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Short communication

External lead contamination of women's nails by surma in Pakistan: Is the biomarker reliable?☆

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ABSTRACT

Adverse health effects of heavy metals are a public health concern, especially lead may cause negative health impacts to human fetal and infantile development. The lead concentrations in Pakistani pregnant women's nails, used as a biomarker, were measured to estimate the lead exposure. Thirteen nail samples out of 84 nails analyzed contained lead higher than the concentration (13.6 μg/g) of the fatal lead poisoning case, raising the possibility of an external contamination. Eye cosmetics such as surma are recognized as one of the important sources of lead exposure in Pakistan. We collected in Pakistan 30 eye cosmetics made in Pakistan, Saudi Arabia and western countries. As the metal composition analysis by energy dispersive X-ray fluorescence spectrometry revealed that some surma samples contained lead more than 96%, the surma might contaminate the nail specimen. Scanning electron microscopy observations showed that lead-containing surma consists of fine particle of galena (ore of lead sulfide) in respirable dust range (less than 10 μm). In addition, relative in vitro bioavailability of lead in the surma was determined as 5.2%. Thus, lead-containing surma consists of inhalable and bioavailable particles, and it contributes an increased risk of lead exposure. Moreover, the relationship between the surma and the lead-contaminated nails by lead isotope ratios analysis indicated the potential of lead contamination in nails by surma. These results suggest that lead in the nails was derived both from body burden of lead and external contamination by lead-containing surma. Therefore, nail is not suited as a biomarker for lead exposure in the countries where surma used, because we may overestimate lead exposure by surface lead contamination in the nail by surma.

1. Introduction

The negative health effects of heavy metal elements such as lead are public health concerns. Joint FAO/WHO Expert Committee on Food Additives (JECFA) reported that exposure to lead has been shown to be associated with a wide range of effects, including various neurological and behavioral effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes, delayed sexual maturation and impaired dental health (JECFA, 2011). And women with a blood lead level greater than 10 μg/dL during pregnancy were at increased risk of delivering preterm or small for gestational age infants. Moreover, prenatal and postnatal exposure to lead even at low concentration could impair neurodevelopment in children, e.g. impediments of cognitive development and intelligence (JECFA, 2011). Sources of lead exposure have been investigated in many environmental media. Some sources of lead exposure are specific to particular regions or cultures (JECFA, 2011). In Pakistan, the many different kinds of objects e.g. leaded petrol, lead-based paints, lead water pipes, lead-acid batteries, lead food cans, traditional remedies and lead containing cosmetics, etc. were identified as the

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sources of lead exposure (Farooq et al., 2008; Kadir et al., 2008).

Surma (also known as Kohl and Kajal) is commonly used as cosmetics of eye makeup. It is widely used by women and children in South Asia, the Middle East and parts of Africa for the purpose of religious and traditional beautification and preventive medicine (Parry and Eaton, 1991; USFDA, 2006). In the United States, surma cannot be imported and is not permitted by regulation. An import alert about eye area cosmetics containing kohl, kajal, or surma was published by United States Food and Drug Administration (USFDA) for detention without physical examination of the product (USFDA, 2014). In contrast, manufacturing of surma is not regulated in Pakistan and estimated lead content varies greatly, from 16 to 70 percent (NIH, 2010). Some surma are also traditionally made at home (Hardy et al., 2008). Many people may be unaware of the lead poisoning risk of surma. The study shows that most mothers who apply surma to their children (54%) did not have any formal education in Pakistan (Rahbar et al., 2002). Consequently, children exposed to surma have increased levels of lead in their blood (USFDA, 2006). Furthermore, researchers have found association between high lead levels in the umbilical cord and the use of surma by mothers in a study of prenatal lead exposure in Pakistan (NIH, 2010).

Investigations of the heavy metal contamination, such as lead and arsenic, of foods and living environments in Pakistan and Japan are ongoing in our laboratory. More recently, the multi-element analysis of keratinized matrices like hair or nail by ICP-MS is commonly used as a biomarker for heavy metal exposure (Goullé et al., 2009). It is considered a useful laboratory method for epidemiological studies, because of its non-invasive nature. In this study, we analyzed lead concentrations in the Pakistani pregnant women's nails to estimate the lead exposure. Unexpectedly some of the results showed high lead concentrations above the concentration in finger nails of the fatal lead poisoning case (Lech, 2006). It was supposed the possibility of an external contamination of the nails. We were focused on the traditional eye cosmetic "surma" and confirmed lead-containing surma consists of inhalable and bioavailable particles. Moreover, we were determined the relationship between lead-containing surma and lead-contaminated nails using the lead isotope ratios analysis to confirm a potential of lead contamination in the nails by surma. Therefore, this study was undertaken to estimate the risk of lead-containing surma and the reliability of nail as a biomarker for lead exposure.

