Heavy metal ion concentration in the amniotic fluid of preterm and term pregnancies from two cities with different industrial output

RADU IONUT NEAMTU[1*], MARIUS CRAINA[1*], GEORGE DAHMA[1], ALIN VIOREL POPESCU[1], ADELINA GEANINA ERIMESCU[1], IOANA CITU[2], AMADEUS DOBRESCU[3], FLORIN GEORGE HORHAT[4,5], DAN DUMITRU VULCANESCU[4,5], FLORIN GORUN[1], ELENA SILVIA BERNAD[2], ANDREI MOTOC [1,6] and IOAN COSMIN CITU[1]

Departments of [1]Obstetrics-Gynecology and Neonatology, [2]Internal Medicine I and [3]Surgery; [4]Multidisciplinary Research Center on Antimicrobial Resistance (Multi-Rez), Microbiology Department, 'Victor Babes' University of Medicine and Pharmacy, 300041 Timisoara; [5]Clinical Laboratory, 'Louis Turcanu' Emergency Hospital for Children, 300011 Timisoara; [6]Department of Anatomy and Embryology, 'Victor Babes' University of Medicine and Pharmacy, 300041 Timisoara, Romania

Received October 11, 2021; Accepted November 10, 2021

DOI: 10.3892/etm.2021.11034

Abstract. The growth and development of the fetus is a complex phenomenon that can be influenced by several variables. High quantities of heavy metal ions in the amniotic fluid have been linked to poor health, especially in industrial, polluted and poor areas. The aim of the present study was to assess the differences in the concentration of these ions between preterm (weeks 15-37) and term pregnancies (starting at week 37). Another objective was to compare pregnancies from two cities with different industry levels. Two sample lots from two Romanian cities were analyzed. A total of 100 patients from Timisoara were compared with 60 from Petrosani, a heavy industry city in Romania. Demographic data were collected, and amniocentesis was performed on all women. Lead (Pb), copper (Cu), nickel (Ni), cadmium (Cd), arsenic (As), iron (Fe) and zinc (Zn) concentrations were assessed. Descriptive and analytical statistics were performed using the Mann-Whitney U test for non-parametric data and the Fisher's exact test for categorical data. In addition, categorical data was represented graphically. In the Timisoara cohort, the differences in heavy metal concentrations between preterm and term pregnancies were not statistically significant. In the Petrosani cohort, however, the concentrations of Zn (P=0.02606) and Cd (P=0.01512) were higher in preterm than in term pregnancies. When comparing the two cohorts as a whole, the concentration of Pb (P=0.04513), Cd (P=0.00002), As (P=0.03027) and Zn (P<0.00001) were higher in the patients from Petrosani than in those from Timisoara. Only Cu concentrations were higher in the Timisoara cohort (P<0.00001). The concentrations of Ni (P=0.78150) and Fe (P=0.44540) did not differ statistically. Thus, amniocentesis is an important diagnostic and exploratory tool in determining differences in the concentrations of elements such as heavy metal ions. Research over a longer period of time should be carried out to examine the relation between heavy metal ions concentration and possible postnatal health outcomes.

Correspondence to: Professor Florin George Horhat, Multidisciplinary Research Center on Antimicrobial Resistance (Multi-Rez), Microbiology Department, 'Victor Babes' University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timisoara, Romania
E-mail: horhat.florin@umft.ro

*Contributed equally

Key words: amniotic fluid, heavy metals, ions, preterm, term, pregnancy, amniocentesis, heavy industry

Introduction

The growth and development of the fetus is a complex phenomenon that can be influenced by several variables. Although electrolytes are present in the amniotic fluid in trace amounts, they are considered essential for the health and well-being of the fetus. Associations between amniotic fluid electrolyte concentrations and fetal development have been made (1). Common ions found in amniotic fluid include sodium, potassium, chloride, calcium, magnesium, phosphate and bicarbonate (2). These ions in the amniotic fluid serve an important role in a normal pregnancy and can aid in prevention and early diagnosis of fetal or maternal pathologies. Through accurate prenatal assessment of the biochemical composition of the amniotic fluid, overall health status and fetal maturity can be evaluated (1-3).

Normally, the volume of amniotic fluid increases steadily until it reaches a maximum of 400-1200 ml at 34-38 weeks. Afterwards, the volume starts to decline. At 40 weeks, the volume of amniotic fluid can measure ~800 ml and continues to decrease as the pregnancy goes on (4-6). Although the composition of the amniotic fluid does not remain constant during pregnancy, the bulk volume at all times (~98%) is water. Other important constituents are urea, creatinine, glucose, proteins, lipids, bile pigments, fetal epithelial cells and mineral ions (7).

Common ions have important uses during pregnancy. Sodium contributes to the regulation of water-electrolyte balance of the amniotic fluid, high chloride reflects possible renal pathologies, high potassium and calcium can be signs of pre-eclampsia, high phosphate indicates reduced antibacterial activity, magnesium and zinc (Zn) assess the risk of fetal growth retardation (8-12). Potassium, phosphate and Zn also affect normal antimicrobial activity (13-15).

