JBACHS
Journal of Basic and Clinical Health Sciences

J Basic Clin Health Sci 2022; 1: 199-205
https://doi.org/10.30621/jbachs.1008609
RESEARCH ARTICLE

ASSESSMENT OF LEAD AND MERCURY LEVELS IN MATERNAL BLOOD, CORD BLOOD AND PLACENTA SAMPLES OF PREGNANCIES WITH INTRAUTERINE GROWTH RESTRICTION

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Received: 26.10.2021; **Accepted:** 24.12.2021; **Available Online Date:** 27.01.2022

Cite this article as: Yuksel D, Yuksel B, Kalafat E, Yuce T, Katlan DC, Koc A. Assessment of lead and mercury levels in maternal blood, cord blood and placenta samples of pregnancies with intrauterine growth restriction. J Basic Clin Health Sci 2022; 6: 199-205.

ABSTRACT

Purpose: Many studies reported that prenatal exposure to lead and mercury are correlated with reduced birth weight and size, and these metals can cause adverse effects on neurodevelopment. In this study, it was aimed to investigate and compare the lead and mercury levels in maternal blood, cord blood, and placenta in pregnant women with IUGR fetuses diagnosed using abnormal Doppler findings and pregnant women with healthy fetuses.
Material and Methods: This study included 75 patients, comprising 41 in IUGR group and 34 in control group. Maternal venous blood, fetal cord blood and placental samples were taken during delivery period.
Results: Mercury levels in maternal blood and fetal cord blood, and lead levels in the placenta were found to be significantly higher in the IUGR group than in healthy subjects. Correlation analysis revealed that measurement values of body weight, body height, and head circumference of fetus might be lower when mercury level was measured higher in maternal blood and fetal cord blood. Furthermore, fetal body weight and fetal body height also would be lower when lead level measured in placenta was higher. Logistic Regression analysis results revealed that mercury levels measured in fetal cord blood could be used as the best marker in predicting low fetal weight, low fetal body height, and low fetal head circumference.
Conclusion: In conclusion, it was thought with this study results that in order to identify the etiology and to give therapeutic prenatal care of the IUGR in a fetus diagnosed as idiopathic IUGR it would be appropriate to measure the level of lead and especially mercury in the fetal cord blood during the prenatal follow-up period.

Keywords: Cord blood, intrauterine growth restriction, maternal blood, placenta, mercury, lead

INTRODUCTION

Intrauterine growth restriction (IUGR) is defined as the failure of the fetus to reach the appropriate intrauterine growth potential as a result of genetic or environmental factors and affects up to 10% of all pregnancies (1-3). IUGR poses a major public health problem as it leads to an increased risk of adverse perinatal outcomes and long-term complications

(4,5). Various causative factors related to IUGR that include maternal, fetal, placental, and genetic factors have been identified in the literature, and it has been suggested that most of these factors change the fetal environment resulting in several detrimental effects on the fetus (5,6).

The prenatal period is the most sensitive period of time in human development and exposure to any toxic factor at this vulnerable stage can result in disruption of normal fetal development and growth (7,8,9). As a result of globally increasing industrialization, heavy metal pollution and trace element deficiencies constitute an important public health concern in many countries (10,11) Exposure to heavy metals both during pregnancy and in the pre-conceptional period may adversely affect the developing fetus through placental transfer during pregnancy and cause various fetal complications (7,8,9,10,12).

Mercury and lead are well-known toxicants that can cross the placenta and accumulate in fetal tissues. It has been reported in many studies that prenatal exposure to lead and mercury are correlated with reduced birth weight and size, and these metals can cause adverse effects on neurodevelopment (13). The literature showed that studies have mostly examined heavy metal levels in low birth weight infants, however, the relationship between the IUGR groups and maternal/fetal cord/ placental heavy metal levels was investigated in very few studies (11).

In this study, it was aimed to investigate and compare the lead and mercury levels in maternal blood, cord blood, and placenta in pregnant women with IUGR fetuses diagnosed using abnormal Doppler findings and pregnant women with healthy fetuses.

MATERIAL AND METHODS

Approval for this research was granted by the Local Research Ethics Committee (Decision Number: 05-221-14/March 24, 2014). All enrolled participants signed written informed consent before participating in the study in compliance with the principles of The Declaration of Helsinki (World Medical Association, Declaration of Helsinki, 1964).

