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Birth Weight in Relation to Maternal Blood Levels of Selected Elements in Slovenian Populations: A Cross-sectional Study

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Abstract: The objective of the present study was to evaluate the relation between maternal blood levels of selected toxic and potentially toxic elements (manganese (Mn), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), lead (Pb) and mercury (Hg)) and birth weight of their new-borns in a Slovenian population, taking into account maternal socio-demographic characteristics and dietary habits. 535 women from 12 regions of Slovenia were recruited at delivery. Maternal blood was collected at 1.5 months after birth. Associations between birth weight and a) predictors obtained through the questionnaires and b) levels of selected elements were tested using bivariate tests and multiple linear regression. Multiple regression models revealed maternal age as an additional predictor for birth weight and confirmed pre-pregnancy body mass, estimated gestational age and gender of the baby as the main predictors for birth weight. Mn in maternal blood was significantly and positively associated with birth weight. The positive association observed between birth weight and Mn in maternal blood could be explained by the essentiality of Mn in foetal development as an important cofactor in enzyme reactions in bone formation and in metabolic regulation for amino acid, lipid, protein and carbohydrate levels.

Key words: Birth weight, toxic elements, essential elements, maternal whole blood, preventive health, Slovenia.

1. Introduction

Concern over problems from exposure to environmental influences on health impairment of the new-born child requires continuous monitoring of the exposure of a population to environmental agents [1]. In response, many developed countries and international bodies have established large-scale human biomonitoring surveys (HBM) [2], which show the concentrations of chemicals and their metabolites in body tissues including, for example, blood, serum, urine, and breast milk. The levels of environmental chemicals in a person's body fluid and/or tissue are indicative of exposure, but not necessarily the cause of a disease or an adverse effect [3, 4].

In this study, some of the most relevant toxic and potentially toxic elements, listed among the 20 top chemicals on the Priority list of Hazardous Substances [5], were addressed. Among the most susceptible groups are pregnant women and children, the latter because of their increased exposure to mixtures of chemicals, increased absorption rates and a diminished ability to detoxify many exogenous compounds, all

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relative to those of adults [6]. The developing foetus is particularly susceptible due to partial lack of the blood-brain barrier [7] and to the rapid and tightly controlled growth of the brain during the pregnancy period [6]. The potential risk to the developing foetus is reflected in the level of contaminants in the maternal blood. It is known that several of these substances are passed from mother to foetus through the umbilical cord and later on to the child through breast milk [8, 9]. Of concern are the long-term, subtle effects that could influence reproductive health, pregnancy outcomes (e.g., birth weight, length, gestational age), reduced resistance against diseases, mental development of the child, or the increased risk of cancer [10]. The toxicity, metabolism and absorption of certain elements are dependent mainly on the chemical species and/or oxidation state. For example, dietary exposure to organic arsenic is unlikely to cause a health risk, but inorganic arsenic is recognized as genotoxic and is a known human carcinogen [11].

Essential elements also have a potential for toxicity and risk assessments for their essential versus toxic effects have been carried out [12, 13]. Trace element deficiencies have been reported in about 40% of the world's population, mostly from developing countries. This is probably a result of poor diet and can lead to endocrine, cardiovascular, skeletal, liver, gastrointestinal, kidney, genetic, and ophthalmologic disorders [14]. Furthermore, effects of toxic elements depend on the interaction between toxic and essential elements, especially when the metabolism of a toxic element is similar to that of an essential element. Exposure to toxic elements is also influenced by lifestyle factors [15].

Birth weight is the main factor influencing infant mortality rates, health of babies and a healthy later life. Birth weight is associated with the gender of the baby, the anthropometry of the mother (e.g., poor pre-pregnancy weight, weight gain during pregnancy, shorter gestational age), parity, multiple birth, complicated pregnancy (e.g., maternal anaemia, pregnancy diabetes, poor antenatal care, problems with placenta, maternal infections), restricted foetal growth, poor nutritional intake of women, and deprived socio-economic conditions [10, 16-19]. Effects are also associated with physically demanding work during pregnancy, environmental tobacco smoke, intensity and duration of cigarette smoking during pregnancy, taking illicit drugs and consuming alcohol [10, 16-20]. In addition, maternal health in relation to pregnancy, the mother's genetic background, age, location of residence and education also influence birth weight [17, 18].

