Associations of blood metals and metal mixtures with myocardial enzyme profile: an occupational population-based study in China

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Abstract

To investigate cross-sectional association between blood metal mixture and myocardial enzyme profile based on an occupational population. We determined creatine kinase (CK) and creatine kinase-MB (CK-MB), lactate dehydrogenase (LD), α-hydroxybutyrate dehydrogenase (α-HBD) and aspartate transaminase (AST) among participants from the manganese-exposed workers healthy cohort (n = 544). Levels of 22 metals in blood cells were determined using inductively coupled plasma mass spectrometry. Least absolute shrinkage and selection operator (LASSO) penalized regression model was utilized for metals screening. Exposure-response relationship between specific metal and myocardial enzyme profile was identified by general liner regression and restricted cubic spline analyses. The overall effect and interactions were evaluated with Bayesian kernel machine regression (BKMR). Manganese was linearly and positively associated with CK ($P_{\text{overall}} = 0.019$, $P_{\text{non-linearity}} = 0.307$), dominating positive overall-effect of mixture exposure (manganese, arsenic and rubidium) on CK level; calcium and zinc were linearly negative associated with LD level ($P_{\text{overall}} < 0.05$, $P_{\text{non-linearity}} > 0.05$) and asserted dominance in the negative overall-effect of metal mixtures (Rubidium, molybdenum, zinc, nickel, cobalt, calcium and magnesium) on LD level. It was interesting to note a “U” shape dose-response relationship of molybdenum with LD level ($P_{\text{overall}} < 0.001$, $P_{\text{non-linearity}} = 0.488$). There was an interaction between age and calcium on LD level ($P_{\text{interaction}} = 0.041$). Besides, there was an interaction between smoking and molybdenum on LD level ($P_{\text{interaction}} = 0.035$). Our study provides evidence that metal mixture exposure affects myocardial enzyme profile. Additional investigations are required to confirm these associations and reveal the fundamental mechanisms involved.

1. Introduction

In terms of mortality, cardiovascular disease (CVD) continues to dominate, despite considerable advances in prevention, diagnosis and treatment (Lim et al., 2012). A dramatic increase was observed in prevalence of CVD, especially in developing countries with low or middle incomes owing to increasing exposure to risk factors related to economic development and rapid, unplanned urbanization. Environmental exposure, such as metals, which an individual is less able to control, can greatly influence development and severity of CVD. Increasing evidence suggests that metals contribute to coronary heart disease, stroke and peripheral artery disease (Bhatnagar, 2006; Cosselman, Navas-Acien, & Kaufman, 2015; Lamas, Navas-Acien, Mark, & Lee, 2016; Navas-Acien, Guallar, Silbergeld, & Rothenberg, 2007; Nigra, Ruiz-Hernandez, Redon, Navas-Acien, & Tellez-Plaza, 2016). Metals, such as lead, cadmium, and arsenic, are considered as the top ten environmental chemicals by the WHO, contributing to the development and progression of cardiovascular disease. Cardiotoxicity is caused by metals inducing key pathophysiological processes for cardiovascular disease, such as oxidative stress, inflammation, nitric oxide bioavailability decrease and cardiac autonomic function alteration.

Increasing evidence suggests a link between metal exposure and cardiac disease, with conduction changes, and altered heart rate variability observed among individual exposed to lead from live or
occupation (Floresi et al., 2014; Kieltucki et al., 2017; Madan, Sharma, Makharia, Poojary, & Deepak, 2007). Chronic exposure to cadmium was linked to hypertension thereby promoting atherosclerosis and impairing cardiac function (Vallee, Gabet, Grave, Blacher, & Olie, 2020). Moon et al. found a significant prolongation of QT interval related to arsenic exposure (n = 1174), leading to malignant arrhythmias (Moon et al., 2018). In addition, arsenic exposure was demonstrated to increase incidence of heart disease among a large strong heart study (Moon et al., 2013). The myocardial enzyme showed sensitivity and specificity over 90% in identifying myocardial injury (Emokpae & Nwagbara, 2017). Moreover, evidence emerging and illustrated the relationship between serum level of creatine kinase (CK) and myocardial damage (Savonitto et al., 2002). Elevated levels of marker enzymes in serum were indicative of myocardial damage (Akila & Vennila, 2016; Priscilla & Prince, 2009; Radhiga, Rajamanickam, Senthil, & Pugalendi, 2012; Saravanan, Ponmurugan, Sathiyavathi, Vadivukkarasi, & Sengottuvelu, 2013; Steen et al., 2006).