Some heavy metal ions can also be traced in the amniotic fluid, and small amounts of these elements are normal. However, large quantities have been shown to have detrimental effects in humans. For example, lead (Pb) is known for its negative effects on neural development (16), while cadmium (Cd) is known for its risk of preterm delivery (17). Usually, this can be seen in people living in highly industrialized, highly polluted and poor areas (18-22). As such, the assessment of these ions in the amniotic fluid is essential to understanding the risks these people might be facing. Heavy metals in high concentrations have the ability to disrupt normal physiological processes. Copper (Cu), arsenic (As), Cd, nickel (Ni), chromium, mercury, manganese and Pb can lead to fetal growth retardation, pre-eclampsia, impaired cognitive development and even cancer (23-28).

The present study had two objectives. The first was to assess the differences of heavy metal ion concentrations in the amniotic fluid between preterm (between weeks 15 and 37) and term (starting week 37) pregnancies. Moreover, the second was to assess whether pregnant women from two cities with different industrial levels from Romania would present different heavy metal ion concentrations in their amniotic fluid.

Materials and methods

Study design. The present retrospective study was conducted in the 'Bega' Maternity Clinic in Timisoara, Romania between April 1st 2020 and April 1st 2021. The study design is in accordance with The Declaration of Helsinki and was approved by the Ethics Committees of the 'Bega' Maternity Clinic (approval no. 260/16IUL2021) and Petrosani Hospital (approval no. 15990/27.07.2021).

Two cohorts of pregnant patients were examined. The first included 100 pregnant women admitted in the 'Bega' Maternity Clinic from Timisoara. The second included 60 pregnant women admitted in the Maternity Clinic of the Petrosani Emergency Hospital. Written informed consent was provided by all 160 individuals for amniocentesis and use of data for research purposes. Amniocentesis was performed on all patients, and each patient cohort was separated in two equal groups.

Participants. The inclusion criteria were as follows: Consenting adult women with single fetal gestation, patients with medical indications for amniocentesis (such as maternal age over 35, unfavorable or uncertain results obtained at screening tests, previous exposure to infectious agents including *Toxoplasma gondii* or cytomegalovirus and known genetic disorders running in the family). The exclusion criteria were: Pregnancies earlier than 15 weeks, pregnant patients with severe anemia, hematological, neoplastic, cardiac or metabolic conditions and patients with previous perinatal complications or fetal disorders.

Petrosani and Timisoara were selected in order to determine whether a mountainous, highly industrial city might harbor higher heavy metal ion concentrations in the amniotic fluid than a city in the plains, with moderate industry. The patients were stratified into four groups: Group 1 (n=50), women from Timisoara with preterm pregnancies (15-37 weeks); group 2 (n=50), women from Timisoara with term pregnancies (≥37 weeks); group 3 (n=30), women from Petrosani with preterm pregnancies; and group 4 (n=30), women from Petrosani with term pregnancies.

Amniotic liquid sampling. All procedures were performed under careful sterile and antiseptic conditions. Before the amniocentesis procedure, an ultrasound evaluation was carried out in order to determine the location of the placenta, fetus and other characteristics of the amniotic fluid.

To enter the amniotic cavity, a spinal needle with a gauge of 20-22 was used under continuous ultrasound guidance. The entry into the amniotic cavity was done firmly in order to prevent rupture of the amniotic membrane and avoiding the placenta. Once the entry into the cavity was confirmed by ultrasound, the amniotic fluid was slowly aspirated. The first 2 ml were discarded, as they may be contaminated with maternal cells. A quantity of 20-22 ml was deemed sufficient, as 18-20 ml were used for genetic, sex and lung development testing (for pregnancies after week 32). The remaining 2 ml were used for the evaluation of heavy metal ion concentration.

After removal of the needle, the mothers were kept under further ultrasound evaluation to confirm proper fetal heart rate. Intramuscular administration of anti-D immuno-globulin was also done for Rhesus-negative women in order to prevent fetal Rhesus disease. After completing the procedure, the mothers were advised to avoid strenuous and sexual activities for the next 48 h.

Detection of heavy metal ions. The working method used for the detection of heavy metals in the amniotic fluid involved flame atomic absorption spectroscopy. Each amniotic fluid sample was nebulized in the gases of the spectrophotometer's flame. Each of the studied metals have well-known specific absorption rates and the spectrophotometer was also equipped with an interference correction system. All measurements are presented in mg/l.

The method follows national guidelines (SR ISO 8288/2001 standards). All reagents used in this determination were of high quality and the water used was ultrapure, with no traces of heavy metals. All spectrophotometer readings were analyzed on a connected computer running GBC Avanta 1.33 (GBC Scientific Equipment). The calibration values used for the readings are presented in Table I.

Data collection. The following clinical and demographical data were collected for each patient: Age of the patient, gestational age, concentrations of Pb, Cu, Ni, Cd, As, Zn, iron (Fe) (in mg/l), femur length (in mm), location of residence (urban or

Table I. Calibration values for the flame atomic absorption spectroscopy.

Metal	Zn	Cu	Cd	Ni	Pb	As	Fe
Maximum error	0.037	0.027	0.012	0.039	0.083	0.027	0.013
R^2	0.999	1.000	0.999	0.998	0.995	0.998	1.000

Zn, zinc; Cu, copper; Cd, cadmium; Ni, nickel; Pb, lead; As, arsenic; Fe, iron.

rural) and smoking status (no smoking, either smoking in the past or quitting the habit once the patient found out about the pregnancy and active smoking). The data were recorded in an Excel file (2016 Office Suite, Microsoft).

