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Original Research

Effects of Elevated Levels of Manganese and Iron in Drinking Water on Birth Outcomes

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Abstract

We examined the impact of elevated exposure levels of pregnant women to manganese and iron through drinking water on pregnancy outcomes. We conducted an epidemiological study among 16,408 pregnant women of Kaunas. We assessed each woman at her residence for exposure to manganese and iron levels measured in four Kaunas public water supply networks. We used a logistic regression to model the association between drinking water quality and birth outcomes controlling the confounding variables. Analysis yielded an increase in adjusted odds ratios (AOR) for term low birth weight (LBW) for moderate exposure level, 1.53 (95% confidence interval (CI) 0.89-2.66); and 1.70 (95% CI 1.07-2.71) for high exposure level. Maternal exposure was associated with a mean reduction of 21 g (SE, 9 g; p=0.02) in birth weight. No associations were observed between manganese and iron levels and preterm birth. These findings suggest that elevated levels of manganese and iron in drinking water are associated with a reduction in birth weight in term-born infants. However, further individual-level epidemiologic studies are necessary to investigate the factors that contribute to the increased sensitivity of some pregnant women.

Keywords: drinking water, manganese, iron, low birth weight, term low birth weight, preterm birth, case control study

Introduction

Epidemiological studies on susceptible population subgroups, such as pregnant women, have become an important issue for public health regulation. Assessment of the impact of exposure of metals though drinking water on pregnancy outcomes provides data for risk characterization and for framing guidelines regarding the level of contaminants in drinking water [1].

Manganese and iron are metals found in water, the quantities of which depend on the geology of an area. A large percentage of the population in Lithuania and other Baltic countries face potential risk of exposure to elevated levels of manganese and iron through drinking water, since approximately 30 percent of the collected groundwater samples exceed the limits set by the Council of the European Union directive 98/83/EC on the quality of water intended for human consumption regarding standards for manganese and iron [2]. Although these are essential nutrients, when present in high concentrations in drinking water they have been found to be associated with various health issues [3].

Experimental studies on fetal development, investigating the toxic effect of manganese, showed that exposure to

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manganese results in a decrease in fetal weight and retardation of the development of the skeleton and internal organs [4]. In addition, manganese caused DNA damage and chromosomal aberrations and was toxic to the embryo and fetus [5]. Epidemiological studies of residents exposed to manganese revealed a neurotoxic effect [6] and Parkinson-like syndrome [7]. In individuals exposed to high levels of this metal, manganese can accumulate in various brain regions, leading to neurotoxicity [9]. It has been postulated that manganese may be involved in the generation of reactive oxygen species and production of oxidative stress [10, 11].

The true health burden of excessive ingestion of manganese is currently unknown. How important maternal exposure to high manganese concentrations through drinking water might be for determining the outcome of pregnancy is also not clear.

Molecular studies of cellular iron toxicity show that iron-mediated tissue damage involves cellular oxidizing and reducing processes and toxic effects on intracellular organelles, in particular mitochondria and lysosomes [12]. Excess iron intake forms an iron-dexorubin complex that can catalyze reactions, generating a variety of free radicals capable of causing cellular damage. DNA is one of the most important targets of iron-produced hydroxyl radical attack that may lead to cell damage, mutation, and malignant transformations [13]. Therefore, exposure to excess iron doses during intrauterine life may have an effect on the alteration of genetic structure of the phenotype, which predisposes susceptibility to a wide range of common diseases [14].

To the best of our knowledge, no epidemiological study has evaluated manganese and iron in drinking water as risk factors for LBW in humans. This epidemiological study was aimed at exploring the possible association between exposure to manganese and iron though drinking water during the prenatal period and LBW as well as preterm birth risk. Individual data on residential addresses, smoking, education, family status, and other risk factors were collected so that we could evaluate the confounders and possible interactions.

