

The Effect of Heavy Metals on Miscarriage

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ABSTRACT Objective: In a world where industrial pollution is increasing day by day, heavy metals are one of the important factors threatening human health. The rates of pregnancy loss and sub-fertility are continuously increasing. This study aims to explain the effects of lead (Pb), cadmium (Cd), and mercury (Hg) levels on the etiology of abortions. **Material and Methods:** The study was designed as a case-control study with two groups: healthy volunteers (n=20) and miscarriage (n=29) group. The pregnant women with similar demographic characteristics were divided into two groups and peripheral venous blood samples of the study subjects were collected. Atomic absorption spectrophotometer was used to examine the blood samples and the levels of heavy metal. **Results:** While the Pb level was found to be higher in the miscarriage group (p=0.038), there was no statistical difference between Cd and Hg levels (p>0.005) in the two groups. On comparing the laboratory values of pregnant women in the control and miscarriage groups, blood progesterone values were observed to be lower in the miscarriage group (p<0.001). **Conclusion:** This study indicates that heavy metals, even below the toxic dose limits, can cause miscarriage. Higher blood Pb levels were found in the miscarriage group while Hg and Cd levels were not different between the two groups. This research points out that the high levels of lead may have an effect on pregnancy loss.

Keywords: Miscarriage; heavy metals; lead; cadmium, mercury

Environmental pollution and industrialization expose humans to heavy metals in every period of their lives. Exposure to heavy metals affects the growth and development of a living being; therefore, it has become increasingly alarming in terms of public health. One of the environmental factors responsible for decreasing the chances of a healthy pregnancy and thus preventing a healthy birth is heavy metals. Both maternal and paternal heavy metal exposure may affect pregnancy. Occupational exposures to heavy metals have been associated with poor obstetric outcomes and adverse effects on female fertility.

Heavy metals have been proved to be environmental pollutants for all living creatures and humans. They may be present in the environment either naturally, or as industrial pollutants resulting from industrial accidents and various other reasons. The levels of heavy metals in blood have been found to be higher in countries with greater industrial development.¹

Miscarriage is defined as a pregnancy that is spontaneously lost before 22 weeks of gestation and below 500 g fetal weight. Spontaneous abortion is largely becoming a public health concern for developing countries.² Early pregnancy loss includes gestational sac that is empty before 12 6/7 weeks of gestation or the presence of an embryo or fetus without cardiac

activity.³ Approximately 10% of the clinically diagnosed pregnancies result in spontaneous abortion.⁴ Chromosomal anomalies are the most common etiologic factors causing spontaneous pregnancy loss. The most important factors that increase the risk of abortion include increased maternal age and previous abortions.^{5,6}

Heavy metals like cadmium (Cd), lead (Pb), and mercury (Hg) have been found to affect reproductive success adversely along with causing environmental pollution.⁷ The mutagenic, teratogenic, and embryotoxic effects of Cd have been well documented in the literature.⁸ Heavy metal exposure during pregnancy has been shown to be associated with pre-eclampsia, preterm delivery, and low birth-weight deliveries while low selenium levels have also been suggested to contribute to preterm labor.^{9,10} It has been reported that exposure to environmental pollutants is associated with ovulation disorder, implantation failure and may also cause pregnancy loss and congenital anomalies.¹¹ Cd is the heavy metal that is used in battery mechanization, pigment technology, and plastic industry. Smokers, as well as those exposed to passive cigarette smoke, may also be exposed to Cd toxicity. The most frequent exposure to Cd, resulting from environmental factors is smoking and some vegetables. Cd levels have been found to be high in the blood and placentas of pre-eclamptic women.¹² Molecular level examinations have revealed that some organisms use specific mechanisms, which may help avoid the toxicity of the Cd in order to overcome its harmful effects. Metallothioneins (MTs) play an important role in these mechanisms. MTs are a family of proteins, rich in cysteine, and range in size from small to medium. In all eukaryotes and some prokaryotes, they have the ability to coordinate metal ions through metal-thiolate bonds.¹³ Cd toxicity inhibits the transmission of zinc (Zn) from the mother to the fetus, induces MT gene expression in specific tissues and causes the Zn-MT complex to change to Cd-MT complex.¹⁴ These changes at the molecular level impair the maternofetal relationship and adversely affect fetal development.

