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Elevated manganese exposure and school-aged children's behavior: A gender-stratified analysis

José A. Menezes-Filho [a,*], Chrissie F. de Carvalho-Vivas [b], Gustavo F.S. Viana [a], Junia R.D. Ferreira [a], Lorena S. Nunes [a], Donna Mergler [c], Neander Abreu [b]

[a] College of Pharmacy, Federal University of Bahia, Brazil
[b] Institute of Psychology, Federal University of Bahia, Brazil
[c] Centre de Recherche Interdisciplinaire sur la Biologie, la Santé, la Société et l'Environnement (CINBIOSE), Université du Québec á Montreal, Canada

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ABSTRACT

High levels of waterborne manganese have been associated with problematic behavior in school-aged children, however to date this has not been reported for children exposed to airborne manganese. The objective of the present study was to examine behavioral traits among children with exposure to airborne manganese from a ferro-manganese alloy plant, located in the metropolitan region of Salvador, Brazil. The study included 34 boys and 36 girls, aged 7–12 years, living in two communities within a 3-km radius from the plant. For each child, hair manganese levels (MnH) and blood lead (PbB) levels were analyzed by graphite furnace atomic absorption spectrometry. The Children's Behavior Check List (CBCL) (Portuguese version validated in Brazil) was administered to parents or caregivers, providing scale scores of internalizing (withdrawn, somatic complaints, and anxious/depressed scales), externalizing (disruptive and aggressive) behaviors and a separate scale for attention problems. Median and range for MnH and PbB were 11.48 μg/g (range: 0.52–55.74); 1.1 μg/dL (range: 0.5–6.1), respectively. Spearman correlation analyses showed that several behavioral indices were significantly correlated with MnH levels for girls, but not for boys: total externalizing behavior (rho = 0.484 vs rho = 0.041) and attention problem scores (rho = 0.542 vs rho = 0.003) coefficients were significantly at $p < 0.001$ level, respectively for girls and boys. No significant correlation was observed with any of the internalizing sub-scales. A linear regression model was fitted with the total externalizing behavior, inattention and total CBCL scores as dependent variables, with log transformed MnH stratified by sex, adjusting for age and maternal IQ. Total externalizing behaviors and attention problem scores were significantly associated with girls' MnH levels but not with boys'. Adjusting for maternal IQ, the β-coefficients for LogMnH associations with total externalizing and attention problems are 8.85 (95%CI 2.44–15.24) and 4.03 (95%CI 1.50–6.56) for girls. For boys, after adjusting for age, the β-coefficients are 0.08 (95%CI 11.51–11.66) and −0.05 (95%CI 4.34–4.25), respectively. The findings of this study suggest a positive association between elevated Mn exposure and externalizing behavioral problems and inattention, with girls presenting more pronounced effects. Future studies on Mn exposure in children should attempt to further elucidate sex and/or gender differences in Mn exposed populations.

1. Introduction

Manganese (Mn) is an essential trace metal, involved in many functions, including bone mineralization, metabolic regulation and protection against oxidative processes (Roth, 2006). It can also be a potent neurotoxin in cases of excessive exposure due to widespread accumulation in the brain (Guilarte et al., 2006). There is an extensive literature dating from 1837 on the neurological and neuropsychological consequences of occupational exposure to Mn among miners and industrial workers (Levy and Nassetta, 2003; Iregren, 1999; Huang et al., 1997; Mergler and Baldwin, 1997). More recently, environmental exposures to air and water-borne Mn have been investigated, particularly among children (for review see: Menezes-Filho et al., 2009; Zoni et al., 2007; Rodríguez-Barranco et al., 2013). Primary school children with elevated Mn exposure consistently display dose-related cognitive deficits (Wasserman et al., 2004, 2006; He et al., 1994; Bouchard et al., 2011; Menezes-Filho et al., 2011; Riojas-Rodríguez et al., 2010; Torres-Agustın et al., 2013). Several studies suggest

* Corresponding author at: Laboratory of Toxicology, College of Pharmacy – Federal University of Bahia (UFBA), Av. Barão de Jeremoabo s/n Campus Universitário de Ondina, 40170-115 Salvador, Bahia, Brazil. Tel.: +55 71 3283 6960; fax: +55 71 3283 6019.
E-mail address: antomen@ufba.br (J.A. Menezes-Filho).

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that there may be sex differences, with girls presenting greater effects compared to boys (Roels et al., 2012; Bouchard et al., 2007; Riojas-Rodríguez et al., 2010; Torres-Agustın et al., 2013).

Excess Mn has also been associated with hyperactivity and other behavior disorders in children (Khan et al., 2011; Yousef et al., 2011; Farias et al., 2010; Bouchard et al., 2007; Ericson et al., 2007). Two studies examined children exposed to elevated water-borne Mn. In a community in Quebec, Canada, with high Mn concentrations in their drinking water, Bouchard et al. (2007) reported higher scores on teacher-rated hyperactive and oppositional behavior among children with higher hair Mn, after adjusting for age, sex, and family income. In Bangladesh, Khan et al. (2011) found that children's internalizing and externalizing behaviors, rated by their teachers, were significantly associated with Mn water concentrations, after adjusting for arsenic and demographic co-variables. Other studies have reported associations between Mn and children's behavior patterns, but the possible sources of external Mn exposure were not investigated (Yousef et al., 2011; Farias et al., 2010). Ericson et al. (2007) measured Mn content in shedding teeth. After adjusting for levels of lead in the tooth enamel, children with high levels of Mn had higher scores on all scales of disinhibitory behavior. To date, no study has examined boy' and girls' behaviors in areas where there is elevated airborne Mn.

