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Prenatal exposure to manganese at environment relevant level and neonatal neurobehavioral development

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ABSTRACT

Background: Effects of prenatal Manganese (Mn) exposure at an environmental relevant level on neonatal neurodevelopment remains unclear.
Objectives: In the multi-center study, we assessed the impact of low level prenatal Mn exposure on neonatal behavioral neurological assessments (NBNA), and explore a threshold umbilical cord blood Mn on neonatal neurological development.
Methods: We investigated 933 mother-newborn pairs in Shanghai, China, from 2008 through 2009. Umbilical cord serum concentrations of Mn were measured and NBNA tests were conducted. The NBNA contains five clusters: behavior, active tone, passive tone, primary reflexes and general assessment with a maximal total score of 40. The score < 37 is defined as low.
Results: The median serum Mn concentration was 4.0 μg/L. Of the 933 infants, 44 (4.7%) had low NBNA. After adjusting for potential confounders, a high level of Mn ($\geq$ 75th percentile) was associated with a lower NBNA score (adjusted $ß = -1.1$, 95% CI: -1.4–0.7, $p < 0.01$) and a higher risk of low NBNA (adjusted OR $= 9.4$, 95% CI: 3.4–25.7, $p < 0.01$). A nonlinear relationship was observed between cord serum Mn and NBNA after adjusting for potential confounders. NBNA score decreased with increasing Mn levels after 5.0 μg/L(LgMn ≥ 0.7). The cord serum Mn $\geq$ 5.0 μg/L had adverse effects on behavior, active tone and general reactions of clusters ($p < 0.001$).
Conclusions: High prenatal Mn exposure even at an environmental relevant level, is associated with poor fetal neurobehavioral development in a nonlinear pattern. A threshold cord serum Mn of 5.0 μg/L existed for lower neonatal behavioral neurological assessments.

1. Introduction

Manganese (Mn) is an essential nutrient to human, but it also has the potential to produce neurotoxic effects when accumulating in an organ, especially the brain (World Health Organization, 1981). Studies of Mn exposure in workplace have demonstrated that exposure to high doses of Mn is associated with irreversible neurodegenerative disorders resembling idiopathic Parkinson disease (Furbee, 2011). More recently, child environmental exposure to Mn has gained interest. Postnatal Mn exposure was found to interfere with development of brain functions (Zoni et al. 2007; Bouchard et al., 2007). However, few epidemiologic data are available on the effects of in utero Mn exposure on child neuropsychological development. In a birth cohort study in France, cord blood Mn was negatively associated with attention and non-verbal memory and boys' manual ability at 3 years, after adjusting for mother's educational level (Takser et al., 2003). In a U.S. study, Mn was analyzed in the enamel of deciduous teeth. Its concentration in tissue formed during the intra-uterine phase was significantly associated with disinhibitory behavior evaluated at 36 and 54 months of age (Erikson et al., 2007).

The history of neonatal neurobehavioral assessment began in early last century. Sarnat (1984); Amiel-Tison (2002) and Dubowitz et al. (2005) gradually developed neurological assessment in the newborn via examining tone and reflexes. After realizing that a newborn can regulate his behavior, Brazelton developed the Neonatal Behavioral Assessment Scale (NBAS), the first truly standardized, comprehensive assessment of newborn neurobehavior (Brazelton, 1973). Canals et al. (2011) confirmed that neonatal self-regulation behaviors were the best predictors of infant development and intelligence and that NBAS could be a useful tool to observe behaviors related to later development in healthy infants. Based on the method of Brazelton and Amiel-Tison for behavioral neurological measurement in newborns as well as

Abbreviations: Mn, Manganese; NBNA, neonatal behavioral neurological assessments; LOD, limit of detection; ICP-MS, Inductively Coupled Plasma Mass Spectrometry

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their own experience, Bao et al. (1991) formulated Neonatal Behavioral Neurological Assessments (NBNA), and subsequently repeated measurements in children showed that the total scores increased with child age and the NBNA had distinct stability and reliability, and was not influenced by geographic location. Therefore, it is convenient for a large survey (Bao et al. 1993).We used NBNA to measure neurobehavioral development of neonates in the study.

To our best knowledge, the potential association between prenatal Mn exposure on neurobehavior of neonates have not been studied, though it is known that neonatal irritability and self-regulation are related to cognitive development and intelligence both short term and long term (Canals et al., 2011). Furthermore, there are no data available suggesting "safe" level of cord serum Mn. We conducted a study of prenatal Mn exposures and neonatal development in a mother-infant cohort in Shanghai, China. The objectives of the present study are (1) to determine the cord blood level of Mn and the corresponding effects on neurobehavioral development in neonates; and (2) to explore possible nontoxic level of Mn for neonatal neurodevelopment.