Lead toxicity is one of the most well-known heavy metal-borne clinical conditions because it

is one of the most easily exposed heavy metals caused by environmental factors. Pb toxicity affects the hematopoietic system, nervous system, reproductive system, urinary system, and blood pressure.¹⁵ It has been shown that exposure to Pb during pregnancy may cause accumulation in the fetal tissues and cause irreversible tissue and organ damage.¹⁶ Pb has been shown to cause premature delivery and premature rupture of the membrane, even at non-toxic doses.¹⁷ An increase in Pb levels in maternal blood has been found to be associated with a decrease in the birth weight of newborns, head circles, and crown-heel length.¹⁸

Another heavy metal, known to be neurotoxic and may also be embryotoxic and fetotoxic during pregnancy, is Hg.¹⁹ The consumption of contaminated seafood, water, and air pollution, use of some creams and teething powder may cause Hg exposure.²⁰ Dental amalgams contain approximately 50% of elemental Hg. In the UK, it is recommended to avoid or remove dental amalgam during pregnancy.²¹ It has been observed that Hg causes organ and tissue damage by disrupting DNA and RNA structure in intra-uterine life.²² Children have been found to be born with anomalies such as microcephaly, cerebral palsy, severe mental retardation, seizure disorders, deafness, and visual impairment even in cases where mothers were not affected after Hg exposure.²³

Approximately 80% of all cases of pregnancy loss occur in the first trimester.⁹ Spontaneous miscarriages may also be a marker of embryotoxicity. This study aims to elucidate the role of heavy metals in pregnancy loss by comparing serum Cd, Pb, and Hg levels in women who have had spontaneous miscarriages even though fetal heart rate was detected in the previous pregnancies, compared to women with healthy pregnancies.

MATERIAL AND METHODS

POPULATION AND DESIGN OF THE STUDY

The study had a case-control design and was conducted between December 2017 and August 2018 at the Department of Obstetrics and Gynecology, Faculty of Medicine, Hitit University, Corum, Turkey.

There were two groups; the first group was defined as the control group and consisted of 20 healthy singleton pregnancies in women aged 18-40 years. The second group (miscarriage group) consisted of 29 singleton pregnancies in women between the ages of 18-40 years, in whom the pregnancy was found to have stopped at the first trimester of fetal heart rate. Prior to the study, the Ethics Committee approval was obtained from the Faculty of Medicine, Hitit University, in accordance with the Helsinki Declaration. The approval date of the ethics committee is 19 December 2017 and the acceptance number is 2017-201. Informed consent was obtained from all participants.

The inclusion and exclusion criteria for the control and the miscarriage groups are defined in [Table 1](#) and [Table 2](#), respectively.

In total, 188 pregnant women were included in the study from the beginning till the end of the investigation. The reasons for the removal of pregnant women from the study were smoking (61), a history of dental amalgam (23), receiving additional medication (16), having worked in industrial jobs with high levels of heavy metal pollution (13), refusing to participate in the study (11), uncoopera-

tive due to language problem (9), and those who voluntary withdrawn from the study (6).

The socio-demographic characteristics, smoking history and medical history of the patients were recorded using the face-to-face questionnaire method. All participants were living in the center of Corum (Turkey). Ultrasonographic evaluations of the pregnant women were performed using the GE Logiq P5 (2015, GE Healthcare, Milwaukee WI) device with a transvaginal probe in the obstetrics clinic. Although the embryo was detected in the ultrasonographic examination, the pregnant women whose fetal heart rate could not be detected were accepted as miscarriage and referred to the Radiology Clinic of Hitit University for being included as ultrasonography controls (n=3). The pregnant women with an audible fetal heart rate were included in the study. The data from thyroid function tests, toxoplasma, rubella, cytomegalovirus (CMV), and herpes simplex virus and other laboratory findings were recorded in the hospital automation system.

MEASUREMENT OF THE SERUM HEAVY METAL CONCENTRATIONS

Peripheral venous blood was obtained after the ultrasonographic evaluation of pregnant women in

TABLE 1: Inclusion criteria for the control and miscarriage groups.