Participants

This prospective case-control study was consisted of 75 participants with a single live pregnancy between 24-38 weeks gestation and the participants were grouped as follows:

- IUGR group (comprised of 41 pregnant women who were already diagnosed with idiopathic IUGR)
- AGA group (Appropriate for gestational age) (consisted of 34 pregnant women with a normal healthy pregnancy as the control group)

In the IUGR group that any other etiological factors could not determine prenatally, intrauterine growth restriction was diagnosed using abnormal Doppler parameters that revealed the estimated fetal weight of >3rd - <10th percentile with cerebroplacental-ratio, and mean uterine artery pulsatility index was <5th and/or >95th percentile (1, 6, 14). The control group was randomly selected from healthy pregnant women who had undergone planned cesarean section at ≥38 weeks with EFW> 10th percentile. Participants were excluded from the study if they had a fetal chromosomal anomaly, fetal anomaly and maternal systemic disease (maternal vascular disease, autoimmune diseases, hypo/hyperthyroidism, gestational diabetes, type 1 and type 2 diabetes mellitus, cardiac disease, immunological disease), placental invasion anomalies, infections, or structural anomalies.

The study parameters consisted of the maternal age, values of the maternal body height, maternal body weight and maternal body mass index (BMI) values, gestational week values, gravida, parity, abortus, values of the fetal body weight, fetal body height, and fetal head circumference, 1-minute and 5-minute Apgar score values, mercury and lead level values measured in the maternal venous blood, fetal cord blood, and placenta.

Blood sample collection

Maternal venous blood samples were obtained just before delivery with cesarean section. After birth, the fetal cord was clamped and cut, then a cord blood sample was collected from the placental side of the cord. After removing the placenta, and placenta samples were taken from 3 different areas throughout the placenta.

All blood samples were centrifuged at 3500 rpm for 10 minutes, then the samples obtained serum and placenta were stored at -80˚C dry air until assay.

Determination of heavy metals in samples

A microwave system (Mars Xpress (CEM), USA) with PTFE microwave digestion vessels was used to perform the acid digestion procedure for the blood and placenta samples. Previously described

procedures were followed for the sample preparation and the instrumental analysis to determine lead and mercury levels in maternal blood, cord blood, and placenta samples (15). Mercury levels were quantified utilizing a spectrometer (Varian Atomic Absorption Spectrometry AA 240) equipped with a vapor generation system (Varian VGA 77) while lead levels were measured using a graphite furnace atomic absorption spectrometer (Varian AA240Z, Victoria, Australia) equipped with a background correction system (Zeeman).

Statistical analysis

To determine the study sample size G power analysis were performed and this analysis showed that to generalize the findings of this study the minimal sample size of IUGR group should be 34 and the minimal sample size of the control group should be 34.(effect size d=0.821, actual power =0.95)

Data obtained in the study were analyzed statistically using SPSS 23.0 version software. The study results showed normal distribution (Kolmogrov-Smirnov test($p > 0.05$). Therefore, the statistical differences of the parametric data between the study groups were analyzed using the Independent Samples t test ($p<0.05$).

In addition, Pearson Correlation test was used to determine the presence of correlation between parameters belonging to patients ($p<0.05$).

The ROC-Curve test was used to determine which study parameters predict the IUGR, and the sensitivity and specificity rates of the parameters were determined by obtaining "cut-off" values. In addition, Logistic Regression test was used to determine the "best parameter" ($p<0.05$).

RESULTS

Maternal body height ($t = -2.020$, $p = 0.048$), gestational week ($t = 9.129$, $p <0.001$), parity ($t = 2.770$, $p <0.001$), fetal body weight ($t = 12.035$, $p <0.001$), fetal body height ($t = 10.627$, $p <0.001$), fetal head circumference ($t = 9.290$, $p <0.001$), and 1-minute Apgar score ($t = -.939$, $p = 0.003$) were found to be different between the groups. However, maternal age, maternal body weight values, maternal BMI values, score values of the gravida, abortus did not differ between the groups ($p >0.05$) (Table 1).