Various associations between trace elements and birth weight have been observed. Studies have shown significant direct correlations between birth weight and maternal levels of zinc (Zn) and copper (Cu) in serum [21]. As for manganese (Mn), it has been suggested that Mn levels in maternal whole blood influence birth weight [22, 23], while the absence of a correlation between birth weight and Mn, Cu, Zn and selenium (Se) in maternal blood has also been reported [24-26]. Levels of Mn in maternal blood have been found to increase throughout pregnancy [27, 28], while lower or higher Mn concentrations in maternal blood has been associated with low birth weight (U-shaped relationship) [29, 30]. Toxic elements (cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg)) may have negative effect on birth weight at relatively low levels of exposure. Xu et al. suggested that the presence of As in maternal blood has a negative influence on birth weight and on gestational age in boys [31]. Studies investigating the effects of Cd exposure during pregnancy have been in conclusive: some studies [32-34] report negative associations, some positive [35], and others no association at all [36, 37]. Exposure to Pb was found to be associated with birth weight either negatively [37, 38], or insignificantly [34-36]. Non-significant correlations have been observed for Hg in maternal blood [35, 36, 39].

The National HBM program was implemented in Slovenia, according to the Chemicals Act in 2003 and

performed in the period from 2007 to 2014 for the first time to estimate the exposure of inhabitants of different are as to selected environmental chemicals and to provide data for an environmental health risk assessment [40]. Attention was on exposure to essential and toxic substances during pregnancy and the breastfeeding period, since these are recognized as being potentially responsible for adverse development and health in children. The main objective of the present study was to examine the relation between birth weight and maternal blood levels of toxic and potentially toxic elements determined within the Slovenian HBM program and taking into account the socio-demographic characteristics and diet of the mothers.

2. Materials and Methods

2.1 Description of Study Population

The study populations included first-time mothers who gave birth to a living baby from 12 different areas across Slovenia, including three urban areas (140 women), three rural areas (109 women) and six potentially contaminated areas due to past industrial activity (245 women) (Fig. 1). Areas of Jesenice, Celje, Idrija with Posočje, Savinjsko-posavska and Zasavje are known to be contaminated with metals, while the area of Bela krajina with polychlorinated biphenyls (PCBs).

2.2 Recruitment and Sampling

The mothers were recruited between 2007 and 2014 through selected maternity hospitals, parenting schools and gynaecologists. Maternal blood, urine, hair and milk samples were collected between 1 to 3 months after delivery. Questionnaires were given to the mothers to obtain information about their lifestyle, living environment, socio-economic status, nutritional habits (frequency of intake), health status of the mother-child pair and breastfeeding status. A total of 535 women signed informed consent, however, 41 women were excluded due to missing blood samples, gestational diabetes, diabetes type I and II, delivery of twins, lifestyle or occupational exposure and missing information on birth weight. The final database contains data from 494 women.

2.3 Sample Preparation and Chemical Analysis of Whole Blood

Venous blood samples were collected using vacutainer tubes containing K_2EDTA (BD Vacutainer® Blood Collection Tubes for Trace Element Testing, Becton Dickinson, US), following regular laboratory procedures,