Smoker status. Information on tobacco use was also collected. Active smokers were defined as patients smoking even during pregnancy. Former smokers were defined as patients that had smoked and gave up the habit in the past, as well as mothers, which quit smoking once the pregnancy was suspected and/or confirmed. Nonsmokers were defined as patients that never smoked.

Statistical analysis. Normal distribution was assessed using the Shapiro-Wilk test. Descriptive statistics for numerical variables include means, standard deviations, medians, interquartile range (IQR, 1st quartile - 3rd quartile) and range. For the comparison of with non-parametric variables, the Mann-Whitney U test was used. For categorical variables, frequency (%) and/or count (n) were included, and Fisher's exact test used to analyze the contingency tables. The α-level was set at 0.05. P<005 was considered to indicate a statistically significant difference. All data were processed using SPSS version 22 for Windows (IBM Corp.).

Results

Overview. In the Timisoara cohort, a total of 100 pregnant women between the age of 20 and 35 were admitted in the maternity during the study period. The mean maternal ages for groups 1 (preterm) and 2 (term) were 27.76±3.83 and 2 7.46±3.58 years, respectively. Mean gestational age was 18.14±1.81 weeks for the preterm group and 38.66±1.04 weeks for the term group. Mean femur length for the preterm group was 24.94±6.32 and 74.55±2.65 for the term group. All demographic data are presented in Table II. The distribution of the area of residence distribution is presented in Fig. 1. Statistical analysis of the area of residence did not reveal a significant difference between the groups (P=0.83694; Fisher's exact test).

The Petrosani cohort consisted of 60 pregnant women between the age of 19 and 35 admitted in the maternity. The mean maternal age was 27.20±4.47 years for group 3 (preterm) and 26.47±4.09 years for group 4 (term). Mean gestational age was 18.33±1.84 weeks for the preterm group and 39.07±0.94 weeks for the term group. Mean femur length for the preterm group was 25.15±5.98 and 76.13±1.76 for the term group. The demographic data for these groups are presented in Table III. The distribution of the area of residence distribution is presented in Fig. 2. Statistical analysis of the area of residence using Fisher's exact test showed no significant difference between the groups (P=0.42956).

Table II. Demographic data for patients from Timisoara.

Parameter	Preterm pregnancies	Term pregnancies
Gestational age, weeks		
Mean	18.14	38.66
SD	1.81	1.04
Median	18.00	39.00
IQR	16.88-19.88	38.00-39.00
Range	6.00	4.00
Maternal age, years		
Mean	27.76	27.46
SD	3.83	3.58
Median	28.00	28.00
IQR	24.88-30.13	26.00-29.00
Range	15.00	15.00
FL, mm		
Mean	24.94	74.55
SD	6.32	2.65
Median	25.30	75.05
IQR	19.80-29.44	72.39-75.63
Range	23.50	11.70

FL, femur length; IQR, interquartile range; SD, standard deviation.

Analysis of the Timisoara cohort. In the Timisoara cohort, there were no statistically significant differences in the heavy metal ion concentrations between pre-term and term pregnancies (Table IV).

Analysis of the Petrosani cohort. In the Petrosani cohort, no statistically significant differences were observed between pre-term and term pregnancies regarding Pb, Cu, Ni, As and Fe concentrations. However, the concentrations of Cd and Zn were significantly higher in the pre-term than in the term pregnancies (Table V).

Analysis between Timisoara and Petrosani. The Timisoara and Petrosani were also analyzed as a whole. No significant differences were observed with respect to Ni and Fe concentrations. However, the median concentrations of Pb, Cd, As and

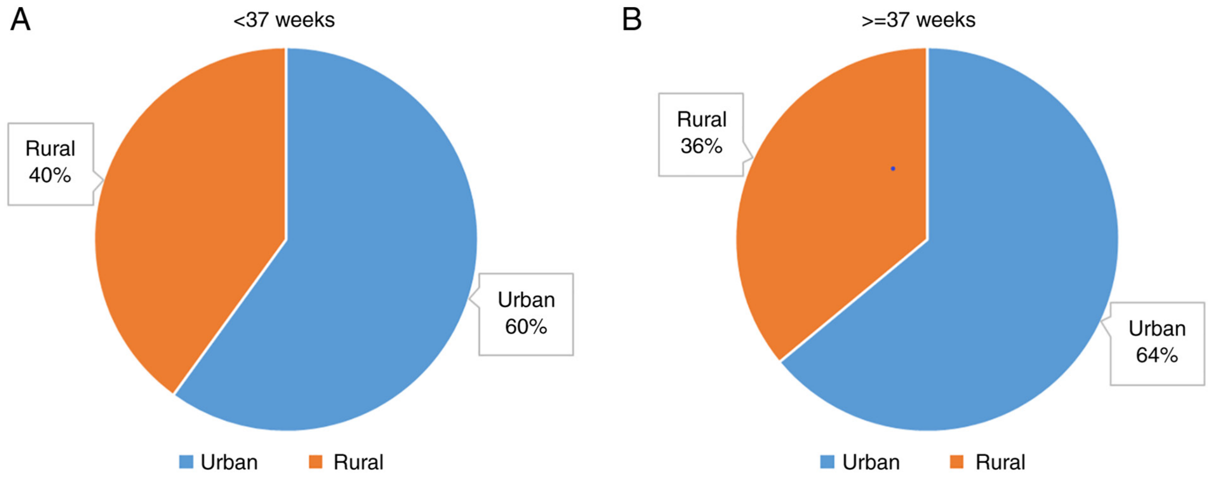

Figure 1. Area of residence of patients from Timisoara with (A) preterm and (B) term pregnancies.