Materials and Methods

Study Population and Birth Outcome

We conducted a population-based epidemiological study in Kaunas city. The study population consisted of a cohort of pregnant women who were residents of Kaunas between January 1, 1995, and December 31, 1998 (n=17,366). The study and its consent procedure were approved by the institutional review board of Kaunas University of Medicine and the Lithuanian Bioethics Committee.

Individual level data on birth outcomes were obtained from the Lithuanian Medical Birth Registry. The database records contain information on maternal sociodemographic variables (birth date, home address at delivery, education, and marital status), data on the course of the current pregnancy, maternal and paternal hazardous habits (smoking, alcohol consumption), and newborn health indicators (birth weight, gestational age, medical pathological data). In this study, multiple births and newborns with no valid information on birth weight, gestational age, or address were excluded from analysis. After exclusions, 16,408 (94.5% of all registered) subjects were eligible for the study. The mean birth weight was 3,489 ± 10 g, the mean gestational age was 39.44 ± 0.02 weeks.

The outcomes of interest were LBW, term LBW, and preterm births (codes P07.0-P07.3, International Classification of Diseases, 10th revision). The group of LBW cases comprised newborns with a birth weight <2,500 g. This group included both premature newborns (<37 weeks of gestation) and small-for-gestational-age (term LBW) newborns. The term LBW group included newborns with fetal growth retardation: their birth weight is below the 10th percentile by gender and gestational age. These primary data were linked to the information on manganese and iron levels in drinking water of residential districts.

Drinking Water Exposure Assessment

The objective of our assessment of drinking water was to assign manganese and iron levels based on the exposure of pregnant women at their maternal residential addresses during their pregnancy. Kaunas municipal water supply system consists of four networks (tree type). Until 2000 all four Kaunas municipal water supply companies did not remove manganese and iron from underground water supplied, and chlorination was not used for water treatment.

The municipal Kaunas waterworks maintain databases of historical public water treatment data and residential addresses of water users. We contacted water companies and collected information on manganese and iron levels corresponding to each street address. To assess the exposure through drinking water, we used manganese and iron measurement data for samples taken from the four water supply networks system of Kaunas city. We computed mean concentrations of metals and linked these levels to residential addresses of pregnant women. We used the Council of the European Union directive 98/83/EC on the quality of water intended for human consumption standard of manganese and iron [2], and Lithuanian recommendations for drinking water standards to classify the quality of drinking water into three categories (Table 1):

- the first category – high-quality water (low exposure to manganese and iron) – average concentrations of manganese and iron did not exceed standards (Mn 100 mg/L, Fe 300 mg/L);
- the second category – moderate quality (moderate exposure) – only manganese level exceeded standards; and
- the third category – water of insufficient quality (high exposure) – average levels of both manganese and iron exceeded standards.

Table 1. Distribution of the study participants and birth outcomes across the manganese (Mn) and iron (Fe) exposure categories.

Exposure category	Mean concentration (μg/L)		All participants	Low birth weight (LBW)		Term LBW		Premature birth	
	Mn±SD	Fe±SD	N=16,408	N=536	I*	N=171	I*	N=658	I*
Low	68±11	97±32	3,377	103	3.05	22	0.65	131	3.88
Moderate	110±10	117±13	3,175	103	3.24	35	1.10	142	4.47
High	375±40	835±108	9,856	330	3.35	114	1.16	385	3.91

*Incidence per 100

Data Analysis

To identify the potential confounding factors, the distribution of known risk factors for LBW, we examined different exposure categories. All factors that were not equally distributed were considered potential confounders. In the analysis of association between exposure to manganese and iron through drinking water and birth outcomes, the following variables were included in the multivariate regression models: maternal age, newborn sex, family status, maternal education, maternal smoking, parental smoking, and infant birth order. To evaluate the association between exposure to manganese and iron and birth outcomes, the risk to deliver LBW, term LBW, and premature newborns was compared among women exposed to different levels (three levels of water quality) of manganese and iron. We used a multivariate logistic regression to model the associations and estimate adjusted OR and their 95% confidence intervals while controlling for selected confounders.

