85,1.06)	0.309	1.08(0.99,1.18)	0.364
	Q3(0.50–1.40)	0.99(0.84,1.03)	0.136	1.01(0.99,1.16)	0.215
	Q4(> 1.40)	1.08(0.81,1.12)	0.910	1.07(0.96,1.11)	0.769
P for trend			0.785		0.557
Mn(μg/L)	Q1(≤ 1.39)	1.00(Ref)		1.00(Ref)	
	Q2(1.39–4.85)	0.85(0.83,1.06)	0.667	0.91(0.81,1.30)	0.385
	Q3(4.85–10.47)	0.97(0.96,0.99)	0.050	1.14(0.80,1.22)	0.460
	Q4(> 10.47)	0.97(0.91,1.17)	0.619	1.16(0.96,1.23)	0.222
P for trend			0.452		0.061
Pb(μg/L)	Q1(≤ 1.32)	1.00(Ref)		1.00(Ref)	
	Q2(1.32–3.28)	1.08(0.97,1.15)	0.793	1.06(0.88,1.04)	0.156
	Q3(3.28–6.60)	1.15(0.99,1.20)	0.248	1.46,0.96,1.09)	0.081
	Q4(> 6.60)	1.12(0.93,1.17)	0.777	0.99(0.96,1.11)	0.654
P for trend			0.492		0.391
V(μg/L)	Q1(≤ 19.54)	1.00(Ref)		1.00(Ref)	
	Q2(19.54–37.97)	1.28(0.99,1.22)	0.132	0.88(0.79,0.90)	0.028
	Q3(37.97–60.97)	1.21(0.99,1.25)	0.538	0.82(0.80,0.92)	0.035

Table 7 (continued)

Metals	Grouping	PR(95%CI)			
		Model I	*P*-value	Model II	*P*-value
	Q4(> 60.97)	1.19(0.95,1.25)	0.967	0.94(0.87,0.96)	0.001
P for trend			0.060		0.007
Zn(μg/L)	Q1(≤ 402.89)	1.00(Ref)		1.00(Ref)	
	Q2(402.89–636.52)	0.92(0.90,1.15)	0.736	0.81(0.80,1.11)	0.517
	Q3(636.52–945.42)	0.92(0.90,1.06)	0.661	0.82(0.78,1.03)	0.634
	Q4(> 945.42)	0.93(0.89,1.08)	0.110	0.89(0.82,1.15)	0.159
P for trend			0.027		0.83

P for trend across quartiles of metals was obtained by including the median of each quartile as a continuous variable in the regression models

Model I: non-adjusted model; Model II: model adjusted for age, sex, ethnicity, education, marital status, annual household income, smoking, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity

Ref reference, *PR* prevalence ratio, *CI* confidence interval, *As* arsenic, *Cd* cadmium, *Co* cobalt, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Pb* lead, *V* vanadium, *Zn* zinc

Table 8 Quantile g-computation-based assessment of the association of metal mixture exposure with osteopenia and osteoporosis

	OR(95%CI)	*P*-value
Osteopenia	1.51(1.28,1.78)	< 0.001
Osteoporosis	1.31(0.61,2.81)	0.490

Adjusted for age, sex, ethnicity, education, marital status, annual household income, drinking, tea consumption, hypertension, diabetes, history of fractures, body–mass index, and physical activity

OR Odds ratio, *CI* Confidence interval

to a mixture of nine metals (namely Ca, Cd, Co, Pb, Mg, rubidium [Rb], strontium, V, and Zn) has been shown to be negatively associated with the risk of OP in elderly Chinese women, with Rb and V being the primary contributors [77]. The NHANES study, which collected data from adults aged ≥ 20 years, associated the simultaneous exposure to a mixture of Cd, Pb, Hg, Mn, Cu, selenium (Se), and Zn with reduced bone density, with Pb, Mn, and Se being the main contributors [78]. Partially consistent with these findings, herein, V exhibited a protective effect on OP, whereas Mn contributed to OP development. Nevertheless, discrepancies among various studies persist, primarily stemming from variations in the study population, the metals included, the biological samples used for testing, and the statistical methods applied. Notably, in Qgcomp, the metals exhibited different directional weights at different stages of the disease. For instance, Pb exhibited a positive weight in association with osteopenia but a negative weight for OP, suggesting that metal may exert differing effects at different stages of the disease. Therefore, further research is essential to

