The JSPS fellowship report

Duration: From May 21, 2022 to May 21, 2023

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Preparation and procedures

In the first step, I asked from the university to paper my research needed, including personal computer, office software, and EndNote, and SAS. After a week, the computer with the requested software was delivered to me. Before conducting the main statistical analysis, the accuracy of the information in Iranian and Japanese birth cohort carefully checked and incomplete information either completed or removed. Then, the data were homogenized between the two countries (such as definitions, measurement units, calculations).

Next, I searched and found the latest related articles for the statistical analysis methods in mixture exposure condition. In addition, I have spent a significant time to become familiar with SAS and R software. After familiarizing with the SAS statistical software, frequency analysis, regression, and comparisons of the information of two countries, Iran and Japan, were performed using this software.

To do the next step and perform more advanced analysis, such as Bayesian Kernel Machine Regression (BKMR) using the Markov Chain Monte Carlo (MCMC) method, I needed to get familiar with R analysis software and learn its formulation language. This statistical program is open source and all analyzes are done by programming using designed packages or creating a new package. In order to perform Bayesian Kernel Machine Regression analysis, there is a package with the same name (bkmr) in the R software, which is the basis for the analysis of several independent factors (simultaneously in one model) on the dependent factor.

In order to make charts and graphs from the statistical analysis results, I learned the ggplot2 package in R statistical software using a book of the same name (written by Hadely Wickham) and did a lot of practical exercises. In the next step, after mastering the R software package and preparing figures and diagrams, the findings were discussed with Japanese colleagues so that the relationship between the independent variables (heavy metal levels in this study) and the dependent variable (weight and height at the time of birth of the baby) were shown graphically in the graphs.

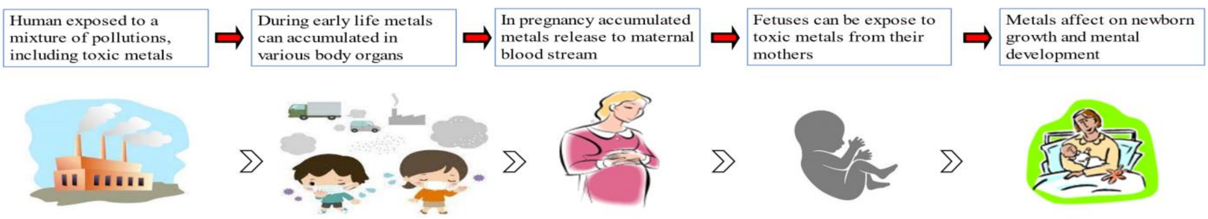
Main study

Human is simultaneously exposed to hundreds of chemicals and pollutants, from industrial activities, polluted environmental, and food chines, which can cause various disorders. Heavy metals are important pollutants that ubiquitous throughout the earth. In the recent decades, new legislations and intensive activities have been decreasing exposure to toxic metals globally. Despite these attempts, chronic and low-levels metals exposure remains a public health problem. Several epidemiologic studies, including our previous studies, have shown many adverse effects induced by heavy metals on human reproductive system, such as pregnancy hypertension, preterm birth, low birthweight, preeclampsia, and impaired infancy neurodevelopment abilities. On the other hand, pregnant women and their fetuses are more vulnerable than general population to adverse effects of these toxicants, even at relatively low-level exposure.

Pregnant women usually encounter to mixture of heavy metals rather than a single exposure, thus, “once at one time” toxicity investigation cannot estimate real effect, interactions among, and cumulative effects of heavy metals on pregnancy outcomes. To estimate mixture metals concentrations influencing on birth size we performed sensitivity analyses using BKMR in the R statistical software, a novel statistical approach, which recently implemented in environmental health studies related to mixtures exposure. The study’s data have been obtained from Iranian and Japanese pregnant women by the same study methods and the same study team.