The mercury level values measured in the maternal venous blood ($t = -2.805$, $p =0.004$), and fetal cord blood ($t = -6.660$, $p <0.001$) were found different between the groups. However, mercury level values measured in the placenta did not differ between the groups ($p >0.05$). On the other hand, the lead level values measured in the placenta ($t = -2.959$, $p = 0.004$) were found different between the groups (Table 1). Interestingly, the lead values measured in the maternal venous blood and fetal cord blood did not differ between the groups ($p >0.05$).

The correlation analysis results showed a negative correlation between fetal body weight and maternal blood mercury level ($r = -0.240$, $p =0.045$), fetal cord mercury level ($r = -0.581$, $p <0.001$), and placental lead level ($r = -0.235$, $p =0.006$). Again, a negative correlation was found between fetal body height and maternal venous blood mercury level ($r = -0.256$, $p =0.041$), fetal cord blood mercury level ($r = -0.548$, $p <0.001$), and placental lead level ($r = -0.284$, p

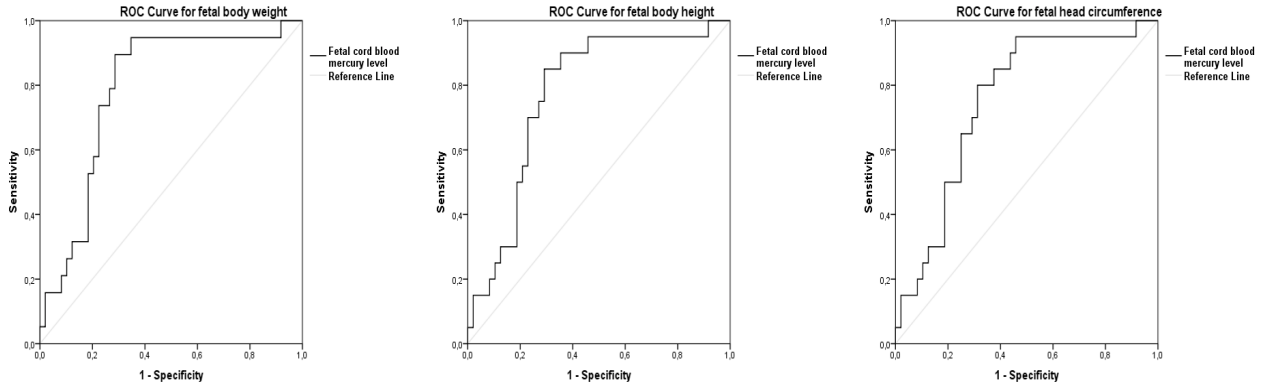

Figure 1. The ROC-Curve plot shows the parameters that can predict the lower fetal body weight, fetal body height, and fetal head circumference

Table 1. This descriptive table demonstrates the maternal and fetal demographic data, and mercury and lead measurement values in the maternal venous blood, fetal cord blood, and placenta (SD: standard deviation).

	AGA	**IUGR**		
Variable	**Mean±SD**	**Mean±SD**	**t**	**p**
Maternal age (year)	28.7±5.6	29.4±5.6	-0.770*	0.444
Maternal body weight (kg)	76.29±11.27	74.25±10.45	0.769*	0.445
Maternal body height (cm)	160.39±5.92	163.17±5.34	-2.020*	**0.048**
Maternal body mass index	29.6±3.6	27.8±3.8	1.765*	0.082
Gestational age (week)	38.65±0.90	33.22±3.47	9.129*	**<0.001**
Fetal body weight (gr)	3181±351.03	1573±678.04	12.035*	**<0.001**
Fetal body height (cm)	52.31±2.14	40.94±5.39	10.627*	**<0.001**
Fetal head circumference (cm)	35.31±2.24	29.68±2.62	9.290*	**<0.001**
Apgar 1	7.46±1.07	6.12±2.30	-2.939	**0.003**
Apgar 5	8.75±0.76	8.30±1.61	-1.145	0.161
Maternal venous blood mercury level (µg/dL)	23.99±7.13	28.19±5.35	-2.805	**0.004**
Fetal cord blood mercury level (µg/dL)	24.71±5.31	131.49±95.12	-6.660	**<0.001**
Placental mercury level (µg/dL)	1.46±1.20	1.80±1.47	-1.095	0.326
Maternal venous blood lead level (µg/dL)	4.89±7.23	7.54±9.71	-1.208	0.199
Fetal cord blood lead level (µg/dL)	10.62±9.90	15.22±13.34	-1.586	0.111
Placental lead level (µg/dL)	0.16±0.88	0.29±0.24	-2.959	**0.004**

(*) t value, Independent Samples t test; p <0.05

=0.023). Fetal head circumference was negatively correlated with the maternal venous blood mercury level (r = -0.290, p = 0.018), and fetal blood mercury level (r = -0.596, p <0.001).