Control group (n=20)	Miscarriage group (n=29)
Age between 18-40	Age between 18-40
Singular pregnancy	Singular pregnancy
Last menstruation date should be on record	Last menstruation date should be on record
Having regular ultrasonographic record during the pregnancy period	Having regular ultrasonographic record during the pregnancy period
Having normal ultrasonographic findings	It should have been confirmed independently by two physicians that there is no fetal heartbeat

TABLE 2: Exclusion criteria for the control and miscarriage groups.

The exclusion criteria (Both groups)	
<ul style="list-style-type: none"> • Presence of dental amalgam • Working in jobs with high industrial pollution • Receiving lithium or metal-containing medication • Taking additional medication except for folic acid • Having high-risk in the dual or triple screening test • Being diagnosed with cervical insufficiency • Having a homozygous thrombophilia gene mutation • Obtaining medication for induced abortion 	<ul style="list-style-type: none"> • Being above 40 years of old • Chronic drug use • Impaired kidney and liver function tests • Smoking • Having one of the Müllerian anomalies • Having two heterozygous thrombophilia gene mutations • Presence of myoma uteri, which disturbs the morphology of the uterus or compresses the endometrial cavity

the control group while it was taken from the patients in the miscarriage group just before the evacuation of uterine cavities. Of the blood, 10 mL was aspirated into spray-coated tubes with sodium heparin and stored at -80°C in a deep freezer.

REAGENTS

Analytical grade chemicals, prescribed for trace element analysis, were used. For the sample preparation, 65% (v/v) nitric acid (HNO_3) (Sigma-Aldrich Corp, St Louis, MO, USA), 70% (m/v) perchloric acid (HClO_4) (Merck, Darmstadt, Germany), and Triton X-100 (Sigma-Aldrich Corp, St Louis, MO, USA) were used. The working standard solutions for Cd, Hg, and Pb (1000 mg mL^{-1}) were obtained from Chem-Lab, Zedelgem, Belgium.

SAMPLE PREPARATION AND DIGESTION

In order to measure Cd level, 1 mL of whole blood sample was accurately measured using a graduated cylinder and automatic pipette (pipette head was washed with solvent) and then added into a propylene tube. Into the same tube, 9 mL of 4:1 $\text{HNO}_3:\text{HClO}_4$ mixture was added. A clean glass bar was used to shake the mixture. After waiting for approximately 10 min for the removal of gas, the mixture was put into a water bath. The mixture was digested in a water bath at 80°C for 2 h and was removed from the water bath when the samples became colorless. After cooling, the colorless solutions were transferred into 10-mL volumetric flasks and made up to the volume with 5% HNO_3 in water. In order to measure Hg and Pb levels, 1 mL of each sample was mixed with 6 mL of HNO_3 solution and 3 mL of Triton X-100 solution. After this, the same digestion method, described for Cd, was followed.²⁴⁻²⁶

APPARATUS

The Optimize Furnace Parameters Wizard with SOLAAR (Thermo Scientific, Cambridge, England) software was used to determine the most suitable temperature and flow rate for analyzing the digested samples for all the elements. The automatic sampler was used to optimize the position of the injection capillaries and to observe the specimen being left in the cuvette. Of each elemental solution, $10\text{ }\mu\text{g/L}$ was used as the main standard. The au-

tomatic sampler was programmed to automatically set the calibration standards in the appropriate range. All samples, blanks, and standards were injected at a constant fixed volume of $10\text{ }\mu\text{L}$, alongside an additional aliquot of $10\text{ }\mu\text{L}$ of the matrix modifier into an Electrographite cuvette. Cd was analyzed at 228.8 nm, Hg was analyzed at 253.7 nm and Pb was analyzed at 217.0 nm; Zeeman background correction was used throughout the process. Peak areas were measured for calibration and subsequent determination of the sample concentrations. The value of R^2 of the calibration curve was higher than 0.995. Finally, after striking with dilution factor, the result was received as ppb ($\mu\text{g L}^{-1}$).²⁴

STATISTICAL ANALYSIS

All statistical analyses were performed with SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA) package program. The distribution of normality was analyzed by the Shapiro-Wilk test. Descriptive statistics were presented as mean \pm standard deviation or median (min-max) according to data distribution for continuous variables. Parametric data of the groups were compared with Student's *t*-test while the nonparametric data were compared with the Mann-Whitney U test. The relationship between the diagnosis and categorical variables was investigated by either chi-square test or Fisher's exact test. A *p*-value <0.05 was considered to be statistically significant.