In the evaluation of associations between different variables and the risk of LBW or premature births, newborns were assigned to two categories (cases and controls): newborns with LBW (<2,500 g cases) and normal birth weight (≥2,500 g controls); premature newborns (<37 weeks of gestation cases) and term-born newborns (≥37 weeks of gestation controls); term LBW newborns (≥37 weeks of gestation, <2,500 g cases) and term normal birth weight newborns (≥37 weeks of gestation, ≥2,500 g controls).

To estimate the incidence rate ratio for the differently exposed subjects, we computed adjusted OR. We adjusted the OR for known and potential risk factors for LBW including maternal age (categories: ≤19 years, 20-29 years (reference group), ≥30 years), years of education (≤12 years, >12 years), maternal and paternal cigarette smoking (nonsmoker, smoker), newborn sex (male, female), and infant birth order (first vs. later). We evaluated the exposure–response association of manganese and iron levels in drinking water and risk of LBW, term LBW, and premature birth using multiple logistic regressions and controlling effects of seven variables selected.

Results

Table 1 shows the distribution of the study participants according to the exposure to manganese and iron levels in drinking water. The mean manganese level of low-exposure zone was 68 μg/L, of moderate-exposure – 110 μg/L, and of high-exposure – 375 μg/L. The mean iron levels ranged from 97 μg/L in the low-exposure zone to 835 μg/L in the high-exposure zone. Most (60.1%) of the pregnant women in our study were exposed to water manganese and iron levels above the standard levels. We assessed the incidence of birth outcomes in three exposure zones. The incidence of LBW and term LBW increased by increasing exposure, while the incidence of preterm birth did not show such tendency.

The factors that affect the development of a fetus may act together and have a cumulative effect. To identify risk factors for LBW and term LBW in the study population, the distribution of variables between cases and controls was examined and crude OR were calculated (Table 2).

The distribution of studied risk factors of LBW and term LBW were similar in both groups. There were significant differences with respect to education, family status, smoking, newborn sex, and birth order in cases and controls. About 61.6% of the LBW infants were born to mothers who were exposed to high levels of manganese and iron. A comparison of women exposed to high levels of manganese and iron with women exposed to low levels of manganese and iron showed crude OR for LBW to be 1.10 (95% CI, 0.88-1.38). Compared with low exposure, increasing exposure levels were associated with an increased risk of term LBW (OR=1.70 for moderate and OR=1.78 for high exposure).

To remove the effect of differences in the prevalence of independent variables, we adjusted the crude OR for the identified predictors of LBW. Table 3 shows the adjusted association between exposure to water manganese and iron and birth outcomes. Adjustment for selected LBW risk factors did not alter the association between exposure categories and LBW risk. Analysis yielded a statistically insignificant increase in adjusted OR for LBW for the moderate-exposure zone compared with the low-exposure zone, 1.01 (95% CI, 0.76-1.34), and 1.11 (95% CI, 0.88-1.39) for the high- to low-exposure water zone.

The analysis data for term LBW indicated the exposure–response relationship: chi-square for the trend was 7.09 (p=0.029); and adjusted OR were as follows: 1(referent), 1.53 (95% CI, 0.89-2.66), and 1.70 (95% CI, 1.07-2.71). We found no association in analysis of exposure-response relationship for preterm birth.

Multiple linear regression analysis showed that maternal exposure during pregnancy was associated with lower

Table 2. Distribution of potential risk factors for low birth weight (LBW) and term low birth weight (Term LBW) among cases (%) of crude odds ratios (OR) and 95% confidence intervals (CI).

