Clin. Lab. 2018;64:XXX-XXX

ORIGINAL ARTICLE

Cadmium and Lead in Women Who Miscarried

Wioleta J. Omeljaniuk [1], Katarzyna Socha [2], Jolanta Soroczyńska [2], Angelika E. Charkiewicz [1], Tadeusz Laudański [3], Marek Kulikowski [3], Edward Kobylec [4], Maria H. Borawska [2]

[1] Department of Public Health, Medical University of Bialystok, Bialystok, Poland
[2] Department of Bromatology, Medical University of Bialystok, Bialystok, Poland
[3] Department of Perinatology, Medical University of Bialystok, Clinical Hospital, Bialystok, Poland
[4] Division of Obstetrics and Pathology of Pregnancy, SPZOZ District Hospital in Bialystok, Bialystok, Poland

SUMMARY

Background: **Cadmium (Cd) and lead (Pb) are toxic elements which, when ingested excessively in food and drinking water, accumulate in selected organs and pass through the placenta barrier to the foetus, showing teratogenic effects. The aim of the study was to determine the concentration of Cd and Pb in blood and placental tissue in women who miscarried.**
Methods: **The study group consisted of 83 women who miscarried. The control group included 35 women in the first trimester of pregnancy and after childbirth. The experimental materials consisted of whole blood and fragments of placental tissue. The concentrations of Cd and Pb were determined using atomic absorption spectrometry (AAS) with electrothermal atomization in a graphite furnace and by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in standard mode.**
Results: **The average concentration of Cd (2.730 ± 2.07 µg/L) and Pb (35.54 ± 11.0 µg/L) in the blood of women with miscarriage was higher in comparison to the level of these toxic metals in the blood of women from the control group (Cd 1.035 ± 0.59 µg/L; Pb 27.11 ± 4.6 µg/L). The average Cd (214.4 ± 514 µg/L) and Pb (199.6 ± 348 µg/L) content in the placenta of women with miscarriage was higher in comparison to the amount of these elements in the placenta of women from the control group (Cd 127.4 ± 85 ng/L; Pb 26.35 ± 7.9 ng/L). Tobacco smoking significantly affected cadmium blood levels and the placental tissue content in women who miscarried.**
Conclusions: **Elevated Cd and Pb concentrations in the blood and placenta of pregnant women may be connected with the occurrence of miscarriage; therefore, the levels of these heavy metals should be monitored in women who plan pregnancy. It seems that determining the level of molar ratio between toxic metal and antioxidant elements can be analyzed as a marker for selection for control examinations as a valuable complement to existing diagnostic procedures in prevention, especially in early pregnancy. Additional diagnostic methods should be established as new tools in perinatal care in order to enable early diagnosis of pregnancy pathology and, especially, to prevent miscarriage.**
(Clin. Lab. 2018;64:xx-xx. DOI: 10.7754/Clin.Lab.2017.170611)

Correspondence:
Wioleta J. Omeljaniuk
Department of Public Health
Medical University of Bialystok
Szpitalna St. 37
15-295 Bialystok
Poland
Phone/Fax: +48 85 6865 056
Email: wioletaomeljaniuk@wp.pl

Manuscript accepted September 10, 2017

KEY WORDS

cadmium, lead, whole blood, placental tissue, miscarriage

INTRODUCTION

Miscarriage (lat. abortus spontaneus) is a term used to describe pregnancy complications involving a spontaneous expulsion of the ovum from the uterine cavity in