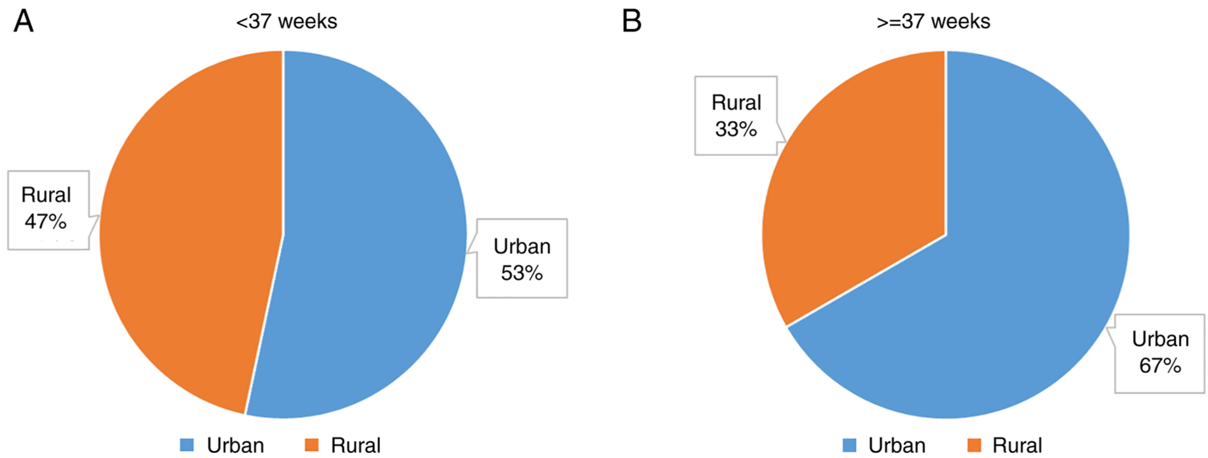

Figure 2. Area of residence of patients from Petrosani with (A) preterm and (B) term pregnancies.

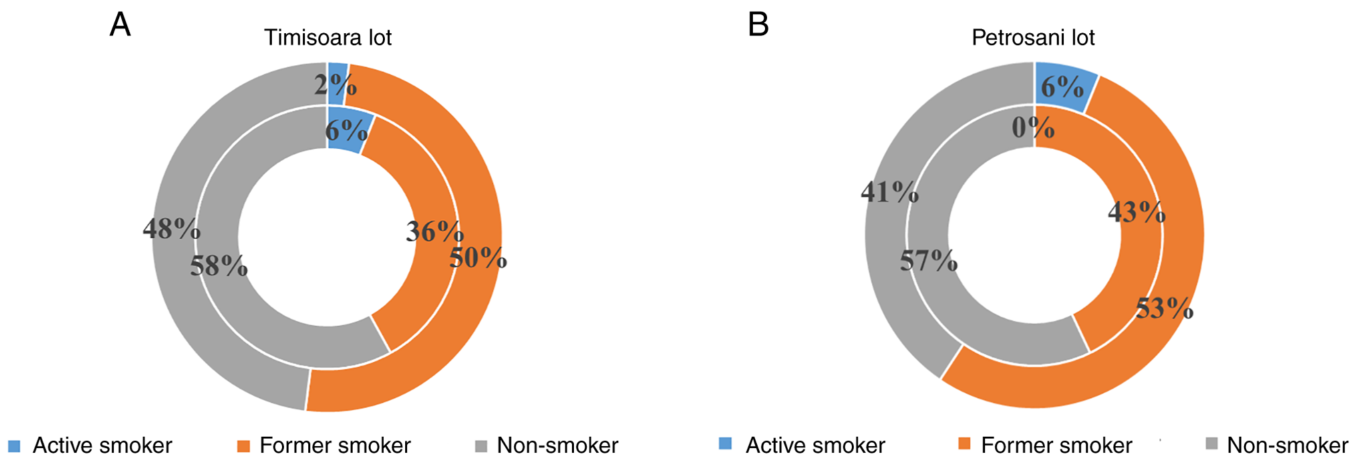

Figure 3. Smoking status of patients from (A) Timisoara and (B) Petrosani. The inner circle represents preterm pregnancies (<37 weeks), while the outer circle represents term pregnancies (≥37 weeks).

Zn were significantly higher in the Petrosani cohort, compared with patients from Timisoara. The median Cu concentration was significantly higher in the Timisoara cohort, compared with that in the Petrosani cohort (Table VI).