RESULTS

Among the control group and miscarriage group, there was no statistically significant difference between age, body mass index (BMI), and the gestational age calculated by the last menstrual date ($p>0.005$). CRL (Crown-Rump Length) values of the pregnant women in the miscarriage group were found to be smaller than those of the gestational weeks calculated by ultrasonographic measurements done at the time they were included in the study. This difference was statistically significant ($p=0.001$, Table 3).

Categorical variables of the study groups, such as blood group, were evaluated by the chi-square test or Fisher's exact test. There was no difference between the control and miscarriage groups in

TABLE 3: Demographic characteristics of groups.

	Control (n=20)	Miscarriage (n=29)	p-value
Age (years)	25.40±3.60 (19.00-31.00)	24.62±4.17 (18.00-33.00)	0.501 ^a
USG (weeks)	12.55±2.89 (7.00-18.00)	9.86±2.62 (6.00-16.00)	0.001 ^a
SAT (weeks)	14.15±2.56 (9.00-18.00)	12.37±1.91 (9.00-17.00)	0.008 ^a
BMI (kg/m ²)	22.14±2.00	22.10±2.10	0.943 ^b

^aMann-Whitney U test, ^bStudent's t-test

USG: Ultrasonography; LMD: Last menstrual date; BMI: Body mass index, CD: Cesarean delivery.

terms of frequency of blood groups and Rh antigen ($p>0.005$). The indirect Coombs test was negative in Rh (-) pregnant women in control and miscarriage groups. There was no significant relationship between the volunteers in both groups with respect to the diagnosis, gravida, parity, living children, and abortions ($p>0.005$). Of the pregnant women, 65% in the control group and 51.7% in the miscarriage group had normal birth; the difference was statistically significant ($p=0.015$).

The infectious parameters for both groups were determined. Toxoplasma IgM, HbS Ag, CMV IgM, Rubella IgM, anti-HCV, and anti-HIV tests were found to be negative. No statistically significant difference was found between the control and miscarriage groups in the anti-Toxoplasma IgG, HBs IgG, CMV IgG, and Rubella IgG tests ($p>0.005$).

There was no statistically significant difference between Cd and Hg levels ($p>0.005$) in the blood of control and miscarriage groups. However, blood Pb levels in the miscarriage group were found to be significantly higher ($p=0.038$, Table 4). When the laboratory values in the control and miscarriage groups were compared, blood progesterone values were observed to be lower in the miscarriage group ($p<0.001$). Additionally, blood calcium levels of the women in the miscarriage group were lower than those in the control group ($p=0.010$) (Table 5).

DISCUSSION

The study aimed to understand the role of Cd, Pb, and Hg in the etiology of pregnancy loss during early

gestational weeks. According to the spectrophotometric analysis, Pb levels were found to be higher in the miscarriage group as compared to that in the control group. On the basis of the analyzed results, we conclude that the possibility of chronic exposure to Pb from outside sources is low and Pb is also one of the factors involved in the etiology of miscarriage.

In this study, the levels of the heavy metals were found to be below the limits suggested by the World Health Organization (WHO) and other organizations as threatening.²⁷⁻²⁹ The authors concluded that the elevation in blood Pb level may be related to miscarriage. There have been reports investigating the Pb level in the blood of pregnant women. In a study, in which Cd and Pb levels were examined in blood and placental tissues, Pb levels were found to be higher in the blood and placental tissues of the pregnant women.³⁰ Similarly, in the

TABLE 4: Heavy metal levels in the groups.

	Control (n=20)	Miscarriage (n=29)	p-value
Lead (μ/L)	44.45±12.49	54.11±17.27	0.038*
Cadmium (μ/L)	0.40±0.05	0.39±0.06	0.704*
Mercury (μ/L)	0.46±0.27	0.47±0.29	0.907*

* Student's t-test

TABLE 5: Laboratory findings.