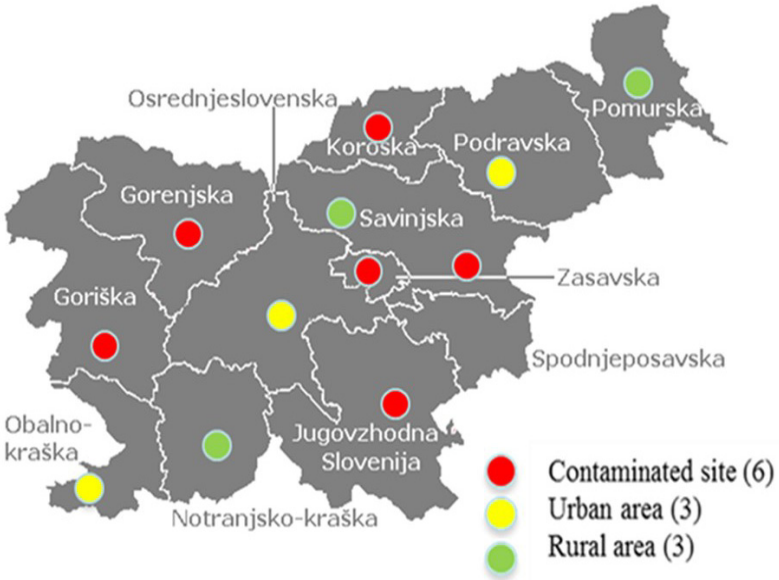

Fig. 1 Sampling locations in Slovenia [40].

and stored at -20 °C until analysis. Selected toxic and potentially toxic elements in the maternal whole blood samples were analysed at the Jožef Stefan Institute, Slovenia.

Whole blood sample is generally recognized as an appropriate matrix for assessment of exposure to Mn, Se, Cu, As, Cd, Pb and Hg. However, in the case of Zn and Cu, serum is a more indicative matrix for their status in the body [41]. Due to cost effectiveness and the limited blood volume available from the mothers, elements were determined in whole blood. For the same reason sample size varied for some elements on account of an insufficient amount of sample and this is the reason why Mn was not determined in participants from three of the selected study areas (pilot phase including one urban, one rural and one potentially contaminated area). To allow comparability with other studies, all values have been converted to ng/mL, using a factor of 1.053 g/mL for women [42].

2.3.1 Determination of As, Cd, Cu, Mn, Pb, Se and Zn in Whole Blood

As, Cd, Cu, Mn, Pb, Se and Zn in whole blood were analysed using an Octapole Reaction System (ORS) Inductively Coupled Plasma Mass Spectrometer (ICP-MS, 7500 ce, Agilent, Tokyo, Japan) equipped with an ASX-510 Autosampler (Cetac), a Babington nebuliser and a Scott-type spray chamber. The ORS was operated in helium gas mode to avoid interference.

Aliquots of 0.3 mL of whole blood sample werediluted ten times with an alkaline solution containing Triton X-100 and disodium ethylene diamine tetraacetic acid (EDTA) in contamination free tubes [43]. An aliquot of an internal standard solution containing Scandium (Sc), Gallium (Ga), Yttrium (Y) and Gadolinium (Gd) was added to each tube and standard addition was used for calibration. The following isotopes were monitored ^{45}Sc, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{69}Ga, ^{75}As, ^{78}Se, ^{89}Y, ^{111}Cd, ^{114}Cd, ^{157}Gd, ^{206}Pb, ^{207}Pb, ^{208}Pb. The instrument was tuned daily using a solution containing Li, Mg, Y, Ce, Tl and Co. On all isotopes were quantified using one central point of the spectral peaks and three repetitions.

Analytical precision was 5%, for Pb, Mn, Cu, Zn and Se, 10% for As and 20% for Cd. Limits of detection (LOD), calculated as three times the standard deviations of the blank sample, were 0.2, 0.4, 0.1, 8, 7, 30, 0.4 ng/mL for Cd, Pb, As, Se, Cu, Zn and Mn, respectively. The accuracy of the results was checked against the certified material Seronorm Trace Elements Whole Blood L-1 (SERO AS, Norway).

2.3.2 Determination of Total Hg in Whole Blood

The concentration of total Hg in whole blood was determined using a Direct Mercury Analyser (DMA-80, Milestone Srl, Italy). A known amount of blood (0.1-0.2 mL) was weighed out into a quartz boat. The sample was first dried and then thermally and chemically decomposed at 650 °C. An oxygen stream then transfers the decomposition products through an amalgamator that selectively traps gaseous mercury, which is subsequently desorbed for quantification. The resulting mercury vapour was analysed using atomic absorption spectrophotometry at 254 nm [44].