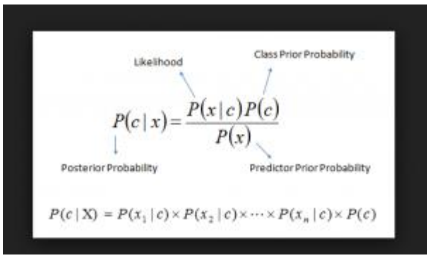
The “once at one time” approach is a dominant pathway in the most epidemiological research. It means effect of only one metal on an outcome (i.e., a disease) investigate at the time in a statistical model. Thus, “once at one time” give an unrealistically estimation to study the association between each metal on human organs to investigate interactions among heavy metals and their cumulative effects on the outcome. Recently, the USA National Institute of Environmental Health Sciences launched a specific initiative, called Powering Research through Innovative Methods for Mixtures in Epidemiology, to encourage methods developments in this direction, and organized workshops and symposiums on the topics. Similarly, we are going to develop a model for different approach in data analysis for exposure to mixture metals and their real effects on pregnancy outcomes. Obtaining such statistical model is a challenging situation because things would only get more complex if we wanted to account for a larger mixture of metals in present on several co-varieties.

As we know, this is the first time to employed mix-metals analysis in two societies according to metals concentration and pregnancy outcomes. In addition, the analyzing method will develop a better statistical model for studying metal mixture effects on pregnancy outcomes and that could be extended to other human health aspects. In the recent years, we have been lunched two birth cohort studies in Iran and Japan, one industrial and one developing country. The study design, blood sampling methods and metals measurement protocol have been done identically in both studies. The whole blood metal measurement has done by ICP-MS in the Faculty of Medicine Juntendo University, Tokyo, Japan. In additional, both studies have obtained socioeconomic, anthropometric characteristics,

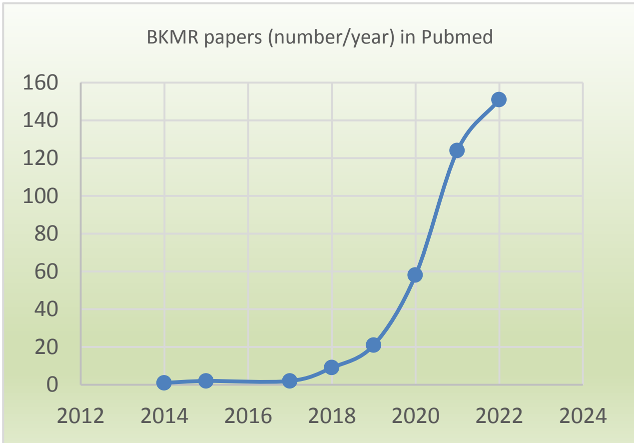


medical/pregnancy history, and pregnancy outcomes from more than 600 participants.

Bayesian analyses: Basically, a Frequentist method makes predictions on the underlying truths of the experiment using only data from the current experiment. On the other hand, Bayesian statistics, take a more bottom-up approach for data analysis. It means that past knowledge of similar experiments is encoded into the analysis, known as a prior. This prior is combined with current experiment data to make a conclusion on the test at hand. In other words, the biggest advantage of Bayesian approaches is using all the information, whether current or prior.



Frequentist	***Bayesian***
Data is a repeatable random sample - there is a frequency	Data is fixed, probably a few samples.
No expression of belief (formally not present) => Objective view on probability.	Approach deals with belief (Formally present) => Subjective view on probability. It helps to update their beliefs in the evidence of new data (thus creating posterior distribution).
Provides us with a point estimate using MLE and Least Squares Estimate.	Provides us with a posterior distribution with high density interval with mean, mode and median stats.
Parameters are fixed and unknown.	Parameters are unknown, random and described probabilistically.
Confidence Interval: Over an infinite sample size taken from population, 95% of these contain the true population value.	**Credible Interval:** A 95% probability that the population value is within the limits of the interval.
Statistical Hypothesis Testing with p-value and significance level is employed to deduce a solution in the decision-making process.	Bayes Factor considered a direct test of null and alternate hypothesis, yielding a measure of strength of evidence.
Less Computationally intensive	Computationally intensive



During the present fellowship research we aimed to investigate prenatal blood metal mixture (As, Cu, Pb, Mn, Rb, Se, and Zn) effects on birth weight and height, using two birth cohort studies in Iran and Japan. Firstly, we run descriptive statistical analyses on participants' characteristics and their blood metals levels, following by comparing these variables between two countries. Then, the linear regression model examined associations between individual blood metals concentrations on birth sizes, adjusted for covariates. Finally, we employed Bayesian Kernel Machine Regression (BKMR), a novel statistical model implemented to analyze the effects of mixed components on outcome, based on exposure-response functions.