whole or in fragments at a period when the baby is unable to survive outside its mother's womb. According to the World Health Organization's (WHO) definition, a miscarriage occurs when a pregnancy terminates before reaching 22 weeks of gestation, with the foetus weight below 500 grams. Miscarriage is a form of pregnancy pathology which prevents successful birth of a mature and healthy baby [1,2].
Miscarriage is the most common complication of pregnancy. Approximately 25 - 30% of women who went through pregnancy have lost one or more pregnancies. The evaluation of the frequency of miscarriages in the general population is made difficult by the fact that many early pregnancies are miscarried before they are diagnosed. It is believed that approximately 10 - 30% of clinically diagnosed pregnancies conclude in spontaneous miscarriage, 80% of which are in the first trimester [3,4]. Multidimensional studies in three main categories, genetics, hormones, and anatomy, may serve to explain most of the cases of spontaneous abortion. The factors responsible for causing the first miscarriage may contribute to further loss of pregnancy. That is why in order to fully understand the phenomenon, studies need to be expanded in the direction of identifying possible infections and poisoning, metabolic disturbances, relating frequently to malnutrition, as well as endocrine and immune disorders.
Both the aetiology and pathogenesis of early pregnancy miscarriage are only partially known, and many aspects of this problem still remain to be explored further. Between 2000 and 2014, there was an observed increase of spontaneous miscarriages in Poland. The average annual number of miscarriages in Poland is 49,476, amounting to 13.0% of all successful pregnancies.
Environmental factors, i.e., chemical agents (medical equipment sterilizers, anaesthetic gases, perchloroethylene, organic solvents), as well as ionizing radiation, environmental pollution (exposure to Cd and Pb, Hg, Ar), air pollution, pesticides, plant protection products, exhaust fumes, pharmacological treatment (cytostatic drugs, antimetabolites, iodine preparations, sulphonamides, antidepressants, and vaccinations) contribute to 8.6 - 37.8% miscarriages [5-9].
Cd and Pb are toxic elements which, when ingested excessively in food and drinking water, accumulate in selected organs, such as blood, liver, kidneys, brain, and bones [10].
These heavy metals are capable of displacing essential micro elements from metalloenzymes. Their interactions with zinc, manganese, copper, iron, magnesium, calcium, selenium, all vital elements to the human body, result in serious morphological and functional changes in various organs [11]. It must be stressed that Cd is infamous for its poor ability to be excreted/eliminated by living organisms and is only slightly excreted with urine and/or bile. The biological half-life of Cd in the human body is 16 - 30 years [11]. The half-life of Pb in the body is from 30 days up to 27 years [12]. Also, both heavy metals are capable of passing through the placenta barrier to the foetus, showing teratogenic effects [11]. However, there is still insufficient data on the direct impact of these two elements on early pregnancy loss.

MATERIALS AND METHODS

The aim of the study was to determine the level of Cd and Pb in blood and placental tissue in women who miscarried and to answer the question of whether dietary habits and tobacco smoking affect the levels of these elements in the bodies of the studied women. The study group consisted of females (n = 83) aged 18 - 44, who experienced early pregnancy loss and were hospitalized in the Department of Perinatology at the Clinical Hospital of the Medical University of Białystok and in the Division of Obstetrics and Pathology of Pregnancy, SPZOZ District Hospital in Białystok. The control group consisted of women (n = 35) that at the time of the study were in their first trimester, aged 18 - 32, who went on to successfully deliver healthy babies (Table 1).
The experimental materials consisted of whole blood and fragments of placental tissue. Whole blood and placental tissue fragments in the miscarriage group were collected during surgery. The blood in the control group was sampled in the first trimester of the pregnancy during a routine check-up. Fragments of the placenta were collected after childbirth. The blood was sampled in accordance with procedures for the determination of elements into vacuum containers by Vacutainer. Next, the blood was deproteinized with nitric acid, 1 mol/L, 1% Triton X-100 was added as a surfactant and diluted with 0.1 mol/L nitric acid. The placenta fragments were collected into polypropylene scintillator containers, mineralized in nitric acid using the microwave method in a closed system (Ertec, Poland), and placed quantitatively in polypropylene containers.
The blood levels of Cd and Pb were determined using atomic absorption spectrometry (AAS) with electrothermal atomization in a graphite furnace, with a *Zeeman* background correction of Cd = 228.8 nm and Pb = 283.3 nm on a Z-2000 spectrometer by Hitachi High-Technologies (Hitachi High-Technologies Corporation, Japan). Standard solutions for the calibration were made from standard solutions of Cd and Pb (Merck, Germany). The accuracy of Cd and Pb determination was controlled using certified reference materials - Seronorm Trace Elements Whole Blood L-2, 1406264 (Sero AS, Norway).
The concentration of Cd and Pb in placenta samples was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in standard mode (NexION 300D, PerkinElmer, USA). ICP-MS conditions are presented in Table 2. Standard solutions for the calibration were made from the standard solution of Cd and Pb (PerkinElmer Pure Plus, USA) The accuracy of Cd and Pb determination was controlled using certified reference materials - Bovine Muscle Powder (Beef) RM

Table 1. Experimental and control group characteristics.