ROC-Curve analysis revealed that fetal body weight could be measured lower healthy subjects if fetal cord mercury level was measured >73.78 µL (area = 0.786, p <0.001, 74% sensitivity, 78% specificity). Fetal body height could be measured shorter than healthy subjects if fetal cord mercury level was measured >40.90 µL (area = 0.771, p <0.001, 75% sensitivity, 73% specificity). Fetal head circumference could be measured lower than healthy subjects if fetal cord mercury level was measured >33.79 µL (area = 0.752, p = 0.001, 80% sensitivity, 70% specificity) (Table 2, Figure 1).

Interestingly, Logistic Regression test results revealed that fetal cord mercury level (B = 0.012, Wald = 7.864, p = 0.005) and placenta lead level (B = 3.891, Wald = 4.666, p = 0.031) could be the best predictors of the risk of lower fetal body weight. Furthermore, fetal cord mercury level (B = 0.010, Wald = 6.829, p = 0.009) and placenta lead level (B = 3.343, Wald=4.436, p=0.035) could be the best predictors of the risk of lower fetal body height. In addition, fetal cord mercury level (B = 0.009, Wald = 6.153, p = 0.013) could be the best predictors of the risk of lower fetal head circumference (Table 2).

DISCUSSION

In recent years prenatal exposure to heavy metals has attracted increasing attention. Studies evaluating fetal exposure to heavy metals and their influences on birth weight have reported that heavy metals can cause fetal toxicity due to the ability to cross the placenta (8). Exposure to lead and/ or mercury during pregnancy causes preterm delivery and low birth weight and has negative effects on fetal growth, and neurodevelopment (16). Although the placenta is a barrier preventing the transfer of toxic metals to the fetus, it cannot prevent the passage of all toxic substances. In addition, toxic metals accumulated in the placenta may impair placental function and prevent the transfer of essential nutrients (17,18,19). All these factors could result in altered growth patterns and in many cases fetal growth restriction (20,21, 22). Related to these issues, previous studies

Table 2. The table shows the study parameters that can predict the lower fetal body weight, fetal body height, and fetal head circumference

		***ROC-Curve* test**				
Prediction	**Variable**	**Area**	**p**	**Cut-off value**	**Sensitivity**	**Specificity**
Fetal body weight	Fetal cord mercury level	0.786	**<0.001**	>73.78	74%	78%
Fetal body height	Fetal cord mercury level	0.771	**<0.001**	>40.90	75%	73%
Fetal head circumference	Fetal cord mercury level	0.752	**0.001**	>33.79	80%	70%
		Logistic Regression test				
Prediction	**Variable**		**B**	**S.E.**	**Wald**	**p**
Fetal body weight	Fetal cord mercury level		0.012	0.004	7.864	**0.005**
	Plasenta lead level		3.891	1.801	4.666	**0.031**
Fetal body height	Fetal cord mercury level		0.010	0.004	6.829	**0.009**
	Plasenta lead level		3.343	1.587	4.436	**0.035**
Fetal head circumference	Fetal cord mercury level		0.009	0.003	6.153	**0.013**

p<0.05

have reported that fetal cord blood lead levels were higher or equal to maternal blood lead levels (23,24). The main aim of this paper was to evaluate lead and mercury levels in maternal venous blood, fetal cord blood, and placenta between fetuses with idiopathic IUGR, identified by impaire uterine or umbilical artery Doppler findings, in comparison with the healthy term pregnant fetuses as controls. The results demonstrated that mercury levels in maternal blood and fetal cord blood, and lead levels in the placenta were found to be significantly higher in the IUGR group than in healthy subjects. Although lead levels measured in the fetal cord blood and maternal venous blood were higher in the IUGR group numerically, these were not statistically significant. The levels of mercury in the placenta showed no difference between the groups. These results were consistent with the findings of previous studies and demonstrate placental permeability to heavy metals, confirming intrauterine fetal exposure (11,21). The higher levels of fetal cord blood mercury and lead compared to maternal levels in the IUGR group indicated that the placenta could not prevent the transfer of heavy metals even at very low levels due to defective placentation in the IUGR group. These findings could suggest that heavy metal exposure could negatively affect fetal growth. (11,20,21). However different results could be reported in some studies, due to the variety of exposure matrices, dietary patterns, environmental factors, and genetic predisposition (11, 25, 26).