2. Materials and methods

A total of 84 nail samples (from both hands and feet) were collected from pregnant women in Gambat, Khairpur district, Pakistan. In addition, 30 eye cosmetics including surma, kohl, kajal and similar products were purchased at local markets and handmade ones were also collected in Karachi, Pakistan. All samples were kept in separate plastic bags in cool and dry environment away from sunlight and fumes before analysis.

2.1. ICP-MS analysis for nail

Nail samples were washed by 70% EtOH, acetone, 2% Triton X100 and water according to a protocol for element determinations in human nail clippings (Sanches and Saiki, 2011). After decontamination process, samples were digested with 1.45 ml of nitric acid Ultrapur-100 (Kanto Chemical Co., Inc., Tokyo, Japan) using microwave digestion system TOPwave (Analytik Jena Japan Co., Ltd, Kanagawa, Japan) according to the instruction manual.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) was performed to determine the lead concentrations in nail samples. ICP-MS analysis was conducted by The National Institute for Environmental Studies (NIES) using Agilent 7500cx (Agilent Technologies Japan, Ltd, Tokyo, Japan). The measurement for lead was carried out by calibration curve method using a lead standard solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and a thallium standard solution (Wako Pure Chemical Industries, Ltd.) for an internal standard. Linearity measured as the correlation coefficient was 1.000 for lead. Low limit of detection of lead was 0.001 ng/g (ppb). Quality control test was performed using NIES Certificated Reference Material No.13, Human Hair, instead of nail. The recovery of lead for the analysis method was 94.2%.

2.2. Observation of surma by scanning electron microscopy ()

A portion of surma was fixed on a holder with carbon adhesive tape for (Nisshin EM Corporation, Tokyo, Japan). Observations of the morphologies of surma were made with JSM-6510LA (JEOL Ltd., Tokyo, Japan).

2.3. In vitro bioaccessibility assay for lead in surma

Determination of lead bioaccessibility in surma was carried out using the standard operating procedure (SOP) for an in vitro bioaccessibility (IVBA) assay for lead in soil (USEPA, 2012). This method is a United States Environmental Protection Agency (USEPA) validated in vitro assay for estimating relative bioavailability (RBA) in environmental media (soil, dust, water, food, air, paint, etc.).

The extraction fluid was used 0.4 M glycine (free base, reagent grade glycine in deionized water), adjusted to a pH of 1.50 $\pm$ 0.05 using trace metal grade concentrated hydrochloric acid. Samples (200 mg) were mixed with the extraction fluid to a solid-to-fluid ratio of 1/100 (mass per unit volume) in a 25 ml lead-free tube. Samples were extracted at 37 °C, 30 rpm in Bio-shaker BR-40LF (TAITEC Corporation, Saitama, Japan) for 1 h ensuring the pH was maintained at 1.5 $\pm$ 0.5. The extracts were filtered with a 0.45 μm cellulose acetate disk filter (33 mm diameter) and stored the filtered samples at 4 °C. The samples were analyzed by ICP-MS Agilent 7500cx in NIES. IVBA and RBA were calculated using the equations on the standard operating procedure manual.

2.4. Lead isotope ratios analysis

Nail samples that had high lead contamination ($n = 13$) were selected (>13.6 μg/g). A total of four ($n = 4$) surma samples were used for the extraction of lead bioaccessibility for comparison of isotopes. Measurement of the lead isotope ratios 207Pb/206Pb and 208Pb/206Pb was performed using ICP-MS Agilent 7500cx in NIES, details can be found in Takagi et al. (Takagi et al., 2008). National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 981, Common Lead Isotopic Standard, was used to correct for mass discrimination. Quality control tests were performed using NIST SRM 2583, Trace Elements in Indoor Dust.

3. Results and discussion

Lead concentrations in nail samples of Pakistani pregnant women ranged from 0.002 to 405 μg/g, geometric mean 0.309 μg/g, arithmetic mean (sd) 11.7 ($\pm$45.6) μg/g, median value 1.77 μg/g. The level of lead in 15% (13 of 84) samples was above the concentration in finger nails (13.6 μg/g) of the fatal lead poisoning case, and 43% of samples (36 of 84) had levels below the lower limit of detection of ICP-MS (Table 1). Reference ranges of lead in finger nail samples from 130 healthy volunteers were reported by Goullé et al. the values of median, 5th and 95th percentiles were 0.52, 0.10 and 3.71 μg/g, respectively (Goullé et al., 2009). Median lead