Parameters	Experimental group (n = 83)	Control group (n = 35)
	Average ± SD (Min - Max)	Average ± SD (Min - Max)
Age (years)	29.90 ± 6.3 (18 - 44)	27.20 ± 3.5 (18 - 32)
BMI (kg/m^2)	22.89 ± 3.6 (17.4 - 34.0)	22.74 ± 3.2 (19.7 - 30.4)
Week of pregnancy	8.96 ± 2.6 (4 - 19)	9.40 ± 2.5 (6 - 14)

SD - Standard deviation, Min - minimum, Max - maximum.

Table 2. ICP-MS conditions used for Cd and Pb determination.

Parameter	Condition for Cd	Condition for Pb
Mass (amu)	109.903 110.904 111.903 113.904	205.975 206.976 207.977
Dwell time per amu (ms)	50	50
Integration time (ms)	1000	1000
Detector calibration mode	Dual	Dual
Replicates	5	5

8414 (Canada Collaborative Materials, USA).
The molar ratio between examined toxic metals and antioxidant elements in the blood and placental tissues, which were presented in our previous studies [13], was also calculated.
The results were then analysed statistically using Statistica v.12.5. software. In accordance with the principles of statistical inference, Mann-Whitney's *U* test was used for two independent groups, as the analysed variables did not follow a normal distribution. The assumed level of significance was $p < 0.05$. The analysis also employed the nonparametric Spearman's rank correlation method. The analysis, focusing on the effects of consumption of various products on the levels of Cd and Pb, used multiple regression, forward stepwise. The patients were surveyed on the frequency of consuming various groups of food products in accordance with the guidelines of The Committee on Food and Nutrition Sciences of The Polish Academy of Sciences [14] and on tobacco smoking.

RESULTS

The average level of Cd in the blood of women with miscarriage was higher compared to the control group (Table 3), and 36% of the patients showed a Cd blood concentration exceeding the maximum value, which is up to 2.7 µg/L [5]. The average Cd content in placental tissue in women with spontaneous miscarriage was higher than in the control group (Table 3).
The average Pb levels in blood and placenta in women who miscarried were higher than in the control group (Table 3).
Among the whole group of patients with miscarriage only 14 reported smoking tobacco during that period, the rest of the group (n = 69) did not admit to smoking. Not a single woman in the control group smoked tobacco. A difference was found in the Cd blood concentration between the smoking women and the non-smoking ones in the experimental group, and between the control group and these two groups. Also, a difference was observed in the Cd content in the placental tissues between the smoking women and the non-smoking ones in ex-

Table 3. Cd and Pb levels in blood and placental tissue in women who miscarried and in pregnant women.

No.		Control group (n = 35)	Experimental group (n = 83)	p-value
		Average ± SD (Min - Max)	Average ± SD (Min - Max)	
Whole blood				
1.	Cd (µg/L)	1.035 ± 0.59 (0.166 - 2.523)	2.730 ± 2.07 (0.711 - 14.69)	< 0.0004
2.	Pb (µg/L)	27.11 ± 4.6 (16.49 - 32.89)	35.54 ± 11.0 (20.34 - 60.94)	< 0.0001
Placental tissue				
3.	Cd (ng/g)	127.4 ± 85 (2.903 - 127.4)	214.4 ± 514 (0.475 - 2294)	< 0.05
4.	Pb (ng/g)	26.35 ± 7.9 (11.75 - 26.35)	199.6 ± 348 (11.21 - 1949)	< 0.0001

SD - Standard deviation, Min - minimum, Max - maximum, p - significance level (< 0.05).

Table 4. Influence of smoking on Cd levels in blood and placental tissues in women who miscarried.

Experimental materials	A/Control group * (n = 35) Average ± SD	B/Non-smoking women with miscarriage (n = 69) Average ± SD	C/Smoking women with miscarriage (n = 14) Average ± SD	p-value
Whole blood	1.035 ± 0.59 µg/L	1.669 ± 1.08 µg/L	3.546 ± 1.77 µg/L	< 0.05 A/B < 0.001 A/C < 0.0001 B/C
Placental tissues	127.4 ± 85 ng/g	172.9 ± 260 ng/g	312.5 ± 323 ng/g	ns A/B < 0.05 A/C < 0.05 B/C

SD - Standard deviation, p - significance level (< 0.05), ns - not significantly, * - non-smoking.

Table 5. Molar ratio between toxic metals and antioxidant elements in blood and placental tissues.