Together with the previous study of our team suggesting that manganese was associated with increased CK level (Huang et al., 2020). It suggests that manganese may be associated with cardiotoxicity, but the specific dose-response relationship in the population remain unclear. Further, we should not neglect the reality of mixed exposure to metals. In this study, the goal was to examine association between metal mixture exposure and cardiac injury in a larger and well-characterized group of manganese-exposed individuals.

2. Materials and methods

2.1 Participants in the study

Participants were from manganese-exposed workers healthy cohort (MEWHC). Additional information was provided previously on this cohort (Lv et al 2014; Zhou et al 2018). Current study was based on occupational health examination conducted in 2021. Face-to-face interview was carried out to gather demographic information, lifestyle information, such as smoking habit, alcohol consumption. In total, 558 individuals were selected from the typical occupation out of the 708 participants. Criteria for exclusion: 1) outliers whose concentrations of metals or myocardial enzyme profile higher than their three times 99th (n = 3); 2) with heart disease, stroke, hyperthyroidism, hypothyroidism, cirrhosis, hepatitis B, chronic hepatitis or liver disease (n = 9); 3) lacked data on myocardial enzyme profile (n = 2). In the end, the current analysis was conducted among the remaining 544 individuals.

Before donating blood, participants were asked to fast overnight. Samples were stored frozen (-80°C). Informed consent forms were signed by all participants. There was approval for this study from the Guangxi Medical University medical ethics committee.

2.2 Metal assessments

A previous protocol was followed to determine 22-metal levels in blood cells (He et al 2022; Xiao et al 2021). The LOD values for 22 metals were (0.001–2.020) µg/L. Metal concentrations lower than LOD
values were estimated by dividing the LOD values by the square root of 2.

2.3 Myocardial enzyme profile assessment

We also detected creatine kinase (CK), creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), lactic dehydrogenase (LD), α-hydroxybutyrate dehydrogenase (α-HBD) levels with a clinical chemistry automatic analyser (Hitachi 7600-020, Kyoto, Japan).

2.4 Covariates

Seniority refers to working years of participants in their occupations. The definition of smoking habit and alcohol consumption were referred to previous study (He et al 2022).

2.5 Statistical analysis

An analysis of descriptive statistics was conducted for describing general and clinical characteristics of participants using medians (\(P_{25}, P_{75}\)) or frequencies (%). Considering the skewed distribution of metals in blood cells, data was log10-transformed, representing as medians (\(P_{25}, P_{75}\)). Metal correlations were evaluated using Spearman's rank-order correlation analysis.

We employed LASSO regression to identify metals in blood cells independently associated with myocardial enzyme parameters. In the LASSO method, the increased in penalty parameter lambda (\(\lambda\)) leads to a more severe penalization of variables. Consequently, a model with fewer coefficients approaching zero was left. Covariates including gender (male, female), age (continuous variable), length of service (continuous variable), BMI (continuous variable), education level (high school and above, middle school and below), alcohol consumption (yes, no), smoking (yes, no) and hypertension (yes, no) were entered into LASSO model without penalty. Using 10-fold cross-validation, we generated an optimal model based on \(\lambda\), where the minimum mean square error (MSE) was observed.