We have been evaluating children's exposure to manganese from the atmospheric emissions of a ferro-manganese alloy plant in the municipality of Simões Filho, Bahia, Brazil since 2007 (Menezes-Filho et al., 2009, 2011). Air Mn levels in $PM_{2.5}$, the respirable fraction; measured during seven days in 2008 in Cotegipe village presented an average 0.151 $\mu g/m^3$ (range 0.011–0.439 $\mu g/m^3$) (Menezes-Filho et al., 2009). These levels are approximately sevenfold higher than that reported for urban air (0.02 $\mu g/m^3$) (ATSDR, 2012) and vary with distance from the plant and wind direction. Median and range hair Mn levels (MnH) were 9.70 $\mu g/g$ (1.10–95.50 $\mu g/g$). We also reported that cognitive function in school-aged children and their mothers was negatively and significantly associated with Mn hair concentrations (Menezes-Filho et al., 2011).

The present study included children from the community of Cotegipe, as well as the neighboring community of Santa Luzia, Bahia, Brazil. The objective was to examine the association between biomarkers of Mn exposure and their behavior, taking into account relevant co-variables.

2. Materials and methods

The two villages, included in this study, are situated in the municipality of Simões Filho, 30 km from the city of Salvador, State of Bahia, Brazil (Fig. 1). Santa Luzia and Cotegipe are small communities of less than 700 people each. Both communities reside in a 3-km radius from the above-mentioned ferro-manganese alloy plant and mostly in a downwind direction.

2.1. Study design and population

A cross-sectional study design was used. All children aged 7–12 years, who attended the community's elementary schools, were invited to participate. The inclusion criteria were: living in one of the communities for at least five years, being officially enrolled and regularly attending one of the schools. The exclusion criteria were: having an estimated IQ below 68 [which is the low limit for borderline intelligence IQ 70 ± 3.2 (Figueiredo, 2002)], clinical diagnostic of any neurologic problems and hearing disability. Estimated IQ was generated from the sum of the standardized scores of the Vocabulary and Block Design subtests of the Wechsler Intelligence Scale for Children and converted into IQ (Mello et al., 2011).

The school principals provided us with a list of the children enrolled and regularly attending classes. We sent out invitations to the parents or legal guardians of all the children in the specified age range. Those who accepted to participate provided written consent and answered a structured questionnaire upon interview. In order to maintain confidentiality, participating children were properly coded and only the principal investigator and field coordinator had access to the data bank. A total of 95 children from the two communities fulfilled the inclusion criteria. Of these, the parents of 78 children (82%) accepted to participate as volunteers. Three children were excluded, one for having estimated IQ below 68, another had hearing impairment and one had Down's syndrome; five refused to provide biological samples. The final study population was composed of 70 children, 74% of all of the children in the specified age range.

The Simões Filho Education Department gave permission to use the school premises and the community leader gave us full support and provided two rooms in the Santa Luzia Community Center where we carried out the interviews. In Cotegipe village, the local elementary school premises were used. The project was approved by the Federal University of Bahia Ethics Committee.

Questionnaires: Trained interviewers applied structured questionnaires to collect socio-economic and life style information of the volunteered families. The socioeconomic classification from the Brazilian Association of Population Studies (ABEP, 2011), based on the possessions of comfort items in the home (washing machine, fridge and vacuum cleaner, car, color TV, bathroom, etc.) was applied. The total ABEP score is ranked into five descending classes (from A to E).

Anthropometry: The same person performed all weight and height measurements. Children took off their shoes but kept their clothes for both assessments. Weight was measured using an upright scale (Urano UP150S), with a capacity to weigh 150 kg in 100-g increments. Height was taken using a measuring board. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. Height-for-age (HA) z-score was calculated using the AnthroPlus software (WHO, 2009) based on the WHO reference 2007 for 5–19 years.

Hair measurements: A tuft of hair of approximate 0.5 cm diameter was cut off as close as possible to the scalp in the occipital region, with a surgical stainless steel scissor and stored in plastic bag until processing. Detailed information on hair sampling, washing procedure and Mn determination by GFAAS are reported elsewhere (Menezes-Filho et al., 2009). Briefly, the first centimeter or the amount available was washed for 15 min in 10 mL of 1% Triton X-100 solution in a 50-mL beaker in ultrasonic bath. Rinsing was performed several times with Type I pure water (Milli-Q, Millipore®). Hair samples were dried wrapped in Whatman N#1 filter paper in a drying oven at 70 °C overnight. Approximately 10 mg of hair was weighed in 50-mL beaker and digested with 2 mL of spectroscopic grade concentrated HNO_3 acid for 2 h on a 90 °C hotplate. The digest was then diluted to 10 mL with Type I pure water in a polypropylene centrifuge tube (Corning®). Acid digested samples and reference material (Human hair from International Atomic Energy Agency, IAEA-085) were analyzed using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). The intra-batch and batch-to-batch precisions were 2.36% and 5.90%. All samples and reference materials were analyzed in duplicates and a difference of 10% or less was considered acceptable.

Blood measurements: Children's venous blood samples were collected from the cubital vein into sodium–EDTA vacuum tubes proper for metal analysis (Vacutainer, Bencton Dickinson, USA). Lead (Pb) is an ubiquitous contaminant and a recognized neurotoxin, associated with effects on cognition and behavior in children at low blood Pb (PbB). PbB concentration was also

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