Findings

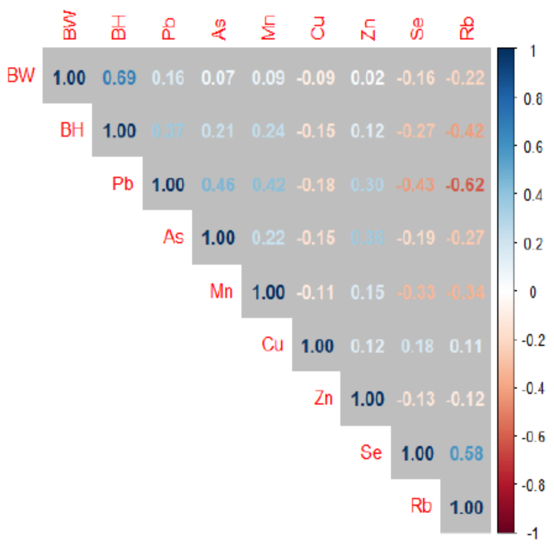
Descriptive statistical analyses were done on participants' demographic characteristics and blood metals levels. There was right skewed (positive skewness) distribution in almost all blood metal concentrations. Thus, we used natural log-transformation of blood metals to improve model fit (reduce outlier effect) for all downstream statistical analyses. Pearson's correlation coefficient was carried out to estimate the relationship among metals and birth sizes. The Student t-test used to compare participants' continuous variables and blood metals concentrations between the two countries. The linear regression model examined the associations between individual metal and birth sizes, one metal at a time, adjusting for covariates that have been previously shown to be associate with birth size and/or blood metal concentrations, including maternal age, pregnancy weight gain, maternal BMI, newborn sex, hematocrit, and gestational age.

To estimate the joint effect of the metal mixture, we performed exposure-response functions using BKMR analysis. This statistical method can fit the high-dimensional nonlinear and nonadditive associations of mixture predictors on an outcome, adjusted for the above covariates. The analysis was done by "bkmr function" via implements the Markov Chain Monte Carlo (MCMC) sampler to fit the BKMR. We set the argument for 50,000 iterations of the MCMC procedure. In addition, we set the argument variable selection (varsel= TRUE) in the bkmr function to calculate the posterior inclusion probability (PIPs; range from 0 to 1) values for each metal at component-wise variable selection. Individual metals with a PIPs ≥0.5 considered important in the model.

To visualize results of the high-dimensional exposure-response functions and inferencing nonlinear and interactive associations between metals and birth sizes, findings presented in four graphical categories. 1) The overall effects of all metals together (from quantiles 25th to 75th) on birth sizes was compared to zero change when metals fixed at their median (50th quantile). 2) Univariate exposure-response function run to reveal relationship between each metal and birth sizes, where all the other metals were fixed at the 50th quantile (median value). 3) Using the bivariate predictor-response function we computed potential interactions between a single metal (predictor fixed at its median) and the second metal placed at its 25th, 50th, or 75th quantiles, on birth sizes. 4) Furthermore, the BKMR analyses calculated the contribution of a single metal on birth size when the metal was fixed at its 25th compared to the 75th quantiles, where all other metals were fixed at their 25th, 50th, or 75th quantiles.

Demographic variables (early pregnancy weight, BMI, height, and pregnancy weight gain) were significantly higher in Iranian women than Japanese (Δ= 11.9 kg, 4.2 kg/m^2, 1.4 cm, and 1.1 kg, respectively; p <0.05). Similarly, Iranian newborn had significantly higher birth weight and height than Japanese babies (Δ= 216 gr and 2.3 cm, respectively, p <0.01). There was no statistically significant difference in newborn gender between the two populations (not shown data). Among the seven metals, concentrations of As, Mn, Pb, and Zn were significantly higher in Iranian than in Japanese participants (Δ = 4.6, 4.0, 26.6, and 599 µg/L, respectively, p <0.01) while concentrations of Cu, Rb, and Se were significantly lower in Iranian than Japanese pregnant women (Δ = 101, 763, and 99 µg/L, respectively, p <0.01).