Additionally, we utilized a general linear model (GLM) to examine relationships between metals screened in LASSO and myocardial enzyme profile. For metals with statistical significance in GLM model (continuous variable), the dose-response relationship between metals and myocardial enzymes was evaluated with restricted cubic spline regression. Nodes were set at 10th, 50th and 90th percentiles with the reference kept at median. In addition, we analysed the association of blood cell metals with myocardial enzymes in a stratified analysis of the total population by age, length of service, BMI, alcohol consumption, and smoking, and further analysed the interaction of these demographic variables with metals affecting myocardial enzymes.

Increasingly, Bayesian kernel machine regression (BKMR) has been used to estimate health effects of mixture exposure. To assess the overall impact of metal mixtures at particular quantiles, the effects of metal mixtures were estimated by comparing from their respective 50th percentiles. The alteration in the estimated impact on each cardiac enzyme due to one metal transitioning from its 25th to 75th percentile while the other metals remain at their 25th, 50th, or 75th percentile, respectively.
All data analysis was performed using SPSS 26.0 and R 4.0.3. All statistical tests were bilateral probability tests, and \( P < 0.05 \) was considered statistically significant.

3. Results

3.1 Demographic and clinical characteristics of participants

The characteristics of the 544 participants are outlined in Table 1, including their demographic and clinical information. Most participants were married or co-habitant. More than half of the participants appeared to be overweight (55.7%). Participants appeared to have a high school education level or above. Most of the participants were current drinkers (75.7%) and current smokers accounted for 47.4%.

3.2 Metal concentrations in blood cells

Except aluminum, the quantitative linear \( R^2 \) of the other 21 metal elements was more than 0.999, which was qualified. Therefore, aluminum was not included in the subsequent analysis in this study. More than 80% of participants had concentrations of 21 metals above the corresponding detectable limits. Table S1 summarizes metals concentrations in blood cells by median along with \( (P_{25}, P_{75}) \) and Fig. S1 presents correlations.

3.3 Associations between metals and myocardial enzyme profile in multi-pollutant model

To screen metal, LASSO model was utilized where putting non-penalization on covariates. A 10-fold cross-validation function was used to select the optimal model where the MSE was the lowest (Fig. 1). For the purpose of investigating the correlations with myocardial enzyme profile, we incorporated all the metals chosen by LASSO into the GLM model.

3.4. Associations between metals and myocardial enzyme profile in multi-metal model

We observed each one-unit increase in log-transformed Mn in blood cells was associated with 50.60 U/L (95% confidence interval (CI) 3.06 to 98.14) increase in CK, after adjusting for potential confounders. We observed each one-unit increase in log-transformed Mo in blood cells were associated with \(-26.28\) U/L (95%CI: -43.04 to -9.52) decrease in LD and \(-14.62\) U/L (95%CI: -27.32 to -1.92) in \( \alpha \)-HBD. Categorical variable analysis also showed significant LD and \( \alpha \)-HBD decrease for Mo in blood cells \( (P_{trend} = 0.004 \) and \( 0.012 \), respectively). We observed each one-unit increase in log-transformed Ca in blood cells was associated with \(-36.65\) U/L (95%CI: -64.03 to -9.27) change in LD. Further, the categorical variable analysis also showed significant LD decrease for Ca in blood cells \( (P_{trend} = 0.005) \). We observed each one-unit increase in log-transformed Zn in blood cells was associated with \(-66.84\) U/L (95% CI: -120.86 to -12.82) change in LD. Further, the categorical variable analysis also showed significant LD decrease for Zn in blood cells \( (P_{trend} = 0.020) \) (Table 2).
3.5 Dose-response relationship between selected metal and Myocardial enzyme profile

The dose-response relationship between metals and myocardial enzymes was analyzed using restricted cubic spline regression with statistical significance in GLM model. Manganese showed a positive and linear dose-response relationship with CK ($P_{\text{overall}} = 0.019$, $P_{\text{non-linearity}} = 0.307$) while calcium showed a negative and linear dose-response relationship with LD ($P_{\text{overall}} = 0.008$, $P_{\text{non-linearity}} = 0.488$). There was a U-shaped dose-response relationship between molybdenum and LD ($P_{\text{overall}} < 0.001$, $P_{\text{non-linearity}} = 0.015$), and a negative linear dose-response relationship between molybdenum and $\alpha$-HBD ($P_{\text{overall}} = 0.010$, $P_{\text{non-linearity}} = 0.125$) (Fig. 2).