Analysis of smoking status. As smoking may affect the concentrations of trace elements such as heavy metal ions in the blood, urine, hair and toenail samples, the smoking status of the patients was also assessed. Fisher's exact test was used

Table III. Demographic data for patients from Petrosani.

Parameter	Preterm pregnancies	Term pregnancies
Gestational age, weeks		
Mean	18.33	39.07
SD	1.84	0.94
Median	18.00	39.00
IQR	17.00-20.00	38.00-39.88
Range	6.00	3.00
Maternal age, years		
Mean	27.20	26.47
SD	4.47	4.09
Median	28.00	26.50
IQR	23.75-30.13	23.88-29.00
Range	16.00	15.00
FL, mm		
Mean	25.15	76.13
SD	5.98	1.76
Median	25.35	75.50
IQR	19.41-30.24	75.49-77.10
Range	22.30	7.90

FL, femur length; IQR, interquartile range; SD, standard deviation.

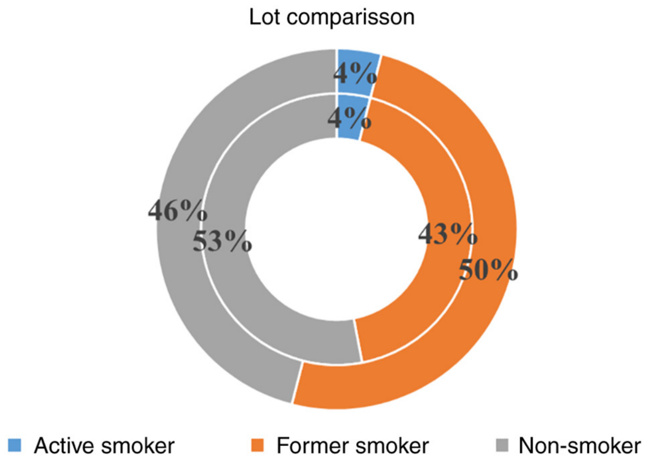

Figure 4. Smoking status of patients from Timisoara (inner circle) or Petrosani (outer circle).

to see if the smoking status was associated with gestational age or with the area of residence. (Table VII; Figs. 3 and 4). There was no association between smoking status and either of these two parameters.

Discussion

Amniotic fluid plays a key role in the development of the fetus. It protects the fetus from mechanical shock and insulates it thermally, whilst helping elements from the maternal plasma reach the fetus, especially in the early pregnancy, before the formation of the placenta and it participates in keeping the antibacterial balance. These properties are the result of the bioactive compounds that take part in its constitution (7).

Since implantation, the extracolemic cavity is produced, forming the amniotic space. The fetus and the amniotic fluid are enveloped by the amniotic sac. At weeks 34-38 the amniotic fluid reaches its maximum value, after which it starts declining as the pregnancy goes on (4-6). However, the concentrations of its bioactive compounds changes as the osmolarity of the fluid decreases. These concentrations are important as they can provide insight into the status of the fetus through amniocentesis.

Although they are found in small amounts, mineral ions can play an important role in the development of the fetus. Common ions are sodium, chloride, potassium, calcium, magnesium, bicarbonate and phosphate (2,7,29). In addition, trace levels of heavy metals, such as Cu, As, Cd, Ni, chromium, mercury, manganese, Zn and Pb, can also be found in the amniotic fluid (1,2,7).

Sodium is involved in regulating the water-electrolyte balance (9,29-31). Chloride can be used to determine possible renal disorders, such as the Barter syndrome (8,9,32). Raised potassium levels may lead to pre-eclampsia and lower antibacterial activity (11,14). Elevated calcium levels can result in pre-eclampsia and spina bifida, while low levels are associated with preterm deliveries (33-37). Low levels of magnesium have been associated with pre-eclampsia and possible fetal growth retardation (12,13,38-40). High bicarbonate may indicate complicated twin-twin transfusion syndromes (41), while phosphate plays a role in antimicrobial activity (42-44).

Heavy metals are metalloids with high density or atomic number, which are usually found in trace amounts in serum. Several have well-known physiological functions, such as heme constitution, hormone production, enzyme regulation or even act as antioxidants (45). Therefore, it is normal for these elements to be found in tissues and bodily fluids. When concentrations are elevated however, certain health risks may appear. In adults, risks range from acute hepatic injury, cardiovascular, neuro-psychological disorders to chronic poisoning or even death (46-48). Considering the risks adults are exposed to, prudence is advisable regarding the risks in children.

Zn is essential for embryogenesis and neurogenesis. Deficiency in this element is associated with fetal growth retardation and neurodegenerative disorders (28,49-51). It may also promote antimicrobial activity (15,17). Due to these concerns, similarly to magnesium and iron, supplementation for both pregnant and lactating mothers is recommended. The average intake is in the range of 9.6-11.2 mg/day (51,52). Another reason to determine the amniotic fluid levels is that oxidative stress, induced by menadione, is further exacerbated by high concentrations (80 μM) of Zn (53). In the Timisoara cohort of the present study, the differences in Zn concentrations between term and preterm pregnancies were not statistically significant. In the Petrosani cohort, the preterm pregnancy group had a median Zn concentration higher than that of the term pregnancy group. The differences between the two cohort were also statistically significant.

