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Manganese in blood cells as an exposure biomarker in manganese-exposed workers healthy cohort

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ABSTRACT

Elevated exposure to manganese (Mn) has long been a public health concern. However, there is currently no consensus on the best exposure biomarker. Here we aimed to investigate the exposomic characteristics of plasma metals among Mn-exposed workers and explore the potential biomarkers of Mn exposure in the blood pool. First, total sixteen plasma metals (Calcium, Magnesium, Iron, Zinc, Copper, Selenium, Lead, Chromium, Arsenic, Manganese, Nickel, Molybdenum, Cadmium, Mercury, Thallium, and Cobalt) were determined among 40 occupationally Mn-exposed subjects. Second, Mn levels in both plasma and blood cells were detected among 234 workers from the manganese-exposed workers healthy cohort (MEWHC), respectively. Analysis of plasma metal exposome showed that the plasma Mn concentrations were positively correlated to plasma Fe (r = 0.361), Ni (r = 0.363), Cr (r = 0.486), and Hg (r = 0.313) (all $p < 0.05$). Mn concentrations in plasma were not significantly correlated to external exposure levels (p_{trend} = 0.200), and it was further confirmed among the 234 subjects (p_{trend} = 0.452). However, Mn concentrations in blood cells progressively increased as the external exposure dose increased (low-exposure group *vs* high-exposure group, median 11.53 μg/L *vs* 20.41 μg/L, p_{trend} = 0.001). Our results suggest that Mn in blood cells, but not plasma, could serve as a potential internal exposure biomarker. Larger validation studies are needed to establish the utility of this biomarker.

1. Introduction

Manganese (Mn) is ubiquitously present in the environment, which is essential for biological systems and processes in human body [1–5]. Human exposure to Mn occurs primarily through inhalation of Mn-contained dust in occupational population. Elevated occupational exposed to Mn would result in manganism characterized by motor and postural signs [6,7]. In recent years, epidemiological evidences have emerged suggesting that Mn-exposed workers might suffer subtle neurological effects, though the exposure level is lower than those historically associated with manganism [8–11]. In general, the symptoms of Mn intoxication are usually progressive and irreversible. However, it would quickly develop among occupationally Mn-exposed workers due to relatively high exposure levels. It makes elevated Mn exposure a relevant public concern, and the biomarker discovery of Mn exposure is growing imperative.

Despite confirmed neurologic deficits caused by elevated Mn exposure, however, no ideal biomarker is available on Mn exposure. Several researches have demonstrated that magnetic resonance imaging (MRI) can be used to visualize Mn accumulation in the brain [12,13]. But it seems not appropriate to apply MRI in a large population for its high price and complex procedure. Urine and blood samples are the most common biological matrices for bio-monitoring. However, Mn excreted in urine represents only a small fraction of Mn in human body. Moreover, Mn concentrations in urine have been reported to be lower than the limits of detection [14,15], which makes further analysis impossible. Mn in blood has been considered as an indicator of current Mn exposure [16,17]. The majority of biomarkers studies related to occupational inhalation of Mn were focus on plasma or serum Mn. Indeed, Mn in plasma (MnP) showed utility to discriminate Mn-exposed

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subjects from the controls in group levels [14,18,19]. However, several studies showed poor relationships between Mn concentrations in plasma and the external exposure levels [20,21]. To date, a consensus has not been reached on which is the best biomarker of Mn exposure. Further investigations are required to explore potential biomarkers of Mn exposure in the blood pool among a larger, well characterized Mn-exposed population.

Exposome is functionally defined as the complex of all chemicals with biological activity in a person's blood including exogenous and endogenous sources [22]. Thus, blood specimen can be used to characterize exposome. Upon absorbed into the circulatory system, the metals bind immediately to the proteins and small ligands and metals are only free at insignificant levels in blood. With respect to blood exposome, the proportions of metals and small molecules were used to characterize it [23]. Given that metals are transported to cells and tissues, the serum/plasma can represent as a distributor of metals. Thus, it is possible to investigate the exposomic characteristic of metals with serum/plasma at a given time. However, few studies are available on the plasma metal exposome. To date, emphasis has been focused largely on single metal exposure, resulting in an underestimation of the health risks in affected population.

Manganese-exposed workers healthy cohort (MEWHC) was started from 2011 [24], which is a unique resource of over 2000 Mn-exposed workers enrolled from a ferro-manganese refinery. It is a longitudinal, prospective and multidisciplinary study designed to explore early health effects, potential biomarkers of exposure and susceptibility, as well as diseases related to occupational Mn exposure. In this study, we measured the blood concentrations of metals in Mn-exposed subjects, investigated the exposomic characteristics of plasma metals, as well as explored the potential biomarkers of Mn exposure in the blood pool.