The analytical precision was 5% and with a LOD of 0.2 ng/mL. The reference material, Seronorm Trace Elements Whole Blood L-1 (SERO AS, Norway), was used to check the accuracy of the results.

2.4 Statistical Analysis

Statistical analysis included descriptive statistics (means, minimum (min), maximum (max), geometric means (GM), confidential intervals (CI) of GM, and percentage), bivariate analysis (Spearman for categorical data and Pearson for continuous), analysis of between-group effects (Mann-Whitney U, Kruskall-Wallis test or ANOVA) and multiple linear regression. Non-normally distributed data (concentrations of elements, baby birth length) were log10-transformed. Concentrations of elements below the LOD were assigned arbitrarily the value of ½ LOD. Multiple linear regression (using the enter all method) was used to evaluate the association between birth weight and all covariates that correlate with the

dependant variable with a level of significance $P < 0.25$. Highly correlated variables were not included in the same model to avoid the effect of multi-collinearity. Predefined confounders were fixed into the models (age of mother, education, pre-pregnancy body mass index (BMI) and residence). *P*-values < 0.05 were considered significant. All statistical analyses were performed using IBM SPSS Statistic Version 23 and STATA/SE 12.1.

3. Results and Discussion

Table 1 gives the characteristics of the study population. The mean age of the mothers was 29 years, the mean pre-pregnancy BMI was 23.1 kg/m^2, the mean estimated gestational length was 39.7 weeks, 50% of women have at least university education, 53.8% of babies were males, and the mean birth length for all babies was 51.4 cm. The mean birth weight of the babies in our study was comparable with those reported elsewhere [23, 30, 35, 38, 45, 46]. A total of 3.4% of the new-borns were below the criterion for "low birth weight" of 2,500 g [17], and 0.2% of new-borns were below 1,500 g, considered as a "very low birth weight" [47]. "High birth weights" (> 4,000 g) [48] were reported for 8.3% of the new-borns.

Table 2 gives the geometric mean concentrations of selected elements in maternal whole blood samples. In general, the level of exposure to toxic elements was low and did not pose a health risk for either the mothers or their babies (Table 2). The percentage of individuals having blood concentrations of Hg, Pb and Cd above the established reference values were 0.81, 0.40, and 3.64%, respectively (Table 2) [49, 51-53]. The GM of Cd in non-smokers was 0.34 ng/mL, 0.41 ng/mL in pre-pregnancy smokers (12%) and 0.63 ng/mL in pregnancy smokers (1.2%). As observed from correlations between blood levels and questionnaire variables, Hg was significantly associated with consumption of fresh sea fish ($r_s = 0.269$, $P < 0.001$) and canned sea fish ($r_s = 0.237$, $P < 0.001$) (data not shown), hence the weak correlations. Furthermore, no relationship was observed between Hg and amalgam fillings ($r_s = 0.017$, $P = 0.739$). Based on the questionnaire data, 41.1% of women reported eating fresh seafood at least once per month (on average less than once per month); 17.2% of mothers reported having noamalgam fillings and 23.3% did not answer this question. On average women had 4.36 amalgam fillings (data not shown).

In the case of Cu, Zn and Se, 2.0, 0.2 and 0.4% of individuals exhibited levels below their lower reference values, while 3.2, 1.4 and 4.9% of individuals had levels of Mn, Cu, Zn and Se above the upper references levels. In contrast, 75.7% of women exceeded the reference levels for Mn [49-51]. The relatively high levels of Mn in the study population were similar to those previously reported in postpartum mothers form Norway [28], in mothers before delivery in South

Table 1 General characteristics of the Slovenian study population.