	A/Control group (n = 35) Average ± SD (Min - Max)	B/Experimental group (n = 83) Average ± SD (Min - Max)	p-value
Blood			
Cd/Zn ratio	0.0009 ± 0.001 (0.0002 - 0.003)	0.0028 ± 0.003 (0.0005 - 0.025)	0.040
Cd/Se ratio	0.0144 ± 0.008 (0.0018 - 0.032)	0.0299 ± 0.029 (0.0016 - 0.224)	0.050
Cd/Cu ratio	0.00047 ± 0.0003 (0.0002 - 0.001)	0.0012 ± 0.001 (0.0001 - 0.006)	0.016
Placental tissue			
Pb/Zn ratio	0.877 ± 0.28 (0.273 - 1.391)	8.018 ± 14.06 (0.394 - 86.78)	0.050
Pb/Cu ratio	8.329 ± 3.01 (3.937 - 12.40)	48.323 ± 80.14 (4.622 - 361.91)	0.050

SD - Standard deviation, Min - minimum, Max - maximum, p - significance level (< 0.05).

Table 6. Effects of food products consumption on the level of Cd in the blood of women with miscarriage.

Factors influencing Cd levels	β factor (standard error)	p-value	Model R^2
Sugar	0.301 (0.117)	0.013	0.21
Potatoes	0.188 (0.129)	0.150	
Cold cuts	0.156 (0.114)	0.177	
Poultry	0.161 (0.121)	0.188	
Fish	0.125 (0.119)	0.298	
Honey	- 0.264 (0.121)	0.033	
Oils	- 0.208 (0.113)	0.070	
Boiled vegetables	- 0.157 (0.116)	0.178	
Milk	- 0.148 (0.115)	0.206	

Table 7. Effects of food products consumption on the level of Cd in the placental tissue of women with miscarriage.

Factors influencing Cd content	β factor (standard error)	p-value	Model R^2
Wholemeal bread	0.308 (0.123)	0.014	0.12
Potatoes	0.234 (0.122)	0.060	
Meat	0.221 (0.118)	0.065	
Meat preserves	0.180 (0.118)	0.134	
Flour based dishes	- 0.186 (117)	0.116	

Table 8. Effects of food products consumption on the level of Pb in the blood of women with miscarriage.

Factors influencing Pb levels	β factor (standard error)	p-value	Model R^2
Cereals, rice	0.247 (0.135)	0.071	0.17
Meat	0.132 (0.117)	0.263	
Wholemeal bread	- 0.378 (0.143)	0.010	
Cheese	- 0.297 (0.119)	0.015	
Pulses	- 0.183 (0.121)	0.137	
Honey	- 0.188 (0.126)	0.139	

perimental group, and between the control group and only the smoking women (Table 4). No relationship was found between tobacco smoking and Pb blood levels and the placental tissue content in women with a diagnosis of miscarriage.

Table 5 shows the molar ratio between toxic metals and antioxidant elements in blood and placental tissues. A difference of molar ratio was found between Cd and Zn, Se, Cu in blood. Also, a difference of molar ratio was observed between Pb and Zn or Cu in placental tissues. The effects of nutrition habits on the Cd and Pb levels in women who miscarried are presented in Tables 6 - 9. Table 6 shows that among all analysed factors, 9 food products influenced 21% of Cd blood levels in women who miscarried. A significant increase of Cd concentration was shown only in the case of frequent consumption of sugar, while frequent consumption of honey decreased Cd blood concentration in the experimental

Table 9. Effects of food products consumption on the level of Pb in the placental tissue of women with miscarriage.

Factors influencing Pb content	β factor (standard error)	p-value	Model R^2
Sweet beverages	0.280 (0.134)	0.043	0.17
White cheese	0.243 (0.123)	0.053	
Poultry	0.232 (0.121)	0.062	
Meat	0.162 (0.118)	0.173	
Potatoes	0.196 (0.143)	0.176	
Raw vegetables	0.151 (0.125)	0.232	
Pulses	- 0.255 (0.137)	0.069	
White bread	- 0.252 (0.152)	0.104	
Flour based dishes	- 0.166 (0.118)	0.167	

group.
The Cd content in the placenta was determined by 5 factors in 12%. A significant effect was observed only in the case of frequent consumption of wholemeal bread (Table 7).
Based on the analysis of data in Table 8 it can be ascertained that frequent consumption of 6 food products may influence the Pb concentration in blood of females with spontaneous abortion in 17% of cases. No food product was found to increase the Pb blood concentration in the experimental group. Frequent consumption of wholemeal bread and hard cheese decreased the level of Pb in the blood of women who experienced miscarriage.
Table 9 presents the effect of 9 food products which had a 17% impact on the Pb content in the placenta of the experimental group, while a positive and statistically significant effect was only observed in the case of sweet beverages.