2. Methods

2.1. Study subjects

This multicenter study recruited 1377 healthy pregnant women who came to the hospital to deliver a term (37–42 weeks of gestation) singleton infant in 10 maternity hospitals in Shanghai, China, from 2008 to 2009, as we previously described (Yu et al., 2013). A total of 48 infants with disorders closely associated with adverse severe neurodevelopment such as traumatic brain injury, meningitis, epilepsy, severe neonatal jaundice were excluded. Of the 1329 infants, 933 parents signed the consent form to do the NBNA test. The study protocol was approved by the Medical Ethics Committee of Shanghai XinHua Hospital affiliated to Shanghai Jiao Tong University School of Medicine (No.2008.7).

After the women had signed the consent form, an in-person interview was conducted to collect information on social and demographic characteristics and potential sources of metal exposure. Anthropometric measurements of the newborns were made by delivery room staff according to a standard anthropometric protocol. Information on gestational age along with characteristics of the birth and newborn were extracted from the medical records.

NBNA was administered when the infants were 3 days old as previously described (Gao et al., 2007). NBNA assesses functional abilities, most reflexes and responses, and stability of behavioral status during the examination. It contains five clusters: behavior (six items), passive tone (four items), active tone (four items), primary reflexes (three items), and general assessment (three items). Each item has three levels (0, 1 and 2). Twenty items have a maximal total score of 40. Neonates with a total score of equal to or more than 37 are considered well developed while below 37 are considered low NBNA (Bao et al., 1991). NBNA assessments were conducted by ten examiners who were rigorously trained and certified by the creator of Chinese NBNA, Professor Bao (Bao et al., 1991).

Umbilical cord blood was collected and serum was separated. All the samples were immediately frozen at $-40\,^{\circ}C$ and shipped in batches to the central laboratory. Serum Mn concentration was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (7500 CE, Agilent) as described previously (Yu et al., 2013). The limit of detection (LOD) for Mn was 0.13 μg/L.

2.2. Statistical analysis

Cord serum concentrations of Mn were expressed as μg/L. Values below LOD were imputed using the default value of ½ LOD (6 of 933 subjects were below LOD). We first examined the distribution of NBNA score and Mn level. The latter was severely skewed towards the left. Thus, we performed log transformation before analysis. We divided study population into four groups based on quartiles of LgMn (Table 1) and performed univariate analysis and multiple regression examining group difference of both mean NBNA score and percent of low NBNA (Tables 2 and 3). We then applied a generalized additive model to estimate the independent relationship between LgMn and NBNA scores, with adjustment for potential confounders (Fig. 1).

Table 1
Characteristics of mothers-infants and the associations with cord serum manganese.

	Lg Mn quartiles				***P* value**
	< P25 (Mn < 2.7 μg/L)	**P25–50 (Mn:2.7–4.0 μg/L)**	**P50–75 (Mn:4.1–9.0 μg/L)**	**≥ P75 (Mn ≥ 9.1 μg/L)**	
N	231	228	239	235	
NBNA Score	39.4 ± 0.9	39.5 ± 0.9	39.4 ± 1.0	38.2 ± 1.6	< 0.001
Behavior	11.8 ± 0.5	11.8 ± 0.5	11.8 ± 0.6	11.2 ± 1.1	< 0.001
Active tone	7.7 ± 0.5	7.8 ± 0.4	7.7 ± 0.6	7.2 ± 0.9	< 0.001
Passive tone	7.9 ± 0.3	7.9 ± 0.3	7.9 ± 0.3	7.9 ± 0.4	0.235
Primary reflexes	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.1	6.0 ± 0.2	0.849
General reactions	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.0	0.050
Birth weight (g)	3454.8 ± 410.7	3396.4 ± 429.1	3347.2 ± 393.3	3415.6 ± 418.2	0.047
Gestational age (d)	275.9 ± 8.6	276.7 ± 7.8	275.8 ± 7.6	275.3 ± 7.1	0.341
Maternal age (y)	27.1 ± 3.9	27.3 ± 4.2	27.0 ± 4.7	28.3 ± 4.0	0.010
Gender(%)					0.396
male	53.7	49.8	57.1	51.1	
female	46.3	50.2	42.9	48.9	
Household incomes (Yuan/m/Person)(%)					< 0.001
< 2000	52.1	51.0	48.0	28.5	
2000–5000	34.3	35.3	34.4	35.7	
> 5000	13.6	13.7	17.6	35.7	
Maternal education(%)					< 0.001
Middle school or lower	39.3	41.3	40.4	21.5	
High school	22.8	418.8	18.3	14.9	
Bachelor degree	33.9	37.2	38.7	54.8	
Higher than bachelor degree	4.0	2.7	2.6	8.8	
Paternal education (%)					< 0.001
Middle school or lower	36.9	34.7	35.2	15.2	
High school	21.2	21.2	18.9	16.6	
Bachelor degree	37.4	38.7	42.5	58.3	
Higher than bachelor degree	4.5	5.4	3.4	9.9	
Maternal occupation (%)					0.001
White collar	22.1	22.2	24.4	13.3	
Technician	27.1	29.2	27.4	15.0	
Blue collar	49.7	47.6	46.7	69.9	
Housewife	1.1	1.1	1.5	1.7	