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Prenatal exposure to low-level methylmercury alters the child's fine motor skills at the age of 18 months☆

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ABSTRACT

Objectives: To compare motor, cognitive and language characteristics in children aged 18 months who were prenatally exposed to low-level methyl-mercury (MeHg), and to analyze the eventual differences in these characteristics in relation to cord blood THg concentration.

Patients and methods: The total number of 205 child-mother pairs was included in the study, and total cord blood mercury was measured in 198 of them. Out of the 198 already measured samples, 47 of them have also been tested for methyl-mercury in cord blood. Data regarding the 47 samples of MeHg levels has been used for calculating the correlation between cord blood THg and cord blood MeHg. MeHg and THg showed a significant correlation ($r=0.95$, $p < 0.05$). One month after the delivery, mothers were asked to complete the questionnaire regarding socioeconomic factors, breastfeeding of their infants, and dietary habits during pregnancy. Neurodevelopmental assessment of motor, cognitive and language skills were conducted on 168 children using The Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III). Regarding the cord blood THg concentration, 135 children were divided in 4 quartile groups. Their neurodevelopmental characteristics have been compared.

Results: The cord blood THg concentration median and inter-quartile range was 2.98 ng/g (1.41–5.61 ng/g). There was a negative correlation between cord blood THg concentration and fine motor skills (rho=−0.22, p=0.01). It is evident that children grouped in 2nd ,3rd and 4th quartile had statistically significant lower fine motor skills assessment related to those grouped in 1st quartile (2nd quartile −1.24, p=0.03; 3rd quartile −1.28, p=0.03; 4th quartile −1.45, p=0.01). The differences in fine motor skills assessments between children in 2nd and 3rd and 3rd and 4th quartile were not statistically significant.

Conclusion: Intrauterine exposure to low-level THg (MeHg) is associated with alterations in fine motor skills at the age of 18 months.

1. Introduction

Neurotoxic effects of mercury (Hg) on human central nervous system (CNS) are well known (Myers et al., 2000a). Human fetuses are particularly affected when exposed to a high dosage (Harada, 1995). Even though severe Hg poisoning is extremely rare, chronic low-level exposure (< 100 ppm in maternal hair) mainly by fish and sea food consumption is very common (Karagas et al., 2012). Thus, methyl-mercury (MeHg), neurotoxic form of Hg, which is produced from inorganic Hg by biomethylation, accumulates in fish. Despite numerous findings regarding neurodevelopmental effects of chronic low and moderate prenatal MeHg exposure were still inconsistent (Karagas et al., 2012; Bose-O'Reilly et al., 2010). Most cited are studies from Seychelles Island and Faroe Islands with completely opposite results regarding the effect of prenatal Hg exposure on child neurodevelopment (Grandjean et al., 1997; Myers et al., 2003b). While

☆ The work described in this manuscript has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The research protocol has been reviewed and approved by the two ethics committee; Ethics Committee of the University of Rijeka and Ethics Committee of the University Hospital Centre Rijeka, Rijeka, Croatia.

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Seychelles study found no negative association, Faroe study found negative association between Hg and child neurodevelopment. Schoeman et al. proposed several reasons in their review which explain why different results might be obtained despite overall similar Hg exposures (Schoeman et al., 2009). The most comprehensible causes include differences in study populations, confounders, outcome measures, Hg concentrations, biological samples and eventually other possible contaminants in fish.

Therefore, a prospective study to investigate the effects of prenatal low level Hg exposure from maternal consumption of fish and seafood on child neurodevelopment was conducted (http://phime.oikon.hr). Here we present the results regarding the impact of the intrauterine exposure to mercury on the development of children at the age of 18 months from Croatian cohort.

2. Study population and methods

Two-hundred-five (205) pregnant women and their children (evaluated in the newborn's age and at the age of 18 months) were included in the study. The pregnant women were permanent residents of the study area for at least 2 years - the coastal city of Rijeka, Croatia and its county (Primorsko-goranska). The basic characteristics of study sample were shown on Table 1. The detailed description of the study protocol, inclusion and exclusion criteria have been described and published elsewhere (Valent et al., 2013a). The research protocol was approved by the Ethics Committee of the University of Rijeka and by the Ethics Committee of the University Clinical Centre Rijeka, Croatia.