At the end of the correlation analysis, it could be seen that measurement values of the body weight, body height, and head circumference of the fetus might be lower than healthy subjects when the mercury level value was measured higher in maternal venous blood and fetal cord blood. Fetal body weight and fetal body height might be measured lower than healthy subjects when the lead level value was measured higher in the placenta. However, the fetal head circumference measurement could not be associated with the placental lead level. With these results, it was thought that higher lead and mercury level values measured in maternal venous blood could negatively affect intrauterine fetal growth and neurodevelopment and it may cause microcephaly and intrauterine fetal growth restriction.

The ROC-Curve analysis and Logistic Regression test results revealed that the mercury levels measured in fetal cord blood could be used as the best marker in predicting the risk of low fetal weight, low fetal body height, and low fetal head circumference which associate with the IUGR. In addition, placental lead level was thought to be a good marker for predicting low fetal body weight and low fetal body height, but it was thought that it could not be used as a predictive marker due to its difficulty in clinical use. On the other hand, it was concluded with this study results that the possibility of heavy metal intoxication should be kept in mind in the condition that the etiological cause could not be identified in the fetus diagnosed intrauterine growth

retardation during the prenatal follow-up period. According to the findings of this study, it would be appropriate to measure the level of lead and, in particular, mercury in the fetal cord blood during the prenatal follow-up period in order to determine the etiology and provide therapeutic prenatal care for IUGR in a fetus diagnosed with idiopathic IUGR. Thus, if a diagnosis of heavy metal exposure can be made in the mother of the fetus diagnosed with IUGR in the prenatal period, it may be possible to protect and treat both the mother and the fetus in the early period.

Limitations

This study had some restrictions. Firstly, this study consisted of a low number of participants and a restricted number of metals. Secondly, this study did not contain the examination of harmful environmental pollutants (such as cadmium, bisphenol) which could affect fetal development. Thirdly, maternal blood levels of prenatal nutrients which are essential for normal development (vitamin, iron, etc.), should be added to this study data. As the lead and mercury concentrations were measured only at the time of delivery and no long term postnatal outcomes were evaluated, it could not be possible to predict the degree of exposure during the whole fetal development process. Furthermore, this study did not describe the living area of the women and therefore it could not describe where they could be more exposed to airborne mercury or lead. Moreover, this study did not contain the dietary properties of the women and therefore it could not identify a dose-response relationship between mercury and lead concentrations. Finally, this study did not contain the urinary mercury concentrations and therefore it could not identify the different spectrum of toxic effects from organic mercury, such as methyl mercury, versus inorganic mercury.

CONCLUSION

In conclusion, it was thought with this study results that in order to identify the etiology and to give therapeutic prenatal care of the IUGR in a fetus diagnosed as idiopathic IUGR it would be appropriate to measure the level of lead and especially mercury in the fetal cord blood during the prenatal follow-up period.

Acknowledgments: The authors would also like to acknowledge Dilek Kaya-Akyuzlu and Fezile Ozdemir for their contributions to the sample preparation process in the Forensic Toxicology Laboratory of the Institute of Forensic Sciences, Ankara University.
Author contributions: The project was constructed by: DY, AK, DCK, TY and EK. BY designed and performed experiments. DY, TY, DCK,EK and BY responsible for data collection and processing. DY,EK and TY analysed the data. DY wrote the manuscript and BY,EK,TY,DCK and AK edited the manuscript.
Conflict of Interest: Authors have no conflict of interest to declare, and don't have relationships with companies that may have a financial interest.
Ethical Approval: The study was approved by the Local Research Ethics Comittee of Ankara University (Decision Number:05-221-14/March 24, 2014)
Funding: This study was financially supported by Ankara University Scientific Research Projects (project number 14L0230010).
Peer-review: Externally peer-reviewed.

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