DISCUSSION

The region of Podlaskie, Poland, is free of heavy industry and therefore the main source of exposure to Cd is through food, which becomes contaminated from pollution and tobacco smoking. Smoking is an additional source of inhaling Cd compounds. The blood Cd concentration in tobacco smokers is several times higher than in non-smokers [11]. Studying the effects of passive smoking in the prenatal period and the first several years of the child's life seems necessary. Tobacco smoking has the worst impact on the foetus development during the final 2 months of pregnancy [10,12]. The range of adverse effects of tobacco smoke on the human body is quite wide. Although pregnancy seems to be the best stimulus for quitting the habit, only one in three women stop smoking during pregnancy. Increasing awareness about the problem may contribute to limiting tobacco smoking among pregnant and breast-feeding women, or indicate the need for possible nicotine substitute and antioxidative therapy in women who are unable to give up the habit [10,12]. Our study showed a significant difference was found in the Cd blood concentration and the placenta content between smoking and non-smoking women.
As pollution of the environment is on the increase, young organisms are exposed to numerous toxic factors, including heavy metals. A child is far more sensitive because of the immaturity of its organs and systems, and, consequently, inefficient mechanisms of toxin excretion. A significant impact on the assimilation of toxic elements is shown by trace elements (Zn, Mg, Fe). Deficiency of these metals, frequent in children, result in increased absorption of Cd [11,17], which was also seen in our present studies.
A pregnant woman's placenta is a weak barrier against foetus intoxication with Cd. The transportation of Cd through the placenta is very slow, and its major part is retained in the tissue. A study conducted on a group of pregnant women in Poland demonstrated a lower concentration of Cd in cord blood of new-born children in comparison to the venous blood of their mothers [11,16-18].
Our study conducted on a group of women with a diagnosis of miscarriage showed a higher level of blood Cd in comparison to pregnant women in the first trimester. Moreover, Cd content in the placenta of women from the experimental group was higher in comparison to the women who gave birth successfully.
A similar relationship was observed in a group of pregnant women with a history of recurrent miscarriage. Their determinations showed a higher blood concentration of both Cd and Pb compared to pregnant women without a history of spontaneous abortion [19]. Their recommendation to the women was to supplement trace elements as a factor that would prevent further miscarriages. Another study [20] also demonstrated an elevated, though statistically insignificant, blood concentration of Cd in women with miscarriage compared to

pregnant women. A significantly higher level of Cd in the blood of women who experienced one or more miscarriage in comparison to women with successful pregnancies was also observed by other researchers [21-23]. The Cd content was found and demonstrated a positive correlation between blood Cd levels in the blood of mothers and in the placenta of women who carried a full pregnancy. Based on their research and an extensive review of literature, the authors suggest preventive screening for heavy metals in the blood of pregnant women [23]. Unfortunately, we were unable to find such a relation in our study.

There is no data on direct effects of Cd on the development of the human foetus. The teratogenic aspects of Cd are related to its interaction with Zn. Apart from a direct impact on the foetus, Cd may cause a Zn deficit by inhibiting the activity of an enzyme responsible for inscribing thymine in the DNA [24,25]. Some researchers [5] recommended that women in early pregnancy supplement Zn, improving circulation in the placenta, whose purpose is to protect the foetus, and enhancing the cadmium extraction from the mother's body. This was the conclusion of studies which demonstrated a negative correlation between Cd extraction and Zn levels in blood serum of women with miscarriage [5]. In our earlier studies we were able to demonstrate a similar relationship, i.e., lower Zn levels in the serum of women who experienced miscarriage, which was related with significantly elevated Cd in the studied women [11,13]. When we analysed the molar ratio between the concentrations of toxic metals and antioxidant elements in blood we noticed an interesting difference between Cd/Zn, Cd/Se, and Cd/Cu in examined groups. It is believed that kind of molar ratio may be a prognostic factor, which should be assessed in patients with inflammatory disease, especially when it concerns antioxidant elements, in the Polish population at risk of deficiency of selenium, among others [26], as we have shown also in our earlier studies [13]. However, these data can suggest the potentially preferred meaning of prevention of deficiency of Se in populations with low levels of Se, which include the Polish population (mean level of Se in serum 70 µg/L). They think also that the low levels of Se and high levels of Cu detected could be a consequence of disease and could potentially be a marker of progression rather than just a risk factor [26]. Of particular interest is the positive correlation between the Cd and Pb content in the placenta of women with spontaneous abortion. It is clear that both metals accumulate in the mother's body and in the placenta which could have a negative impact on the course of pregnancy or the health of the child. In our previous study, [13] we found a negative correlation ($r = -0.248$; $p < 0.026$) between the concentration of Pb and Mn in the blood of women with early pregnancy loss. Also, a positive correlation ($r = 0.573$; $p < 0.0001$) was found in the Cd and Pb content. The analysis of the correlation between the Cd and Mn content in the placenta of women from the experimental group showed that it was negative ($r = -0.266$; $p < 0.015$), while the Mn content in the tissue was analysed. The results indicated a negative correlation between the placenta Cd content and Mn in women who miscarried. This may be related to the reduced antioxidant potential of the studied women [13], where Mn plays a significant role as a component of manganese superoxide dismutase (MnSOD). MnSOD is an enzyme whose centre of activity features manganese ions, and its main function is dismutasing O^{2-} [11,13].