Cu is an essential cofactor for several metalloenzymes. It also has antimicrobial and antiviral properties. Average intake varies from 0.6 to 1.6 mg/day, although this can be higher

Table IV. Heavy metal concentrations in patients from Timisoara (n=100) with preterm and term pregnancies.

Metal	Mean	SD	Median	IQR	Range	P-value[a]
Pb						
Preterm	0.0409	0.1582	0.0001	0.0000-0.0136	1.012	0.74300
Term	0.0570	0.1944	0.0002	0.0000-0.0138	1.012	
Cu						
Preterm	0.4736	0.5068	0.2840	0.0829-0.9263	1.662	0.59780
Term	0.5810	0.5106	0.3690	0.0780-1.0339	1.738	
Ni						
Preterm	0.4426	0.6478	0.0131	0.0000-0.9954	2.057	0.09952
Term	0.6863	0.7292	0.7175	0.0000-1.2206	2.787	
Cd						
Preterm	0.0067	0.0354	0.0001	0.0000-0.0001	0.238	0.69680
Term	0.0103	0.0429	0.0001	0.0000-0.0001	0.240	
As						
Preterm	0.9131	1.0294	0.5530	0.0000-1.7294	3.769	0.17080
Term	0.7219	0.9954	0.0020	0.0000-1.4208	3.767	
Zn						
Preterm	0.0776	0.1518	0.0145	0.0000-0.0674	0.658	0.98310
Term	0.0534	0.1329	0.0140	0.0000-0.0369	0.742	
Fe						
Preterm	0.3072	0.3624	0.1504	0.0414-0.4477	1.500	0.13470
Term	0.3332	0.3126	0.2333	0.1769-0.3369	1.382	

[a]Mann Whitney's U-test. IQR, interquartile range; SD, standard deviation.

during pregnancy and breastfeeding (54). Deficits may lead to neurological and immunological abnormalities of the fetus (54). By contrast, high Cu concentrations may lead to diverse abnormalities. For example, high concentrations in maternal serum have been linked to pre-eclampsia (55,56), and elevated levels in the amniotic fluid have been associated with fetal growth retardation (28). When considering genetics, Cu is has been implicated in two major diseases, namely Wilson and Menkes diseases. In both cases, accumulation of Cu in the fetus can usually be observed prenatally (57-60). In the present study, the differences between term and preterm pregnancies were not statistically significant, both for patients from Timisoara and from Petrosani. When comparing the two cohorts, the Timisoara cohort presented higher median concentrations, with the differences being statistically significant.

Ni is essential for gut bacterial growth, hormone production, iron absorption and as part of RNA and DNA. Recommended dietary allowance for women is 400-600 μg/day (61,62). Large doses of Ni or prolonged exposure can cause harmful effects such as genotoxicity, hematotoxicity, teratogenicity, immunotoxicity, carcinogenicity and allergic reactions (62,63). Smokers and families with lower socio-economic status might be exposed to higher doses (62-64). Rodent models have shown that the transfer of Ni through the placenta occurs mainly from the mother to the fetus, resulting in its accumulation in the amniotic fluid or fetal organs (65,66). In the present study, the differences between term and preterm pregnancies were not considered statistically significant for both cohorts. There were no statistically significant differences in Ni concentrations between the two cohorts.

Fe is an important molecule that serves several functions in the body, with the most important role being in oxygen transportation, as part of the heme structure (67). Another important role is combating infections (68). Normal intake for iron is 16 mg/day for women and 7-11 mg/day for children <14 years (69). Fe deficiency is a common worldwide problem leading to anemia. Children and pregnant women are at a high risk for this condition and may need supplementation. During pregnancy, total intake should reach 27 mg/day, while during lactation, the recommended intake falls to 10 mg/day (70).

Deficiency and anemia in pregnant women has been associated with low birth weight, preterm delivery and potential fetal anemia (71,72). During intra-amniotic infection or inflammation complications, the amniotic levels of Fe are not significantly increased compared with healthy individuals. This may be due to hepcidin upregulation, which may result in hypoferremia in maternal serum, as hepcidin redirects Fe to macrophages correlated with the infection or inflammation episode (73,74). Common symptoms of Fe deficiency anemia include paleness, fatigue, reduced cognitive performance and diminished immune responses. Infants experience these as well, along with an increased risk of cognitive and psychomotor developmental deficit (75).

Excess Fe can be observed after repeated blood transfusions or in congenital cases of hemochromatosis. Mutations

Table V. Heavy metal concentrations in patients from Petrosani (n=60) with preterm and term pregnancies.

