Environmental Pollution xxx (2018) 1–7

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Concentrations of selected heavy metals in placental tissues and risk for neonatal orofacial clefts☆

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ARTICLE INFO

Article history:
Received 19 March 2018
Received in revised form
19 July 2018
Accepted 23 July 2018
Available online xxx

Keywords:
Mercury
Cadmium
Lead
Arsenic
Orofacial clefts

ABSTRACT

Orofacial clefts (OFCs) have multifactorial etiologies. Prenatal exposure to heavy metals can induce OFCs in animal models, but evidence from studies of human subjects is scarce. We examined whether concentrations of mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As) in placental tissues are associated with risk for OFCs in offspring. This population-based case-control study included 103 newborns affected by OFCs with available placental tissues and 206 controls randomly selected from 509 non-malformed newborns with available placenta samples, recruited in five rural counties in northern China. Socio-demographic information was collected using a structured questionnaire in face-to-face interviews. The concentrations of Hg, Cd, Pb, and As in placental tissues were analyzed using an inductively coupled plasma-mass spectrometry in helium mode. The median concentrations of Hg (7.4 ng/g), Cd (57.1 ng/g), and Pb (96.1 ng/g) were all statistically significantly higher in OFC cases than in controls (Hg 5.5 ng/g, Cd 38.6 ng/g, and Pb 67.9 ng/g, respectively); no differences were observed between the two groups in median concentrations of As. Concentrations above the median for all subjects were associated with a 2.33-fold (95% confidence interval [CI] 1.33–2.09) increased OFC risk for Cd and a 3.08-fold (95% CI 1.74–5.47) increased risk for Pb. The risk for OFCs increased with concentration tertiles, with an adjusted odds ratio of 3.06 (95% CI 1.36–6.88) for the second tertile and 8.18 (95% CI 6.64–18.37) for the highest tertile of Cd, and 3.88 (95% CI 1.78–8.42) for the second tertile and 5.17 (95% CI 2.37–11.29) for the highest tertile of Pb. The association between Hg concentration and OFC risk was borderline nonsignificant after adjusting for confounding factors. Prenatal exposure to Cd and Pb, as reflected by their concentrations in placental tissues, is associated with an increased risk for neonatal OFCs.

1. Introduction

Orofacial clefts (OFCs) are a group of birth defects affecting the lip and palate and present as cleft lip (CL), cleft lip and palate (CLP), or cleft palate (CP) (Mossey et al., 2009). Epidemiological data show that OFCs occur in 1 out of 700 live births (Panamonta et al., 2015). Children affected by OFCs require multi-disciplinary interventions and substantial medical and economic assistance (Berk and Marazita, 2002; Wehby and Cassell, 2010). Moreover, OFCs are associated with an increased risk for death (Christensen et al., 2004; Ngai et al., 2005). Therefore, OFCs increase the psychological and socioeconomic burdens on individuals, families, and society and predispose affected infants to morbidity and mortality.

During embryogenesis, the migration of neural crest cells contributes to the formation of embryonic primary and secondary palates, which develop into the lip and palate regions between 4–6 weeks and 6–12 weeks of gestation, respectively (Mossey et al., 2009). Immediately at the end of the sixth week of gestation, the embryogenesis of the lateral nasal process has a peak in cell division that renders it susceptible to harassment (Sperber, 2002). Any disturbance due to exposure to environmental teratogens around this critical time can lead to deformities of the lip and/or palate. However, despite extensive study, the etiology of OFCs remains

☆ This paper has been recommended for acceptance by Prof. W. Wen-Xiong.

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https://doi.org/10.1016/j.envpol.2018.07.112
0269-7491/

abstruse and elusive. It is generally accepted that environmental or genetic factors, or their interactions, contribute to the formation of OFCs.

As well-known toxic elements, the adverse effects of exposure to mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As) on reproductive outcomes have attracted much attention. High levels of Hg can adversely affect a developing fetus and cause organ damage (Friedrich, 2017), while elevated As can induce abnormal embryogenesis (Abdul et al., 2015). The toxicity of Pb is well documented; previous studies have found that maternal Pb exposure during pregnancy increases the risk of developing congenital malformations, such as neural tube defects (NTDs) and congenital heart defects (Cengiz et al., 2004; Carrillo-Ponce Mde et al., 2004; Liu et al., 2015). With respect to Cd-induced embryotoxicity, various animal models have demonstrated that Cd treatment during organogenesis can cause OFCs in offspring (Gale and Layton, 1980; Holt and Webb, 1987; Soukupova and Dostal, 1991; Salvatori et al., 2004). However, evidence from human-subject studies that have focused on the potential relationship between exposure to toxic metals and OFCs in offspring is limited. Therefore, it is of considerable interest to investigate the association between maternal exposure to toxic metals during pregnancy and the risk for OFCs in human populations.

The placenta is responsible for the circulation of nutrients and waste products between fetus and mother; it also acts as a detoxifying barrier during embryo/fetus development. It is well established that Hg, Cd, Pb, and As in the mother's blood can cross the placenta and may accumulate in it in various amounts (Abdul et al., 2015; Grandjean and Landrigan, 2006; Kippler et al., 2010; Esteban-Vasallo et al., 2012). Therefore, the placenta can provide information on fetal exposure to certain heavy metals (Slikker, 1994).