Some researchers [27,28] observed that the highest amounts of Cd are found in root vegetables and legumes, and cereals grown in heavily industrialized areas. However, our studies have shown that an increase of Cd concentration in the blood of women who miscarried is only seen in the case of frequent consumption of sugar. Interestingly, frequent consumption of honey significantly decreased Cd blood concentration in the experimental group. This may be related with honey's strong antioxidant properties, which lowered the blood Cd content. It is worth taking a closer look at the relationship between frequent consumption of wholemeal bread and an increase of Cd in the placenta of our patients. Dark, wholemeal bread is made from multigrain flour, made from paddy cereals. Consequently, it contains all the minerals accumulated in the hull, including various impurities.

Pregnant women are exposed to environmental Pb at work or where they live and through tobacco smoking (by the women themselves or through second-hand smoke) [30]. Our study, however, did not find a relationship between tobacco smoking and Pb blood levels and the placental tissue content in women who miscarried. On the other hand, some researchers [23] found a significant positive relationship, but only in the case of blood lead content of the studied women.

Pb and other components of tobacco smoke contribute to increasing the risk of pregnancy complications: premature birth, preterm water breaking, IUGR (*intra uterine growth restriction*), or LBW (*low birth weight*). Tobacco smoke disrupts the structure and function of the placenta, leading to hormone inefficiency and nutrient transport disorders (including micro elements) [11,31]. Another study [29] indicated that the increase of Pb concentration results in prolonged pregnancy and has a negative impact on the birth weight of the foetus.

Our studies showed an increased concentration of blood Pb in women who miscarried in comparison to women in early pregnancy. Also, the lead content in the placenta of women from the experimental group was higher in comparison to the women who gave successful birth. Additionally, we observed a negative correlation between the Pb concentration in whole blood and Mn level in serum of women with miscarriage. This may be related to the reduced antioxidant potential of the studied women, with Mn playing a significant role as a component of manganese superoxide dismutase (MnSOD) [13]. Another study [20] showed a similar relationship, where the blood Pb concentration in women who miscarried was higher (insignificantly) in comparison to women with successful pregnancies. However, in some studies [32] comparing the average concentration of blood Pb in women with spontaneous abortion to women in early pregnancy, did not find any statistically significant difference. They also suspect that low concentrations of Pb in the blood of women in early pregnancy does not pose a risk of miscarriage [32]. Some researchers [22,23] confirm that the average concentration of Pb in the blood of women who had a miscarriage is significantly higher compared to women in week 20 of pregnancy. However, the Pb content in the placenta is the result of numerous complex biochemical reactions and various factors relating to the mother's body, which are not necessarily affected by the woman, but could also result from Pb levels in maternal blood and also in the bones [23]. When we analysed the molar ratio between the contents of toxic metals and antioxidant elements in placental tissues we noticed an interesting difference only between Pb and Zn or Pb and Cu in the examined groups. It is believed that kind of molar ratio may be a prognostic factor, which should be assessed in patients with inflammatory disease, especially when it concerns antioxidant elements in the Polish population at risk of deficiency of selenium, among others [26], as we have shown in our earlier studies [13].