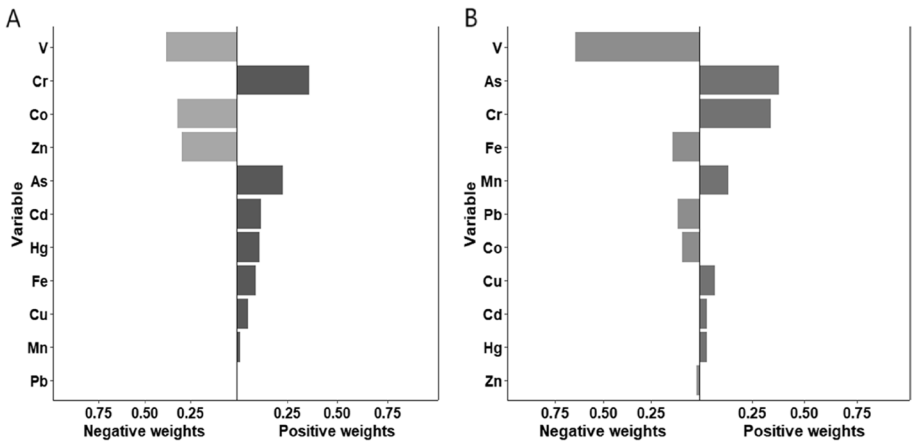

Fig. 6 Sensitivity analysis of the quantile g-computation model with weighted indices for each metal. **A** Correlation of metal mixture exposure with osteopenia. **B** Correlation of metal mixture exposure with osteoporosis. Translogarithmic conversion was performed on urinary metal concentrations. Models were adjusted for age, sex, ethnicity, education, marital status, annual household income, drinking, tea consumption, the prevalence of hypertension, the prevalence of diabetes, history of fractures, body–mass index, and physical activity. As, arsenic; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Hg, mercury; Mn, manganese; Pb, lead; V, vanadium; Zn, zinc

comprehensively analyze the relationship between metal mixture exposure and bone health.

Additionally, the potential inverse causal relationship between urinary metal concentrations and the risk of osteopenia and OP warrants further investigation. For instance, Cd exposure in humans is primarily assessed using Cd concentrations in the blood and urine as biomarkers, where urinary Cd primarily reflects Cd accumulation in the kidneys and indicates renal damage and OP in later stages and blood Cd indicates acute exposure. Both blood and urinary concentrations are crucial for determining the bone density. Urinary Cd concentrations have been suggested as a more reliable risk factor for OP and osteopenia compared with blood Cd concentrations [33]. However, presently, evidence regarding whether cadmium induces OP or if OP leads to increased Cd excretion is inconclusive. Consequently, large-scale, high-quality prospective studies are needed to clarify the relationship between metal concentrations, OP, and bone loss, along with the exploration of the underlying complex physiological mechanisms.

This study boasts several notable strengths. Firstly, the baseline survey data from the CMEC study was utilized to ensure that information was gathered by trained investigators. Furthermore, stringent quality control measures were implemented during questionnaire administration, physical examinations, and laboratory testing, enhancing the reliability of the collected data. Finally, Qgcomp was applied to examine the effect of metal mixture exposure on BMD. However, certain limitations of this study must be acknowledged. The cross-sectional design of this study hinders the establishment of a causal relationship between urinary metal concentrations and BMD. Furthermore, herein, metal concentrations were evaluated in only sentinel urine samples, neglecting potential external metal exposures from the environment and other biological specimens such as the plasma and hair. Consequently, future research should address these limitations and focus on establishing a causal relationship between metal concentrations and BMD, along with investigating the holistic effects of metal exposure from multiple sources.

Conclusion

In conclusion, this cross-sectional study involving participants aged 30–79 years from the Chinese Dong and Miao communities showed that exposure to metal mixtures is positively associated with the prevalence of osteopenia and OP in these ethnic minorities. Considering the ubiquitous nature of metal exposure in daily life and the notable health implications of OP and osteopenia, follow-up studies in large-scale cohort is warranted to elucidate the potential biological mechanisms underlying the observed associations between metal mixture exposure and abovementioned bone diseases.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
All authors contributed to the study conception and design. Conceptualization, YZ; methodology, YZ and CC; investigation, SW and YZ; experiments, SW and JZ; statistical analysis YZ, CN, and YH; writing original draft preparation, YZ; writing—review and editing, YZ, YH, and FH; administration, FH; all authors have read and agreed to the published version of the manuscript.

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Data availability
The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Sichuan University Medical Ethical Review Board (K2016038) and the Research Ethics Committee of The Affiliated Hospital of Guizhou Medical University (2018[094]). Informed consent was obtained from all subjects involved in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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