Metal	Mean	SD	Median	IQR	Range	P-value[a]
Pb						
Preterm	0.0728	0.2249	0.0020	0.0000-0.0284	1.124	0.20840
Term	0.1579	0.3048	0.0190	0.0000-0.0865	1.202	
Cu						
Preterm	0.0386	0.0519	0.0265	0.0054-0.0468	0.265	0.78420
Term	0.0260	0.0200	0.0185	0.0109-0.0409	0.074	
Ni						
Preterm	0.5743	0.7706	0.0990	0.0000-1.2199	2.198	0.47120
Term	0.7173	0.8653	0.1765	0.0000-1.4485	2.898	
Cd						
Preterm	0.0135	0.0463	0.0003	0.0000-0.0020	0.235	0.01512[b]
Term	0.0070	0.0368	0.0001	0.0000-0.0001	0.202	
As						
Preterm	1.0785	0.9899	0.7525	0.2260-1.7548	3.004	0.66710
Term	1.1075	1.2392	0.7632	0.0086-1.8127	4.743	
Zn						
Preterm	0.2202	0.2975	0.0845	0.0344-0.3295	1.259	0.02606[b]
Term	0.0604	0.053	0.0425	0.0199-0.0989	0.177	
Fe						
Preterm	0.3377	0.4297	0.1235	0.0704-0.5135	1.747	0.16690
Term	0.4119	0.3316	0.3760	0.1406-0.6029	1.500	

[a]Mann Whitney's U-test, [b]statistically significant. IQR, interquartile range; SD, standard deviation.

of the High Fe^{2+} protein family and other Fe transport proteins lead to iron build-up in the organism, resulting in liver cirrhosis, hepatocellular carcinoma, heart disease and impaired pancreatic function (76). Although these symptoms usually appear in later life, infants with Fe overload may be at risk of developing brain and hematopoiesis alterations (77).

In the present study, the differences in Fe concentrations between term and preterm pregnancies were not considered statistically significant in the Timisoara or Petrosani cohorts. Nor were there any differences between the two cohorts.

Cd is an element still being investigated, along with Pb and As. It is commonly found in cigarettes. Present only in very small amounts in the body, no physiological role has been established yet. As a toxic element, previous studies have looked into its effects on cardiovascular function (78,79), obesity and ghrelin regulation (80,81), possible cancer occurrence (82,83), reproduction and pregnancy (84-87). Rodent fetal experimentation has suggested cytotoxicity problems, progesterone disorders, microRNA expression changes, elevated oxidative stress and DNA damage (84-86). The toxic effects from Cd, such as high oxidative stress, cytotoxicity and apoptosis have also been shown in humans (87). Prenatal exposure has been linked with lower birth weights, preterm deliveries and even possible spontaneous abortion (88-90). Elevated amniotic fluid levels have been also linked with pre-eclampsia (26,91). Children with such prenatal history might also be prone to cardiometabolic disorders (92). In the present study, the differences in Cd concentration between term and preterm pregnancies were not considered statistically significant in patients from Timisoara. In the Petrosani cohort, the median concentrations for the preterm pregnancy group were significantly higher, compared with the term group. Compared with patients from Timisoara, the Petrosani cohort presented significantly higher median concentrations.

As is an element known since ancient times, where it was frequently used as a poison. Very small amounts do have some physiological functions, interacting with the metabolism of selenium and methionine; the normal dose is in the range of 12-40 μg/day (93,94). Except for the derivative arsenic trioxide (As_2O_3), which has antitumor properties, the levels of As should be maintained in the recommended levels, as high doses can lead to neuronal insulin signaling disruption and the development of malignancies, severe gastrointestinal toxicities, diabetes, cardiac arrhythmias or even death (95-97). Related neurological problems include lower IQ levels, attention-deficit/hyperactivity disorder and autism spectrum disorders (98,99). Elevated maternal levels have been associated with maternal hypertension preterm deliveries, low birth weight and even possible spontaneous abortion (23,24,100,101). In the present study, the differences between term and preterm pregnancies were not considered

Table VI. Comparison of heavy metal concentrations in the amniotic fluid between the Timisoara (n=100) and the Petrosani (n=60) groups.

Metal	Mean	SD	Median	IQR	Range	P-value[a]
Pb						
Timisoara	0.0489	0.1765	0.0001	0.0000-0.0130	1.012	0.04513[b]
Petrosani	0.1136	0.2671	0.0030	0.0000-0.0468	1.202	
Cu						
Timisoara	0.5281	0.5339	0.3225	0.0805-1.0230	1.738	<0.00001[b]
Petrosani	0.0322	0.0392	0.0190	0.0090-0.0433	0.265	
Ni						
Timisoara	0.5645	0.6970	0.0010	0.0000-1.1390	2.787	0.78150
Petrosani	0.6480	0.8089	0.0504	0.0000-1.3100	2.898	
Cd						
Timisoara	0.0085	0.0392	0.0001	0.0000-0.0001	0.239	0.00002[b]
Petrosani	0.0101	0.0413	0.0001	0.0000-0.0010	0.235	
As						
Timisoara	0.8175	1.0120	0.2310	0.0000-1.5270	3.768	0.03027[b]
Petrosani	1.0751	1.1116	0.7400	0.0685-1.7029	4.743	
Zn						
Timisoara	0.0664	0.1462	0.0140	0.0000-0.0560	0.742	<0.00001[b]
Petrosani	0.1394	0.2249	0.0530	0.0238-0.1418	1.266	
Fe						
Timisoara	0.3202	0.3370	0.2219	0.0982-0.4240	1.500	0.44540
Petrosani	0.3735	0.3793	0.2660	0.0883-0.5653	1.768	

[a]Mann Whitney's U-test, [b]statistically significant. IQR, interquartile range; SD, standard deviation.