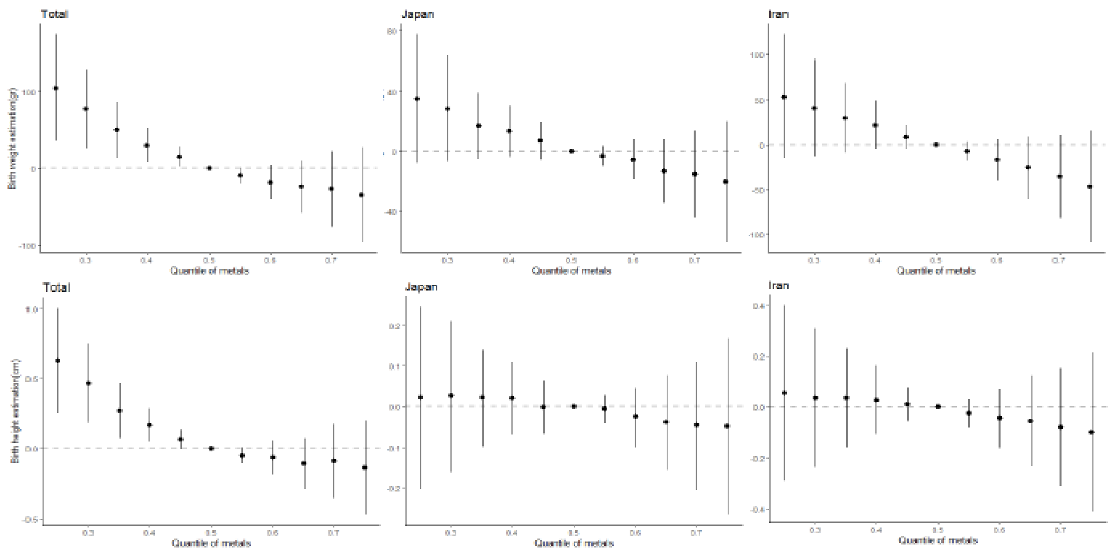
Pearson correlation coefficient analysis found a positive significant relationship between birth weight and height (*r* = 0.69, p <0.01). Among metals, the strongest negative correlation was found between Rb levels with birth weight and height (r = -0.22 and -0.42, respectively, p <0.05), followed by Se (r = -0.16 and -0.27, respectively, p value < 0.05). There was a positive correlation between Pb and birth height (r = 0.37, p <0.01) .



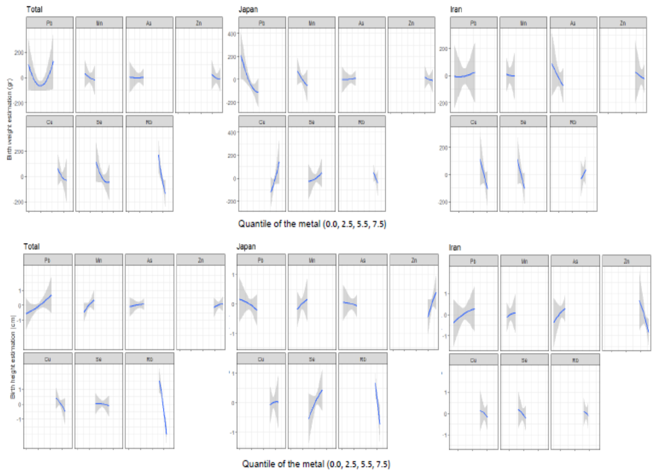
Simple and adjusted linear regression models were fitted separately for each metal. Among seven measured metals, Rb and Se exhibited significantly negative associations with birth weight, in both models (coefficients = -309 and -173 for simple vs -290 and -125 for multiple regression, respectively, p value <0.05), while Cu, Rb, and Se were significantly negatively associated with birth height (coefficients = -1.8, -1.6, -3.3 for simple vs -0.9, -3.0, -0.9 for multiple regression, respectively, p <0.05). In addition, the analysis found significant positive associations between Mn and Pb levels with birth height in unadjusted and adjusted models (coefficients = 2.0 and 1.1 vs 0.9 and 0.7, respectively, p <0.05).

We investigated linearity and interactions of mixture metal exposure on birth sizes using the BKMR analysis. The MCMC methods estimated PIPs for each metal (Figure 2). The highest important metal in the model was Rb with PIPs of 0.95, 1.00, 0.70 for birth weight in total data and birth height in the total and Japanese data, respectively.