Characteristics of study population		N	Mean (min-max)	% of participants
Women	Age at delivery (years)	493	29.0 (19-39)	
	Pre-pregnancy body mass index (kg/m^2)	483	23.1 (16.6-44.8)	
	Estimated gestational length (weeks)	473	39.7 (28-42)	
	Education: primary	35		7.4
	secondary	200		42.6
	university	235		50.0
Children	Gender: male	266		53.8
	female	228		46.2
	Birth weight (kg)	494	3.38 (1.40-5.17)	
	Birth length (cm)	493	51.4 (44-59)	

N = number of samples, min = minimum level, max = maximum level.

Table 2 Concentrations of elements (ng/mL) in maternal whole blood of the study population and respective reference values.

Elements	N	GM	95% CI of GM	Range (min-max)	Reference value/range	% below reference value	% above reference value
Mn (ng/mL)	370	**17.2**	16.7-17.8	5.8-35.2	**4-14** [27]	0.00	75.7
Cu (ng/mL)	494	**1,077**	1,057-1,084	657-2,004	**800-1,400** [51]	2.02	3.24
Zn (ng/mL)	494	**6,731**	6,644-6,819	3,010-11,733	**3,500-9,100** [50]	0.20	1.42
As (ng/mL)	494	**0.929**	0.866-0.997	0.201-16.798	**< 20** [51]	-	0.00
Se (ng/mL)	494	**94.7**	93.2-96.3	53.9-175.9	**60-130** [49]	0.40	4.86
Cd (ng/mL)	493	**0.346**	0.328-0.365	0.20-3.084	**< 1.0** [53]	-	3.64
Pb (ng/mL)	494	**16.8**	16.2-17.4	4.2-71.9	**< 70** [53]	-	0.40
Hg (ng/mL)	493	**1.11**	1.03-1.19	0.20-10.17	**< 5.8** [55]	-	0.81

CI = confidence interval, GM = geometric mean, min = minimum level, max = maximum level, N = number of samples.

Table 3 Correlation between birth weight and selected variables.

Correlations with baby birth weight (kg)			
Variable	**r_s**	***P*-value**	**N**
Residential location*	-	0.140	448
Age of mother (years)-groups	-0.043	0.346	493
Pre-pregnancy body mass index-groups	0.092	0.042	483
Education-groups	-0.033	0.469	470
Estimated gestational age (week)-groups	0.325	0.000	473
Gender of baby	-	0.033	494
	r_p	***P*-value**	**N**
Log10 Mn (ng/mL)	0.178	0.001	370
Log10 Cu (ng/mL)	-0.074	0.102	494
Log10 Zn (ng/mL)	0.004	0.938	494
Log10 As (ng/mL)	0.018	0.689	494
Log10 Se (ng/mL)	-0.042	0.347	494
Log10 Cd (ng/mL)	0.053	0.239	493
Log10 Pb (ng/mL)	0.076	0.091	494
Log10 Hg (ng/mL)	-0.040	0.376	493
Baby length (cm)	0.717	0.000	493

*potentially contaminated, rural, and urban. GM = geometric mean, *P*-value shows the level of significance, N = number of samples.

African coastal communities (from part "Rural 2") [54], in pregnant woman from Southwest Quebec [46], in the Mother and Children's Environmental Health birth cohort study in Seoul, in Cheonan, and Ulsan in the Republic of Korea at delivery [30] and in pregnant women from Costa Rica [25]. The concentrations of essential elements at 6 weeks postpartum may have changed according to physiological and metabolic changes during pregnancy and during the postpartum/lactation period, as was demonstrated by Hansen et al. who found that concentrations of Mn, Se, Zn and As increased postpartum [28].

Of the variables tested, pre-pregnancy BMI, estimated gestational age, gender of the baby and baby length were associated significantly with birth weight (Table 3). Among the selected elements, significant correlations were observed between the birth weight and Mn levels, and marginally significant correlations between Pb and Cu levels in the maternal blood samples (Table 3). No statistically significant correlations were observed between birth weight and consumption of different food items.

A positive association between Mn in maternal blood and birth weight was confirmed by the linear regression model, in which the Mn levels were adjusted for the relevant predictors (Table 4). Among

Table 4 Multiple linear regression between birth weight and selected variables.