We hypothesized that higher levels of selected heavy metals in placental tissues would be associated with increased risks for OFCs in offspring. We tested this hypothesis by measuring the concentrations of Hg, Cd, Pb, and As in placental tissues from a group of OFC cases and a group of non-malformed newborns using a case-control study conducted in a rural area of China, where the birth prevalence rate of NTDs is among the highest in the world: 31.5 per 10,000 births in 2014 (Liu et al., 2016).

2. Material and methods

2.1. Study design and subjects

We used a case-control study design. As described elsewhere (Ren et al., 2011), newborns or terminated fetuses with any major external structural defects, including OFCs, NTDs, congenital hydrocephalus, limb defects, and so forth were recruited from five rural counties (Pingding, Taigu, Shouyang, Xiyang, and Zezhou) of Shanxi Province in northern China, where a population-based birth defects surveillance program was established to monitor major external structural birth defects in 2002. Diagnoses of newborns/fetuses with major birth defects were done through physical examination or prenatal ultrasound examination by county healthcare workers. Photographs were taken for verification purposes after consent had been obtained from the mother. Once a newborn/fetus with a major birth defect was identified as a case, a healthy newborn with no congenital malformation was selected as a control to match the case by residence of the mother (the same county), date of the last menstrual period (±4 weeks), and newborn sex. Detailed information on demographic, obstetric, lifestyle, and environmental exposure was collected by trained healthcare workers through face-to-face interviews before patient discharge from hospital, within 1–10 days (median 1 day) of delivery, using a structured questionnaire. Placental tissues as well as maternal venous blood samples, scalp hair, umbilical cord, and cord blood samples were requested upon informed consent.

To the best of our knowledge, no previous studies have investigated the associations between placental concentrations of Pb, Cd, Hg, and As and the risk for OFCs. One study (Jin et al., 2013) conducted on the same population found that higher placental levels of Hg were associated with an elevated risk for NTDs. Assuming that the placental concentrations of Hg in the present study were similar to those of that previous study, the numbers of cases and controls needed to produce statistically significant results in the present study would be 91 each (Power Analysis and Sample Size, version 11, NCSS, LLC.; Mean [Std.]: NTDs 5.46 [11.87] ng/g wet weight, controls 1.35 [0.94] ng/g wet weight; $\alpha = 0.05$ two-side; $\beta = 0.1$). To maximize the sample size, we included all 103 OFC cases with available placental tissues as the case group, and twice as many newborns ($n = 206$), who were randomly selected from all 509 non-malformed newborns with available placental tissues, as the control group.

2.2. Placental tissue collection and laboratory assessment

Placental tissues were collected immediately after delivery and placed in a labeled (identification code) polyethylene plastic bag by local healthcare workers. Because washing and blotting when samples were fresh was not feasible at local rural hospitals due to a lack of deionized water, placental samples were stored at −20 °C at the hospitals and then transported on dried ice to our laboratory, where they were maintained at −20 °C until analysis. Before laboratory assessment, the placental tissues were thawed at 4 °C, and approximately 6 g wet tissue was cut within 3 cm around the point of cord attachment on the fetus side, using a titanium tool to avoid external metal contamination. The samples were rinsed three times with deionized water to dislodge the blood in the tissues, blotted on clean tissue paper, and transferred to polyethylene plastic bags. All supplies used for sample collection were prescreened to avoid contamination. Then the samples were freeze-dried (ALPHA2–4 LD plus, Christ, Germany) for 24 h to remove water. Approximately 0.2 g (CP225D, Sartorius, Germany) dried placental tissue was added to 2.0 mL nitric acid (UP-grade) and 0.5 mL hydrogen peroxide (UP-grade), digested in quartz digestion vessels under a high-pressure microwave digestion system from room temperature to 160 °C for 5 min, 160–200 °C for 5 min, and 200 °C for 20 min, 1300 W, 40 bar (Ultra WAVE, Milestone, Italy). Acid digested placenta tissue samples were then diluted to 15 mL with deionized water, and this solution was further diluted 1:4 before analyzed by inductively coupled plasma-mass spectrometry (ICP-MS, 7700×, Agilent, USA), which was performed in helium mode. A blank solution was prepared and carried at the same time alongside each of the 15 placenta samples to check for possible contamination during the digestion procedure and sample operation. The placenta samples, in each case and control group, were randomly inserted into the sample orders before laboratory analysis and proportionately selected in each run of the assay to keep balance between cases and controls. The operators were masked to the group (i.e., case or control) of samples.

Rhenium (GSB 04–1745–2004) was used as the internal standard to correct for sample introduction and plasma effects and check the instrument stability during the ICP-MS. The certified standard from Chinese national reference materials (GSB 04–1729–2004 for Hg, 1000 μg/mL; GSB 04–1767–2004 for Cd, Pb, and As, 100 μg/mL) was used for the generation and validation of the standard curves. To guarantee the accuracy of the results, a standard material made from pig liver (GBW10051) with known concentrations of Hg, Cd, Pb, and As was prepared and carried alongside each batch of the placenta samples. All correlation

Please cite this article in press as: Pi, X., et al., Concentrations of selected heavy metals in placental tissues and risk for neonatal orofacial clefts, Environmental Pollution (2018), https://doi.org/10.1016/j.envpol.2018.07.112