Cord blood total Hg (THg) concentration has been measured in 198 of examinees, and MeHg measurement on 47 examinees out of this same group (47 subjects have been tested for both THg and MeHg levels). These data was used to calculate the correlation between cord blood THg and MeHg in our study group.

THg determination: THg in blood was determined by thermal combustion at 650 °C, amalgamation and atomic absorption spectrometry using a Direct Mercury Analyzer (Milestone, USA). The procedure has been described in detail elsewhere (EPA Method 7473, 1998). About 0.200 g of blood was weighed in a sample boat. The reference material (RM) Seronorm Trace Elements in Whole Blood L-1 (LOT No: MR4206) was used to check the accuracy of the results for THg in blood and the value found (2.2 ± 0.18 ng/ml) was in good agreement with the reference value (2.2 ± 0.2 ng/ml). The limit of detection (LOD) of the method calculated as three times the standard deviation of the blanks was 0.02 ng/g blood, while the limit of quantification (LOQ) calculated as ten times the standard deviation of the blanks was 0.07 ng/g blood. The estimated uncertainty for THg in blood samples at levels higher or equal to 1 ng/g was 7% (k=2).

MeHg determination: About 200 mg of blood sample was weighed directly in a 30 ml screw capped Teflon vial in which acid leaching using mixture of 5% H_2SO_4, (p.a.), 18% KBr (p.a.) and 1 M solutions of $CuSO_4$ (p.a.) performed. MeHgBr was extracted into CH_2Cl_2 and back extracted into aqueous phase. MeHg was then ethylated, and purged as methyl-ethyl-mercury (MeHgEt) onto a Tenax trap followed by thermal desorbed at 180 °C. MeHgEt was then separated on an isothermal GC column. Hg species were converted to Hg(0) by pyrolysis at 600 °C and measured by a cold vapour atomic fluorescence detector (CV AFS). The procedure has been described in detail elsewhere (Horvat et al., 1993; Liang et al., 1994).

The accuracy of the results for MeHg in mother's blood or cord blood and MeHg was checked by analysing RM lyophilised whole human blood PT-WB1 obtained from a non-exposed population which was used a quality control material. MeHg in PT-WB1 was determined by the laboratories participating in the PHIME interlaboratory comparison. The determined value (6.2 ± 0.3 ng/g) was in good agreement with the assigned value (6.3 ± 0.5 ng/g). The estimated uncertainty of MeHg values in blood samples was 12% (k=2). The LOD of the method for MeHg determination in blood calculated on the basis of three times the standard deviation of the blanks was 0.02 ng/g blood, while the LOQ calculated as ten times the standard deviation of the blanks was 0.07 ng/g blood.

After the delivery, mothers were asked to complete the questionnaire regarding socioeconomic factors, breastfeeding of their infants, dietary habits during pregnancy including detailed food frequency

Table 1
General characteristics of the study populations of mother-child pairs.

n	**205**
Boys, n (%)	102 (49.7%)
Girls, n (%)	103 (50.3%)
Gestational age (weeks) mean (min-max)	39.2 (34–41)
Birthweight (g) mean (min-max)	3591 (2400–4820)
Mother's age at delivery (years) mean (min-max)	30 (19–42)
BMI (kg/m^2) before pregnancy mean (min-max)	22.8 (16.9–40.7)
Education	
Primary school, n (%)	5 (2%)
Middle school, n (%)	122 (60%)
High school, n (%)	20 (10%)
University degree, n (%)	58 (28%)
Residential environment	
Centre, n (%)	55 (27%)
Periphery, n (%)	109 (53%)
Rural, n (%)	41 (20%)
Breastfeeding in the 6th weeks of life	
Exclusive or predominant, n (%)	142 (69%)
Partial or formulae, n (%)	63 (31%)
Cigarette smoke during pregnancy	
Yes	25 (12%)
No	180 (88%)
No. of amalgam fillings	
up to 3, n (%)	196 (96%)
3–9, n (%)	9 (4%)
10 or more, n (%)	0 (0%)
Consumption of sea fish	
Less than once per month, n (%)	11 (5%)
1–3 times per month, n (%)	67 (33%)
At least once per week, n (%)	127 (62%)