Risk factors	Total N=16,408 LBW cases 536			Total N=16,408 Term LBW cases 171		
	%	OR	95% CI	%	OR	95% CI
Age						
20-29 years	58.6			62.0		
≤ 19; ≥30 years	41.4	1.46	1.23-1.74	38.0	1.25	0.92-1.71
Family status						
Married	77.6			78.9		
Single	22.4	2.57	2.08-3.16	21.1	2.30	1.58-3.33
Education						
> 12 years	35.6			38.0		
≤ 12 years	64.4	1.62	1.36-1.94	52.0	1.45	1.06-1.97
Maternal smoking						
Nonsmoker	90.3			83.6		
Smoker	9.7	4.70	3.46-6.37	16.4	8.19	5.39-12.43
Paternal smoking						
Nonsmoker	50.0			56.1		
Smoker	50.0	1.75	1.47-2.08	43.9	1.35	0.99-1.82
Newborn sex						
Female	45.7			632		
Male	54.3	1.31	1.11-1.56	36.8	1.89	1.38-2.59
Birth order						
First	54.7			56.7		
Later	45.3	0.71	0.60-0.85	43.3	0.66	0.49-0.90
Exposure						
Low	19.2		1	12.9		1
Moderate	19.2	1.07	0.81-1.41	20.5	1.70	0.99-2.90
High	61.6	1.10	0.88-1.38	66.6	1.78	1.13-2.81

infant birth weight. The mean birth weight of the LBW infants was 14 g lower for the high exposed group (p=0.11) and that of the term LBW infants was 21 g lower (p=0.02) compared to the low exposed group. There was no statistically significant difference in infant gestational age for moderate and high exposure levels.

Discussion

The results of this population-based case-control study show that exposure to manganese and iron through drinking water during pregnancy slightly increases the risk of LBW. This effect was not explained by preterm births and other measured predictors of LBW, such as age, education, or tobacco smoking, since we controlled the effect of these covariates during multivariate analysis. The study indicated the exposure-response relationship between the exposure to manganese and iron through drinking water and term LBW, indicating fetal growth retardation. This suggestion was tested by controlling the effect of selected risk factors. The adjustment somewhat reduced the excess risk of term LBW associated with exposure metals studied, but the risk increased. The adjusted OR for term LBW increased by 53% in the moderate-exposure group and by 70% in the high-exposure group (chi-square for trend was 7.09; p=0.029). We found no consistent pattern of association between exposure to metals and preterm birth. Such results imply that intrauterine growth retardation, but not lower gestational age, may explain the effect of water contamination by manganese and iron concentrations, exceeding standards, on birth weight.

Table 3. Adjusted odds ratios (AOR) for low birth weight, term low birth weight (Term LBW) and preterm birth by manganese and iron exposure category.

Exposure category	Low birth weight		Term LBW**		Preterm birth	
	AOR*	95% CI	AOR*	95% CI	AOR*	95% CI
Low	1		1		1	
Moderate	1.01	0.76-1.34	1.53	0.89-2.66	1.13	0.89-1.45
High	1.11	0.88-1.39	1.70	1.07-2.71	1.03	0.84-1.27
	β^1 g (SE)	P	β^1 g (SE)	P	β^2 weeks (SE)	P
Low	Ref		Ref		Ref	
Moderate	- 9 (11)	0.40	- 6 (11)	0.60	0.03 (0.04)	0.43
High	-11 (9)	0.22	- 19 (9)	0.04	- 0.01 (0.01)	0.61
	β^{1}* g (SD)	P	β* g (SD)	P	β^{2}* weeks (SE)	P
Low	Ref		Ref		Ref	
Moderate	-3 (10)	0.8	1 (11)	0.89	0.04 (0.04)	0.37
High	-14 (8)	0.11	- 21 (9)	0.02	-0.03 (0.01)	0.33

*Adjusted for age, family status, education, maternal and paternal smoking, newborn sex, birth order.
**Chi square for trend 7.09, df = 2, p = 0.029.
β^1 represent the difference in mean birth weight and β^2 – gestational age at birth (both continuous variable) for exposure category in each row adjusted for gestational age.
β^{1}* and β^{2}* as above, adjusted for family status, maternal and paternal smoking, and newborn sex.
SE standard error of the difference between the means.