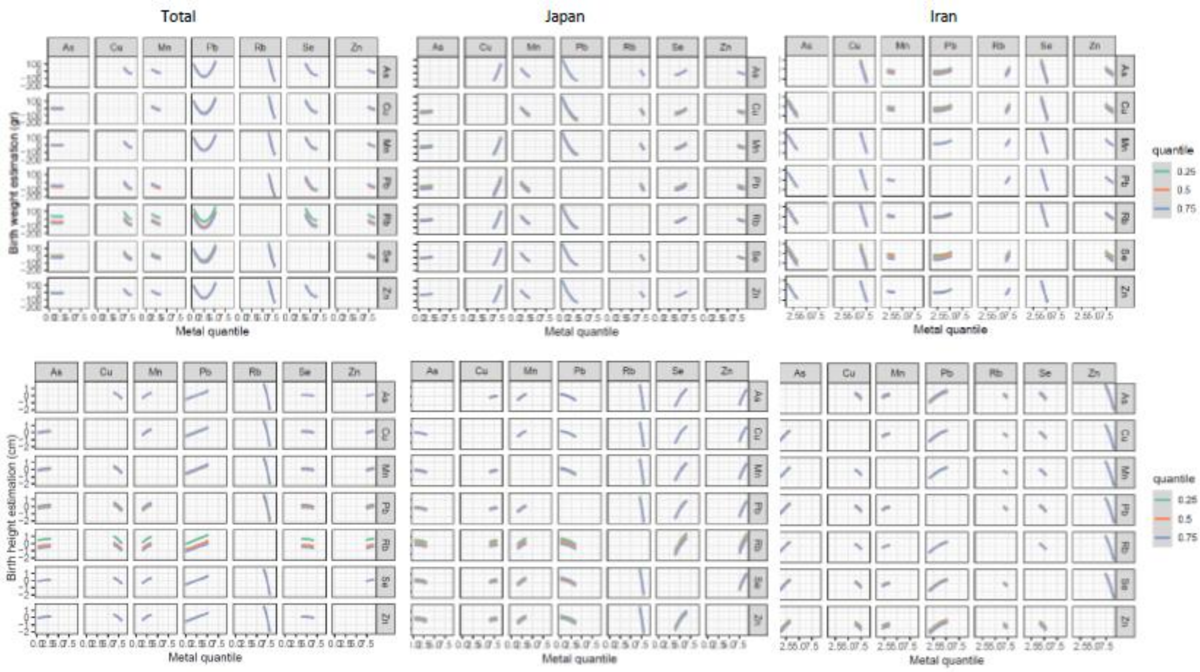
The BKMR overall function (seven metals combined) showed a decreasing trend in birth size in almost all models, which the largest and significant ones were found in the total data. The model estimations showed a significant decreasing in birth weight (mean±SD) from 105±35 gr to -34±32 and birth height from 0.63±0.19 cm -0.13±0.17, while metal mixture levels increased from their 25th to 75th quantiles.



Univariate exposure-response function disclosed a sharp linear relationship between Rb and decreasing birth weight and height in the total data. Similar relationship was found between Rb with birth height in the Japanese data. There were inverse relationships in Cu and Zn levels with birth size between Japanese and Iranian. The other univariate exposure-response between metals and birth sizes, such as U-shape relationship between Pb and birth weight in total data, shown in the.