The development of civilization and increased emissions of lead to the environment leads to significant long-term pollution of living and industrial areas, and in particular, contamination of farmlands near zinc, lead, and copper mills. The region of north-eastern Poland, where this study was conducted, does not house any form of heavy industry. A huge role is also played by the dynamic growth of the automotive sector. Significantly higher levels of lead were found in the soil near express roads. It is worth noting that the situation improved since the introduction of unleaded petrol, yet still jet fuel contains Pb compounds. Pb levels in plants depend on soil contamination and type of crop (cereals, vegetables, fruit).

In our study, no food product was found to increase the Pb blood concentration in the experimental group. Frequent consumption of wholemeal bread and hard cheese decreased the level of Pb in the blood of women who miscarried. However, frequent consumption of sweet beverages increased the Pb content in the placenta of the studied women. These types of drinks contain sweeteners, colorants and phosphorus compounds which contribute to the accumulation of heavy metals in the body. Also, the production method of such food products promotes contamination.

The foetus is only partially protected against the adverse effects of some toxic metals, including cadmium and lead. It still remains unknown, however, what levels of Cd and Pb could cause mutations and innate disorders in the foetus, and what exactly the mechanism of these changes is.

CONCLUSION

1. The average content of Cd and Pb in the blood and in the placenta of women with miscarriage was higher in comparison to the level of these toxic metals in the experimental materials of women from the control group.
2. A difference was found in the Cd blood concentration and content in the placental tissues between the smoking women and the non-smoking ones in the experimental group.
3. Elevated Cd and Pb concentrations in the blood and placenta of pregnant women may be connected with the occurrence of miscarriage; therefore, the levels of these heavy metals should be monitored in women who plan pregnancy.

Acknowledgement:
We thank the participants for their willing cooperation.

Authors' Information:
EK has passed away.

Declaration of Interest:
The authors declare they have no actual or potential conflicts of interests.

References:

1. Rai R, Regan L. Recurrent miscarriage. Lancet 2006;368;9535: 601-11 (PMID: 16905025).
2. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. Acta Obstet Gynecol Scand 1977;56;3:247-53 (PMID: 560099).
3. Weeks A, Gemzell DK. Spontaneous miscarriage in the first trimester. BMJ 2006;332:1233-4. https://doi.org/10.1136/bmj.332.7552.1223
4. Christiansen OB, Nielsen HS, Kolte A, Pedersen AT. Research methodology and epidemiology of relevance in recurrent pregnancy loss. Semin Reprod Med 2006;24:5-16 (PMID: 16418973).
5. Gerhard I, Waibel S, Daniel V, Runnebaum B. Impact of heavy metals on hormonal and immunological factors in women with repeated miscarriages. Hum Reprod Update 1998;4;3:301-9 (PMID: 9741713).
6. Garcia-Enguidanos A, Calle ME, Valero J, Luna S, Domínguez-Rojas V. Risk factors in miscarriage: a review. Eur J Obstet Gynecol Reprod Biol 2002;102;2:111-9 (PMID:11950476).
7. Green RS, Malig B, Windham GC, Fenster L, Ostro B, Swan S. Residential exposure to traffic and spontaneous abortion. Environ Health Perspect 2009;117;12:1939-44 (PMID: 20049215).
8. Handal AJ, Harlow SD. Employment in the Ecuadorian cut-flower industry and the risk of spontaneous abortion. BMC Int Health Hum Rights 2009;9:25 (PMID: 19814818).

9. Nakhai-Pour HR, Broy P, Bérard A. Use of antidepressants during pregnancy and the risk of spontaneous abortion. CMAJ 2010; 182;10:1031-7 (PMID: 20513781).

10. Krzywy I, Krzywy E, Pastuszak Gabinowska M, Brodkiewicz A. [Lead - is there something to be afraid of?] Ann Acad Med Stetin 2010;56,2:118-28 (PMID: 21469290). [Article in Polish.]

11. Krzywy I, Krzywy E, Peregud-Pogorzelski J, Łuksza K, Brodkiewicz A. Cadmium - is there something to fear? Ann Acad Med Stetin 2011;57,3:49-63 (PMID: 23383548).

12. Nusbaum ML, Gordon M, Nusbaum D, McCarthy MA, Vasilakis D. Smoke alarm: a review of the clinical impact of smoking on women. Prim Care Update Ob Gyns 2000;7;5:207-14 (PMID: 11 025273).

13. Omeljaniuk WJ, Borawska MH, Socha K, et al. Antioxidant status in women who had a miscarriage. Adv Med Sci 2015;60;2: 329-34 (PMID: 26233636).