Model						
Dependent variable	**N of observations**	**R^2**	**Adjusted R^2**	***P*-value**	**95.0% Confidence Interval for β**	
Baby birth weight (kg)	319	0.280	0.224	0.000		
Independents variable:		***β* coefficient**		***P*-value**	**Lower Bound**	**Upper Bound**
Residential location	**Rural (A)**	Reference				
	Urban (B)	0.167		0.028	0.018	0.316
	Potentially contaminated (C)	0.098		0.144	-0.034	0.230
Age (years)	**< 25**	Reference				
	25-29	-0.170		0.054	-0.343	0.003
	≥ 30	-0.180		0.052	-0.362	0.001
Pre-pregnancy BMI	**under weight**	Reference				
	normal weight	0.264		0.021	0.040	0.488
	over weight	0.362		0.003	0.123	0.602
Education	**Primary**	Reference				
	Secondary	-0.071		0.520	-0.286	0.145
	Tertiary or more	-0.058		0.599	-0.276	0.159
Estimated gestational age (weeks)	**< 37**	Reference				
	37-39	0.764		0.000	0.540	0.988
	≥ 40	0.880		0.000	0.658	1.101
Intake coffee, tea	**< 1 × per month**	Reference				
	≥ 1 × per month	-0.030		0.662	-0.164	0.104
	≥ 1 × per day	-0.008		0.893	-0.126	0.110
Intake of alcohol	**never**	Reference				
	< 1 × per month	0.147		0.013	0.031	0.263
	≥ 1 × per month	0.158		0.024	0.021	0.295
Intake of tinned sea food	**< 1 × per month**	Reference				
	1 - 3 × per month	-0.009		0.874	-0.122	0.103
	≥ 1 × per week	0.051		0.451	-0.081	0.183
Intake of eggs	**< 1 × per week**	Reference				
	1 × per week	-0.017		0.796	-0.144	0.111
	> per week	0.049		0.462	-0.082	0.180
Gender of the baby	**male**	Reference				
	female	-0.117		0.021	-0.215	-0.018
Log10 Mn (ng/mL)		0.523		0.014	0.107	0.939
Log10 Cu (ng/mL)		-0.296		0.480	-1.120	0.527
Log10 Cd (ng/mL)		-0.047		0.665	-0.258	0.165
Log10 Pb (ng/mL)		-0.055		0.711	-0.344	0.235
(Constant)		2.677		0.041	0.106	5.247

N = number of samples, *P*-value shows the level of significance, R^2 = R Square = gradient of the regression line and the strength of the relationship between a predictor and the outcome; β coefficient = gradient of the regression line and the strength of the relationship between a predictor and the outcome, BMI = body mass index.

the predictors tested, the regression model revealed statistically significant correlations between birth weight and pre-pregnancy BMI, intake of alcohol before pregnancy, the baby's gender, as well as a marginally significant correlation between birth weight and the age of the mother. A higher pre-pregnancy BMI was associated with a higher birth weight, which agrees with the findings of other studies [16, 17, 56]. Furthermore, the differences in gender-related birth weight (boys were heavier) is also in accordance with

an earlier report [17]. Teenagers and young women have been reported to have lighter babies [17, 18], but in the present study the opposite was observed. The reason for this could be the narrower age interval (19-39 years) included in the present study. The model also shows that babies from urban areas had higher birth weights than those from rural areas (Table 4).

The positive association observed between birth weight and Mn in maternal blood could be explained by the essential role of Mn in foetal development. Mn is an important cofactor in enzyme reactions in bone formation and in metabolic regulation of levels of amino acid, lipid, protein and carbohydrate [25, 27]. However, in contrast to the present study, only non-significant or non-linear correlations between birth weight and Mn have been reported [22, 23, 25, 26].