3.6 Stratified analysis

Stratified analysis showed age significantly modified the association between Ca and CK ($P_{\text{interaction}} = 0.041$) (Table S2). Moreover, smoking status significantly modified the negative association between Mo and LD ($P_{\text{interaction}} = 0.035$); the negative association between Mo and LD were stronger among current smoker [$\beta$ (95% CI) = -28.83 (-44.8, -12.85)] (Table S5). Other variables did not modify the association between remaining metals and myocardial enzyme levels (all $P_{\text{interaction}} > 0.05$) (Table S2,3,4,6).

3.7 Bayesian kernel machine regression analyses

We evaluated overall effect of metal mixtures at a particular quantile as compared to all exposures fixed at $P_{50}$. BKMR model showed a significantly positive over-all effect of three metal mixtures on CK level (Fig. 3C). Interestingly, we found a negatively over-all effect of seven metal mixtures on LD level though it was not statistically significant as credible interval contained zero.

4. Discussion

Manganese was linearly and positively associated with CK and dominated positive overall-effect of metal mixtures (manganese, arsenic and rubidium) on CK level; calcium and zinc were linearly negative associated with LD level and dominated the overall negative effect of metal mixtures (Rubidium, molybdenum, zinc, nickel, cobalt, calcium and magnesium) on LD level. Interestingly, we found a “U” shape dose-response relationship between molybdenum and LD level. There was an interaction between age and calcium on LD level. Besides, there was an interaction between smoking and molybdenum on LD level.

In the present study, we found manganese was significantly associated with elevated serum CK levels, which is consistent with previous findings (Huang et al., 2020). Anonna et al (Anonna et al., 2020) suggest the pathogenesis of ischemic heart disease is related to elevated levels of manganese in the body. In addition, a case-control study in a Chinese population showed high levels of Mn and Fe were associated with increased risk of developing coronary heart disease in pregnant women and risk of
coronary heart disease in the offspring (Wang et al., 2022). Animal studies have shown high level of manganese exposure can impair myocardial integrity and function in rats (Miller, Caton, & Finley, 2006). In addition, previous studies on animal models in rats, cats, and dogs have shown that manganese affects cardiovascular function mainly by affecting myocardial contraction (Agata, Tanaka, & Shigenobu, 1992; Kostial, Landeka, & Slat, 1974; Tanaka et al., 2002; Wolf & Baum, 1983). The mechanism needs to be further explored.

Zinc is one of essential metals (Prasad, Halsted, & Nadimi, 1961) and a component of many metalloenzymes, including angiotensin-converting enzymes, Cu/Zn-superoxide dismutase, and transcription factors. In the current study, zinc was negatively correlated with serum LD levels, suggesting zinc have a protective effect on cardiac function. zinc reduces oxidative stress and inflammation, and zinc supplementation may help prevent atherosclerosis and endothelial damage (Reiterer et al., 2005) and halt the development of cardiovascular disease (Sarmento, Silva, Sbruzzi, Schaan, & Almeida, 2013). In addition, Takamasa Kido et al. showed serum AST and LD levels were significantly higher in zinc-deficient rats compared to the other groups, suggesting that zinc deficiency can exacerbate the inflammatory response (Kido, Hachisuka, Suka, & Yanagisawa, 2021). Previous vitro and animal studies reported the benefits of zinc supplementation in the control of destructive cellular mechanisms (Fortmann, Burda, Senger, Lin, & Whitlock, 2013). Gulnur et al. showed that zinc supplementation improved arrhythmias, promoted myocardial healing, and prevented protein kinase C degradation (Karagulova, Yue, Moreyra, Boutjdir, & Korichneva, 2007).