14. Gronowska-Senger A (red.), Araucz M, Komitet Nauki o Żywieniu Człowieka: Przewodnik metodyczny badań sposobu żywienia: praca zbiorowa (in Polish), 2013 Wyd. Komitet Nauki o Żywieniu Człowieka Polskiej Akademii Nauk. ISBN 836330509X, 9788363305093).

15. Neumeister B, Böhm BO. Klinikleitfaden Labordiagnostik. Urban & Fischer Verlag/Elsevier GmbH, 2015. https://shop.elsevier.de/klinikleitfaden-labordiagnostik-9783437222344.html

16. Chen L, Ren WH, Zhu SL, et al. [Effects of chronic cadmium loading on the testis and endocrine function of reproduction in male rats]. Sheng Li Xue Bao 2002;54;3:258-62 (PMID: 12075 475). [Article in Chinese.]

17. Winiarska-Mieczan A, Gil G: Evaluation of infant exposure to lead and cadmium in ready-to-use food. Bromat Chem Toksykol 2007;40:137-44. http://www.ptfarm.pl/pub/File/wydawnictwa/bromatologia/2_07/s137-144.pdf

18. Mead MN. Cadmium confusion: do consumers need protection? Environ Health Perspect 2010;118:528-34 (PMID: 21123140).

19. Ajayi OO, Charles-Davies MA, Arinola OG. Progesterone, selected heavy metals and micronutrients in pregnant Nigerian women with a history of recurrent spontaneous abortion. Afr Health Sci 2012;12;2:153-9 (PMID: 23056021).

20. Tolunay HE, Şükür YE, Ozkavukcu S, et al. Heavy metal and trace element concentrations in blood and follicular fluid affect ART outcome. Eur J Obstet Gynecol Reprod Biol 2016;198:73-7 (PMID: 26802253).

21. Saad AA, Hegazy NH, Amer N, et al. The role of cadmium exposure on spontaneous abortion. World Journal of Medical Science 2012;7;4:270-5. https://www.idosi.org/wjms/7(4)12/11.pdf

22. Adam KM, Abdaltam SA, Noreldeen AM, et al. Relationship between maternal blood lead, cadmium, and zinc levels and spontaneous abortion in Sudanese women. Public Health Research 2015;5;6:171-6. http://article.sapub.org/10.5923.j.phr.20150506.01.html

23. Al-Saleh I, Shinwari N, Mashhour A, Mohamed Gel D, Rabah A. Heavy metals (lead, cadmium and mercury) in maternal, cord blood and placenta of healthy women. Int J Hyg Environ Health 2011;214;2:79-101 (PMID: 21093366).

24. Martelli A, Pousselet E, Dycke C, Bouron A, Moulis JM. Cadmium toxicity in animal cells by interference with essential metals. Biochemie 2006;88:1807-14 (PMID: 16814917).

25. Czeczot H, Majewska M. Cadmium - exposure and its effects on health. Farm Pol 2010;66;40:243-50. http://ptfarm.pl/pub/File/Farmacja%20Polska/2010/04-2010/02%20%20Kadm.pdf

26. Lener MR, Scott RJ, Wiechowska-Kozłowska A, et al. Serum concentrations of selenium and copper in patients diagnosed with pancreatic cancer. Cancer Res Treat 2016;48;3:1056-64 (PMID: 26727715).

27. Satarug S, Baker JR, Urbenjapol S, et al. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett 2003;137:65-83 (PMID: 12505433).

28. Ostrowska P. Cadmium - occurrence, use and methods of recycling. Mineral Resources Management 2008;24;3;3:255-60. https://www.min-pan.krakow.pl/Wydawnictwa/GSM2433/ostrowska.pdf

29. Starek A. [Health effects of occupational exposure to chemical substances in smokers]. Med Pr 2002;53:73-7 (PMID: 12051 156).

30. Bellinger DC, Stiles KM, Needleman HL. Low-level lead exposure, intelligence and academic achievement: a long term follow up study. Pediatrics 1992;90;6:855-61 (PMID: 1437425).

31. Levine F, Muenke M: VACTERL association with high prenatal lead exposure: similarities to animal models of lead teratogenicity. Pediatrics 1991;87;3:390-2 (PMID: 2000280).

32. Vigeh M, Yokoyama K, Kitamura F, Afshinrokh M, Beygi A, Niroomanesh S. Early pregnancy blood lead and spontaneous abortion. Women Health 2010;50;8:756-66 (PMID: 21170817).