Table VII. Smoking status of patients from the Timisoara and the Petrosani groups.

Patient group	Active smoker, n	Former smoker, n	Non-smoker, n	P-value[a]
Timisoara (all patients)	4	43	53	0.7193
Petrosani (all patients)	2	28	30	
Timisoara				
Preterm	3	18	29	0.26855
Term	1	25	24	
Petrosani				
Preterm	0	12	16	0.23577
Term	2	17	13	

[a]Fisher's exact test.

statistically significant, both for patients from Timisoara and from Petrosani. When comparing the two cohorts, the Petrosani cohort presented significantly higher median concentrations of As.

Pb is well-known for its high toxicity and has no known physiological roles, and as such, avoiding contact with this metal is advised. Pb poisoning affects the kidneys, the cardiovascular system, reproduction and especially the neurological and psychologic systems, as it can pass the blood-brain barrier (102-106). In children, the neurological and psychological effects can be drastic, as their brains and mind undergo much development. This can lead to problems such as lower IQ levels, attention-deficit/hyperactivity disorder and antisocial disorders (107-109). Other pediatric disorders due to elevated lead concentrations in the mother's serum could be reduced glomerular filtration, asthma, immunological and dermatological disorders (110-113). Perinatal effects of high Pb concentrations are low birth weight, preterm delivery, pre-eclampsia and pregnancy hypertension (90,114-117). Umbilical cord blood analysis has also shown that Pb affects DNA methylation and has also confirmed the reduced intellectual abilities of children coming from pregnancies with exposure to Pb (118,119). In the present study, the differences in Pb between term and preterm pregnancies were not statistically significant in either cohort. Compared with the Timisoara cohort, the patients from Petrosani presented significantly higher median concentrations of Pb.

For both cohorts, the differences between the preterm and term pregnancies were minimal. Indeed, no statistically significant differences were observed in the Timisoara cohorts, and the patients from Petrosani only showed higher concentrations of Zn and Cd in the preterm pregnancy group. More importantly, the comparison between the two cities showed that patients from Petrosani, a well-known industrial region, had higher concentrations of Zn, Cd, Pb and As. This is in agreement with other international studies showing that people living in industrialized regions are susceptible to accumulation of these elements, even if they mostly measured the concentrations either in the mother's serum, urine, toe-nails, hair, fetal placenta and cord blood (16-22,90,115,120,121). These elements are important as their concentration may be further increased by tobacco and are secreted in colostrum and breast milk (122). These elements are also some of the more toxic metalloids. Concern regarding their levels and possible health problems has also been expressed in other studies (46,47,92,99,111,113,123).

The smoking status of mothers can influence concentrations in blood, urine, hair or toe-nail samples (124-127). However, in order to avoid this, the patients in the present study were separated in three groups. Active smokers were defined as patients smoking even during pregnancy. Former smokers were defined as patients that had smoked and gave up the habit in the past, as well as mothers, which quit smoking once the pregnancy was suspected and/or confirmed. Nonsmokers were defined as patients that never smoked. There was no association between smoking status and gestational age, nor with any of the two cities in particular. Therefore, smoking status may not be an interfering element with respect to the concentration of heavy metal ions.

There are some limitations to this study. Larger sample lots might help produce finer, more accurate results. The design of the study may result in unequal follow-up, as it involved two maternity clinics from different cities. All mothers were recommended supplements such as Elevit 2 (Bayer, Germany) or Femosun (Sun Wave Pharma, Ascendis Health, South Africa), which may interfere with some of the recorded elements, especially Fe. Another limitation, which was not analyzed, may be the frequency of smoking, represented by the number of cigarettes smoked by an individual per day. Only the smoker status was assessed under the labels previously described.

In conclusion, bioactive components found in the amniotic fluid are important and can be monitored through amniocentesis. This tool enables healthcare professionals to assess the condition of the developing fetus. Common ions have been largely studied in the past. Heavy metal ions require more attention as minimal differences in concentration might influence the fetal development. Cd and Pb are elements with high toxicity and almost no physiological function. Thus, the authors recommend that these elements be avoided, especially by pregnant women and children. Metalloids such as Fe, Cu, Zn or Ni are to be discussed by expecting mothers with their healthcare provider, in order to check if any supplementation is needed. More in-depth research should be done in order to outline the effects of these elements and to determine how they affect antenatal outcomes and childhood development in the long run.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

RIN, GD, AD and AM contributed substantially to the conception and design of the study, the acquisition, analysis and interpretation of the data, and were involved in the drafting of the manuscript. AVP, AGE, DDV, MC and ESB contributed substantially to the analysis and interpretation of the data and were involved in the drafting of the manuscript. IC, ICC, FGH and FG contributed substantially to the interpretation of the data and were involved in the critical revisions of the manuscript for important intellectual content. FGH, AM and MC are responsible for confirming the authenticity of all the raw data and supervision. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study design followed the international regulations in accordance with The Declaration of Helsinki. The study was approved by the Ethics Committees of the 'Bega' Maternity Clinic, (approval no. 260/16IUL2021) and Petrosani Hospital (approval no. 15990/27.07.2021). Patient informed consent for publication of the data associated with the manuscript was obtained.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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