In contrast with the literature [20], non-significant correlations between maternal smoking and birth weight were observed in the present study, probably due to the low frequency of smoking among the participating mothers (1.2% of women smoked during pregnancy and 12.4% before pregnancy). Although it is known that some food items appear to be beneficial for an appropriate birth weight [57, 58], our study failed to reveal any significant associations between birth weight and the food items that were included in the study questionnaire. The only association was between birth weight and alcohol intake before the pregnancy, but it was positive and also questionable due to the low levels of alcohol intake in the study population (average consumption was less than once per month).

4. Limitations

A disadvantage of the Slovenian study presented here is that the gestational age was estimated from the information obtained from the mothers, i.e., the estimated gestational age was calculated from the date of birth and predicted date of birth reported by the mothers, since clinical information from the maternity hospitals was not available. Another limitation of the study is the time-period in which blood samples were collected (1.5 months postpartum). Since literature data suggest that food intake during pregnancy can affect the levels of elements in maternal blood during and after pregnancy [59], the levels of selected elements in postpartum maternal blood samples could be used for estimation of pregnancy exposures.

5. Conclusions

The present study is the first of its kind to examine exposure to such a wide range of trace elements in relation to infant birth weight while taking into account the socio-demographic characteristics and dietary habits of the mothers. Exposure to toxic elements was low and comparable to that in other populations. Most mothers did not exceed the given reference levels. The birth weight was in agreement with other literature studies. The results of this study also show that maternal age, pre-pregnancy BMI, estimated gestational age, length of baby and marginally the gender of baby all influence the birth weight. Despite low, environmentally relevant, exposure to the selected potentially toxic elements, a significant and positive correlation was observed between birth weight and the levels Mn in blood from Slovenian mothers. Additional evaluations and further studies are needed to focus on this specific and other potential associations.

6. List of Abbreviations

AMAP: Arctic Monitoring and Assessment Program; ANOVA: Analysis of variance; As: Arsenic; ATSDR: Agency for Toxic Substances and Disease Registry; BMI: Body mass index; Cd: Cadmium; CDC: Centres for Disease Control and Prevention; Cu: Copper; LOD: Limit of detection; DMA: Direct Mercury Analyser; EDTA: Ethylenediaminetetraacetic acid; Ga: Gallium; Gd: Gadolinium; GM: Geometric mean; HBM: Human biomonitoring study; Hg: Mercury; ICP-MS: Inductively Coupled Plasma Mass Spectrometer; K: Potassium; max: Maximum level; min: Minimum level; Mn: Manganese; N: number of samples; NMEC:

National Medical Ethics Committee; ORS: Octapole Reaction System; *P*: *P*-value shows the level of significance; Pb: Lead; PCBs: Polychlorinated biphenyls; R^2: R Square = gradient of the regression line and the strength of the relationship between a predictor and the outcome; r_p: Pearson's correlation coefficient; r_s: Spearman's correlation coefficient; Sc: Scandium; Se: Selenium; WHO: World Health Organization; Y: Yttrium; Zn: Zinc; β: β coefficient = gradient of the regression line and the strength of the relationship between a predictor and the outcome.

7. Declarations

7.1 Ethics Approval and Consent to Participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The human biomonitoring study was approved by the Republic of Slovenia National Medical Ethics Committee (NMEC) with numbers of accordance 42/12/07 and 53/07/09. Participation of women was voluntary; informed consent was obtained from all individual participants included in the study. All samples and related information were coded and anonymised. The participants had the right to withdraw from the study at all stages of the study period.

7.2 Consent for Publication

Not applicable.

7.3 Availability of Data and Materials

The dataset used in this article was obtained from the National Human Biomonitoring Program in Slovenia. The complete dataset has not yet been published but is expected to be available within five years.

7.4 Competing Interests

The authors declare that they have no competing interests.

7.5 Funding

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7.6 Authors' Contributions

MJ and DM measured concentrations of selected elements in maternal blood samples by ICP-MS. MP, AS, JST and MJ carried out measurements of total mercury in maternal blood samples. MJ performed the statistical analysis. MJ, JST, DM, MH, ABK, LK, MK and JØO conceived the study, and participated in its design and coordination as well as helping to draft the manuscript. All authors read and approved the final manuscript.

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