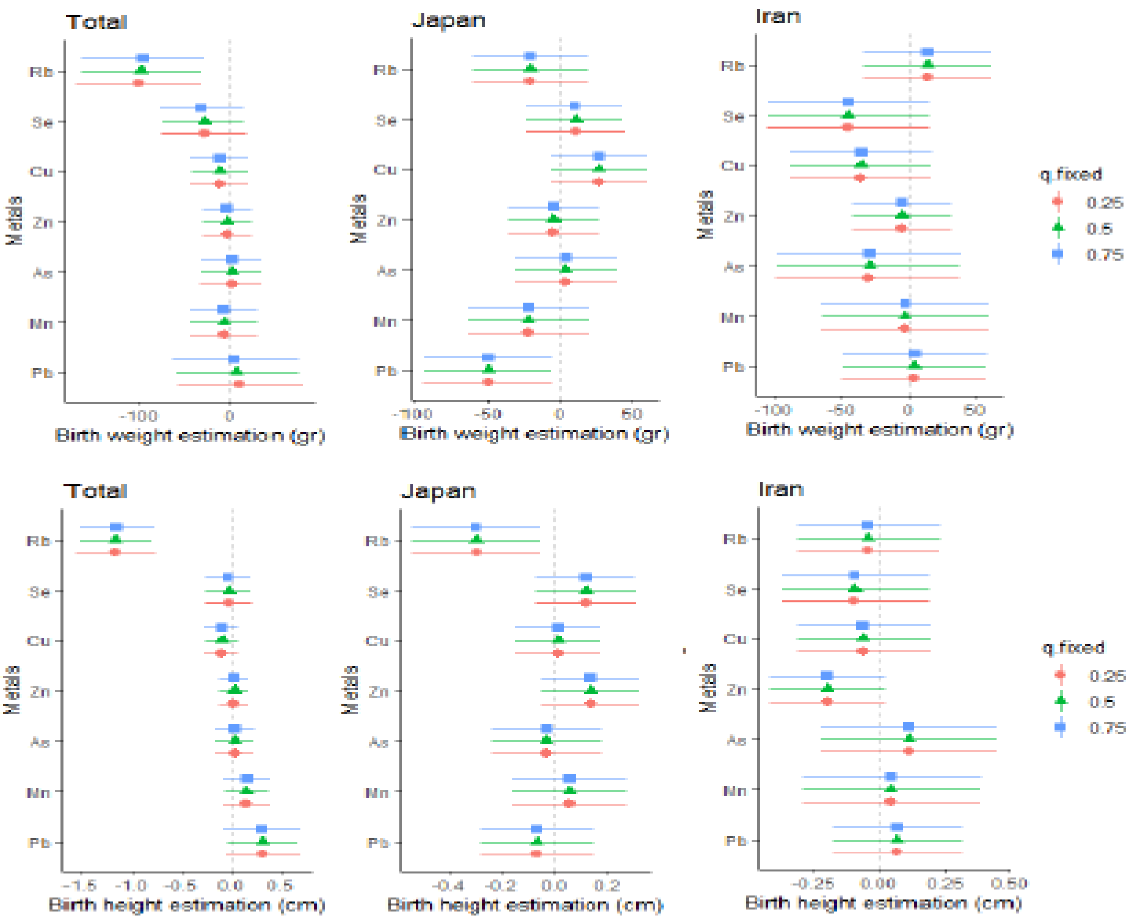


The bivariate predictor-response function, as same as univariate function, showed Rb levels as the most important predictor for both birth weight and height in the total and Japanese data set. The inverse relationship of Zn with birth height, between Japanese and Iranian, was confirmed in this model as well. As figure slop did not change significantly when metal was at different quantiles (other elements at their median), there may not be any interaction among those metals.



The single predictor function analysis found only Rb had a significant negative association at all three quantiles, when other metals fixed at their 25th, 50th, and 75th quantiles, with birth weight (mean estimation: -101, -98, and -95 gr, respectively) and birth height (-1.17, -1.16, and -1.15 cm, respectively) in the total data set. In addition, negative associations between Rb with birth height (-

0.30 cm) and Pb with birth weight (-50 gr) was observed in the Japanese data, identically at all quantiles.



The present work may involve some weakness. First, we combined two birth cohort data from different countries with varsity in subjects' characteristics and blood metals levels. Variable variations can increase the study power to generalize findings to the target population-pregnant women. On the other hand, the study findings may be influenced by these significant variations between the two populations. Second, the present study used the first trimester blood metals concentrations for the exposure assessment, due to fetuses' anthropometric characteristics (i.e., weight and height) develops from early pregnancy and a higher probability to induce adverse pregnancy outcomes when toxicant exposure taking place at the early gestation. However, fetuses' growth continues during the substantial trimester. In addition, many of blood metals' have only a few days to weeks half-life, and the concentrations change during subsequent trimesters, according to exposure levels and/or bioavailability. Therefore, it would be proper to use several blood metals measurements throughout pregnancy, instead of only one measurement. Third, almost all study participants were not occupationally exposed to metal, thus, they might be encountered from their diet and polluted environment. However, the complete sources of prenatal metals exposure were not clearly understood, as these studies did not collect information about the metal concentrations in their foods and/or environment.

Conclusion

The present study investigated prenatal blood metals effects on birth size in apparently healthy women with singleton pregnancy. The overall function of the BKMR estimated continuously decreasing birth weight and birth height in response to increasing prenatal blood metals levels. All the statistical analyses found a strong negative relationship between blood Rb levels, as the best single predictor, with birth weight and height. Similar to other potentially toxic metals (i.e., Pb and As), Rb has not been known to have any physiological activities on the human reproductive system, thus, pregnant women should avoid it “as little as possible”.