The limited data available from biologically based dose-response studies pointed to the need for evidence of the relationship between chronic exposure to environmental manganese and iron and birth outcomes. Kaunas city was selected for the study because of elevated concentrations of manganese and iron in groundwater, water treatment without chlorination, about 60 percent of the population using drinking water containing high concentrations of these metals, and low rates of migration in and out of the study area.

Some recent studies have reported associations between increased levels of manganese in drinking water and cognitive behavioral problems in children [15]. A few studies in adults have reported neurological dysfunction associated with increasing levels of manganese in drinking water, whereas other studies found no neurological disorders [18]. The nature of cellular damage caused by manganese has been attributed to its capacity to generate cytotoxic levels of free radicals and manganese-related neurodegeneration [7].

Our study results support the experimental studies on drinking water in pregnant rats, which showed that pups from the highest dose group exhibited a significantly decreased weight gain as compared to the other groups [21]. The authors indicated that neonatal subjects are at higher risk for manganese-induced toxicity than adults when compared under similar exposure conditions.

The results of this population-based epidemiological study also indirectly support experimental reproductive studies [4]. The data indicate the effect of fetal growth retardation, which may be attributed to general systemic toxicity of high metal concentrations. Experimental research provides evidence of a sub-chronic exposure via drinking water to a mixture of water-contaminating metals' dose- and time-dependent toxicity in rats [24]. The toxicity manifested as a decrease in body weight, indicating the effect of growth retardation associated with the biotransformation of xenobiotics and generation of free radicals which results in oxidative stress [25].

When present in high doses, iron may also produce neurological effects [26]. Iron is preferentially toxic to cells with high mitochondrial activity and may inhibit the mitochondrial respiratory enzymes NADH-cytochrome c oxidoreductase and succinate dehydrogenase [27]. Long-term iron toxicity may involve iron-mediated oxidative damage to the mitochondrial genome leading to progressive dysfunction [28] and DNA damage [29].

To the best of our knowledge, there is only one recent study on humans that investigated exposure to manganese through drinking water and its impact on infant mortality [30]. The authors reported a possible association between exposure to manganese and all-cause infant mortality. However, given the methodological limitations of this study, the association needs to be confirmed through future work.

The strength of our study includes data of population with equal access to medical care and all main pregnancy outcomes of Kaunas city; this should reduce possible confounding effects of social and demographic variables on

study results. Other strengths of our study include the population-based ascertainment of cases and controls, the large sample sizes, control of the main LBW risk-assessment confounding variables, and use of water-monitoring data blinded to case status for individual residence. Nevertheless, this study has been limited by crude estimates of water exposure. The use of municipal monitoring data for assessing exposure to manganese and iron has limitations, which is attributed to the fact that water samples taken at a water treatment plant a few times per year may not adequately reflect exposure if there is spatial variation across a distribution system. The effect estimates that what we observed may be biased owing to non-differential misclassification of exposure through drinking water since we estimate the category of exposure for a particular residential address. A potential limitation is the lack of data on individual quantity of water consumed. In this study, we were also unable to investigate constituents other than manganese and iron that could be present in drinking water supplies [31]; however, there was no exposure to chlorination by-products.

On the basis of our epidemiological and other experimental data, we might postulate that high manganese and iron levels in drinking water might have an effect on human fetal growth retardation in susceptible women, since exposure to xenobiotics is associated with common toxicity to the embryos and fetus, DNA damage, and xenobiotic-metabolizing genes polymorphism [8]. This further suggests that interaction between metabolic genes and exposure to manganese and iron through drinking water, even at lower levels, could lead to elevated risk of fetal growth retardation. Therefore, we support other authors' suggestions [32] that carefully planned individual-level epidemiological studies are necessary to further investigate this relationship and to set the manganese and iron guidelines for drinking water.

Conclusions

Chronic exposure to manganese and iron through drinking water was associated with increased risk to deliver term LBW newborn. However, we could not exclude the possibility that these findings may also be attributable to other environmental and genetic risk factors. Further research is needed to investigate the interaction between maternal exposure to manganese and iron through drinking water and birth outcomes.

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