Molybdenum is also an essential trace element playing an important role in a variety of physiological activities. Animal experiments have shown molybdenum had a protective effect on the heart muscle. Previous studies have found reduced serum molybdenum in patients with arrhythmias or unexplained angina pectoris. In addition, people dead with myocardial infarction found to have low molybdenum content in the myocardium, the less molybdenum the more damage occurred. Li Suyun et al. showed molybdenum reduced the leakage of creatine phosphokinase (CPK) and LD from cardiac myocytes on mice, thereby reducing myocardial damage (Shuyun Li, 2003). Wang Fan et al. showed molybdenum was effective in reducing the detection rate of myocardial necrosis and decreasing the area of necrosis in experimental rats, and that consumption of grains high in molybdenum significantly reduced the incidence of Keshan disease in the population (Wang Fan, 1982). Li Xinmin et al. showed improving myocardial mitochondrial respiratory chain function and oxidative phosphorylation efficiency is one of the possible ways in which molybdenum protects the myocardium (Li Xinmin, 1996).

For this research, we utilized two different methods of analyzing multiple pollutants (LASSO and BKMR models) to address the limitations of conventional strategies. By incorporating BKMR, we were able to consider nonlinearity and the possibility of complex interactions among metals in relation to myocardial enzyme profile. Nevertheless, our study had several limitations. The inherent limitation in this cross-sectional study is the presence of reverse causation. Furthermore, the sample size was relatively small, thereby restricting the capacity to analyze the findings. Furthermore, there may still be residual confounding factors that cannot be ignored, such as variations in eating patterns.
5. Conclusions
Our study provides evidence that metal mixture exposure affects myocardial enzyme profile. Additional investigations are required to confirm these associations and reveal the fundamental mechanisms involved.

Declarations

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Authors Contributions
Writing - original draft, Writing - review & editing: Xiaoting Ge, Junxiu He, Yuan Zheng; Investigation, Data curation: Hong Cheng, Yu Bao, Sencai Lin, Sihan Hu, Haiqing Cai, Xiuming Feng, Qinghua Fan, Lin Wang; Methodology: Fei Wang, Chaoqun Liu, Xing Chen; Conceptualization, Writing - review & editing, Funding acquisition: Xiaobo Yang

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Data Availability
Data will be made available on reasonable request.

Declarations

Ethical Approval
The study was approved by the medical ethics committee at Guangxi Medical University.

Consent to Participate
All authors agreed to participate.

Consent to Publish
All authors agreed to the publication.

Competing Interests
The authors declared no conflict of interest.
References


Tables

Tables 1 to 2 are available in the Supplementary Files section

Figures
Figure 1

Prediction mean square error (left) and the path of estimated coefficient (right) of metals in LASSO model. We predicated an optimal model using 10-fold cross-validation, according to $\lambda$ where mean square error (MSE) was the minimum (vertical red solid line).

A:CK; B:CK-MB; C:LD; D:α-HBD; E:AST;
Figure 2

Restricted cubic spline results for the association of single metal and cardiac enzymes in all participants.
Figure 3

Overall effect (left) and single metal effect (right) of metal mixture exposures on myocardial enzyme profile among the participants

left: Overall effect of the metal mixtures on myocardial enzyme profile. (b) Difference in myocardial enzyme profile for increase (25th to 75th) in specific metal when the remaining metals kept at their 25th,
50th and 75th percentile, respectively. Mode was adjusted for gender (men/women), age (continuous), seniority (continuous), BMI (continuous), current smoker (yes/no), current drinker (yes/no).

**Supplementary Files**

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- supplymentarymaterial20230626.docx
- Table12.xlsx