2. Material and methods

2.1. Study population

In this prospective cohort study, a total of 274 Mn-exposed individuals were selected from the participants in MEWHC. A detailed description of the inclusion and exclusion criteria can be found elsewhere [24]. In brief, we first randomly selected 40 Mn-exposed workers for exploring the plasma metal exposomic characteristics. In the second-stage study, we intended to explore potential biomarkers of Mn exposure in the blood pool among a larger population. Taken for granted that smelting workers whose job assignments are more stable and whose exposure scenarios are relatively less complicated, 234 male smelting workers were recruited for further study. The program included questionnaires (self-reported demographics and life styles including smoking habits and alcohol consumptions) and the collection of fasting peripheral venous blood samples (5 mL). Plasma was separated from peripheral venous blood. Plasma and blood cells samples were stored and kept in a deep freeze at −80 °C until analysis. All subjects provided written informed consents to participate in the study. All study protocols were reviewed and approved by the medical ethics committee of Guangxi Medical University.

2.2. Monitoring of airborne metals concentration

Environmental exposure information was obtained through air monitoring in this ferro-manganese refinery both with personal and station air samplers. For typical occupations, the air sampling procedure was performed according to the standard specification "Specifications of Air Sampling for Hazardous Substances Monitoring on the Workplace" (GBZ159-2004). In addition, air samples in the smelting branch factory were collected with a station air sampler (JH-1FC, Wuhan, China) for another three consecutive days. Temperature and barometric pressure were documented during the sample collection procedures. Air flow was pumped at a flow rate of 100 L/min for 2 h a day after the smelting started. The mean values of all samples were presented in this report.

For airborne Mn analysis, the detection procedure was performed using a flame atomic absorption spectrometer according to the China National Standard Operation Protocol (GBZ/T160.13-2004) for occupational safety surveillance. Mn time weighted average (Mn-TWA) values were calculated following an 8-h work shift. Mn cumulative exposure index (Mn-CEI) was calculated using TWA, short term exposure limit (STEL) and seniority of the participants.

In addition, the contents of metal compounds collected in the smelting branch factory were detected by Agilent 710 inductively coupled plasma optical emission spectrometry (Agilent Technologies). The setup of the instrumental conditions were: high-frequency power = 1.20 kW, plasma flow rate = 15 L/min, Auxiliary Flow = 1.5 L/min. The filters were digested with 20 mL of a mixture of nitric acid (HNO_3) and hydrochloric acid (HCL). This solution was heated for 25 min under reflux in a heating block at 200 °C. After cooling to room temperature, 10 mL of ultrapure water was added to the dry residues. After 30 min' standing, the extract was diluted to 50 mL with ultrapure water. The contents of metals in air were determined using a curve established with certified ICP grade standards. Quality control (QC) measures included analyses of the initial calibration verification standard, duplicate samples every 10 samples and a procedural blank. The limits of detection (LOD) for the airborne metals were in the range 0.990–270.590 μg/L.

2.3. Determination of 16 plasma metals

Forty workers were randomly selected from the participants in MEWHC. A plasma sample of 100 μL was diluted to 2 mL with 1.2% HNO_3. Levels of metals compounds were determined by an Agilent 7700X inductively coupled plasma mass spectrometry (Agilent Technologies). Analyses were conducted through external calibration with an internal standard containing ^{6}Li, ^{45}Sc, ^{89}Y, ^{115}In, ^{159}Tb and ^{209}Bi for 16 metals, respectively. The parameters of the Agilent 7700X were available as below: radio-frequency power = 1500W, plasma flow = 15 L/min, helium flow in collision cell = 5.0 L/min, sampling depth = 8.0 mm, resolution = 0.6–0.7 amu.

QC measures included analyses of the initial calibration verification standard, a mixed-element standard solution, and a procedural blank. ClinChek®-Control (Plasma Control lyophilised/Kontroll plasma lyophilisiert) was used as the QC sample. Relative standard deviation (RSD) was less than 5%. Results were given as the average of sixteen replicate measurements. LOD for the plasma metals were in the range 0.0003–1.0007 μg/L.

2.4. Biological monitoring of Mn and iron

In order to confirm whether Mn in plasma could serve as Mn exposure biomarker and to explore other potential biomarkers of Mn exposure in the blood pool, a total of 234 male smelters were enrolled in further study.

We conducted assays on Mn levels with an AA-6300 atomic absorption spectrophotometer (Shimadzu, Japan). The setup of the instrumental conditions were: λ = 279.5 nm, current lamp = 10 mA, and slit width = 0.2 nm. The microwave heating program presented four steps (Temperature/ °C; hold/min): 1 (150–250 °C; 30s), 2 (800 °C; 10s), 3 (2200 °C; 3s), 4 (2400 °C; 2s). For Mn determination, plasma (40 μL) and blood cell samples (40 μL) were diluted 40 folds with a matrix modifier containing 1.0% Triton X-100, 1% HNO_3, and 200 μg/L palladium chloride, respectively. Samples were analyzed using a HNO_3-matched calibration curve created with different weights of certified reference material GBW9 (E) 080157 (National Institute of Metrology, Beijing, People's Republic of China). Mn recovery was 95.1–106.7%, with > 90% precision. The average limits of detection of Mn in blood cells and plasma were 0.17 μg/L, respectively. All samples were above