	Control (n=20)	Miscarriage (n=29)	p-value
Hb (g/dL)	11.54±1.24	11.44±1.08	0.583
Glucose (mg/dL)	89.53±8.62	90.20±8.59	0.790
Creatine (mg/dL)	0.68±0.12	0.68±0.13	0.834
TSH (uIU/mL)	1.71±0.34	1.95±0.59	0.053
Progesterone (ng/mL)	40.45±5.22	10.30±2.11	<0.001*
Hba1c	5.22±0.54	4.93±0.47	0.069
AST (U/L)	24.89±4.23	24.35±4.031	0.986
ALT (U/L)	21.29±2.96	21.37±3.76	0.933
Na (mmol/L)	139.39±3.05	138.41±3.56	0.421
K (mmol/L)	3.96±0.24	3.89±0.32	0.768
Cl (mmol/L)	99.36±3.83	98.36±3.26	0.348
Ca (mmol/L)	8.75±0.17	8.93±0.29	0.010*

* Student's t-test

Hb: Hemoglobin; TSH: Thyroid-stimulating hormone; Hba1c: Hemoglobin A1c; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Na: Sodium; K: Potassium; Cl: Chloride; Ca: Calcium.

present study, the blood Pb levels were also higher. In another study, placental tissues were examined and heavy metal levels of the placental tissues were compared. The transplacental transition of heavy metals is variable in pregnancy.³¹ Therefore, in the present study, heavy metal levels in the placenta and aborted material were not compared.

When the miscarriage group was compared with the control group, no statistically significant difference between Cd levels in the mother's blood was observed. However, an earlier report based on the clinical data of pregnant women with high Cd levels showed more recurrent miscarriages.³² In a study in which blood and placental tissues were evaluated together, Cd levels were higher in the miscarriage group.³⁰ High blood levels of Pb, Cd, and Hg have been shown to reduce pregnancy rates in a study examining the effect of heavy metal levels on fertility outcomes.³³

When serum Hg levels of the miscarriage group and the control group were compared, no significant difference was found between them. The literature reports studies stating that high maternal Hg levels result in poor obstetric outcomes. In a study examining Hg levels in maternal blood, it was found that the developmental scores of the children born to mothers with high blood Hg levels were lower during 35-48 months.³⁴ The mothers with high Hg levels gave birth to shorter babies (in terms of length) and with lower birth weights.³⁵

We found blood progesterone levels to be higher in the healthy pregnancies. Although, there has been no prescribed limit of progesterone level to identify abnormal pregnancies, the best limit in early gestational weeks has been shown to be 10 ng/mL.³⁶ A study examining early risk factors for miscarriage showed a higher risk of lower serum progesterone levels (<12 ng/mL).³⁷ In the present study, serial progesterone level measurements were not performed and, instead, single value measurements were considered.

One of the limitations of the present study was that the variables related to the living areas of the participants were not considered. For example, although there were comprehensive inclusion and exclusion criteria for the participants, it was not

recorded for the patients whether they have lived in a heavy metal contaminated area. Further, it was controlled that the patients were not smokers but we did not inquire if they had been exposed to passive cigarette smoke at home or in their social lives. The low number of participants (total number of pregnant females was 49) could be considered as another limitation of the study. Further, it was not possible to search for organ anomalies, genetic screening and culture of embryos in post-curettage pathologies of the miscarriage group. Therefore, inadequate pathological evaluation of the aborted material is also a limitation of the study.

CONCLUSION

Exposure to heavy metals may adversely affect the health of pregnant women. Even if the levels of heavy metals in the blood are below the toxic dose limits, it can cause miscarriage. This study found higher blood Pb levels in the miscarriage group. Further research in a larger population sample is required to confirm these results and to elucidate the mechanism of the interaction between heavy metals and miscarriage.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Engin Yıldırım; **Design:** Engin Yıldırım; **Control/Supervision:** Engin Yıldırım; **Data Collection and/or Processing:** Engin Yıldırım; **Analysis and/or Interpretation:** Mehmet Kürşat Derici; **Literature Review:** Engin Yıldırım; **Writing the Article:** Engin Yıldırım; **Critical Review:** Mehmet Kürşat Derici; **References and Fundings:** Engin Yıldırım, Mehmet Kürşat Derici; **Materials:** Engin Yıldırım, Mehmet Kürşat Derici.

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