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Change of the levels of trace elements and heavy metals in threatened abortion

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Abstract

Backround: This study aimed to investigate the impact of changes in the serum levels of trace elements and heavy metals in threatened abortion, which is a common complication in early pregnancy. Earlier studies have shown that changes in the serum levels of some trace elements and increased serum concentrations of heavy metals are associated with spontaneous abortion; however, this relationship has not been fully clarified.

Methods: The patient group consisted of 45 pregnant women diagnosed with threatened abortion. There were 40 healthy non-pregnant women in the control group. Blood samples were obtained from the patients and the healthy controls, and the concentrations of serum trace elements and heavy metals were measured using atomic absorption spectrophotometry.

Results: Statistically significant decreases were found in the serum zinc (Zn), iron (Fe), magnesium (Mg) and manganese (Mn) levels, and significant increases were found in the serum copper (Cu), cadmium (Cd) and lead (Pb) levels in cases of threatened abortion in comparison to the controls ($p < 0.001$). However, there was no difference between the groups in terms of the serum cobalt (Co) levels ($p > 0.001$).

Conclusion: Changes in the balance of some essential trace elements (especially decreases in Fe and Zn) and elevated concentrations of some toxic heavy metals in the blood may be important diagnostic and prognostic parameters for threatened abortion

Keywords: Heavy metals; Threatened abortion; Trace elements

1. INTRODUCTION

Threatened abortion is a common complication in early pregnancy (before 20 weeks of gestation). It is usually diagnosed with mild to moderate vaginal bleeding in the presence of a closed cervix. Nearly 25% of pregnant women have some degree of vaginal bleeding during the first trimester, and about 50% of those pregnancies end in spontaneous abortion.[1,2] Threatened abortion has been shown to be associated with an increased incidence of antepartum hemorrhage, preterm labor, and intrauterine growth retardation.[1,3] Previous pregnancy loss or history of a baby with congenital abnormality increases the fetal loss possibility of a patient with first trimester bleeding. Moreover, thrombophilia, diabetes mellitus, hypothyroidism, infertility treatment, maternal age, uterine structural anomalies, and environmental factors (such as nutrition) are risk factors for spontaneous abortion.[4,5]

Essential trace elements are involved in various biochemical pathways, and they play a crucial role in fetal growth and development during pregnancy.[6] The concentration of these trace element is altered during pregnancy due to the requirements of the growing fetus and changes in the mother's physiology. Alterations in the concentrations and homeostasis of each of these micronutrients during pregnancy may be closely related to various disorders, including miscarriage, preterm delivery, stillbirth, intrauterine growth restriction, fetal malformations, premature rupture of membranes, and other adverse pregnancy outcomes.[7,8] Zinc (Zn) is one of the important essential trace elements; it plays a crucial role during pregnancy, especially during the embryonic stage because of ongoing cellular multiplication, differentiation, and organogenesis. Zn is an essential constituent of over 300 enzymes that participate in carbohydrate and protein metabolism, nucleic acid synthesis, and antioxidant functions.[9] Alteration in Zn homeostasis may have devastating effects on the outcome of a pregnancy, including fetal growth restriction or embryonic or fetal death.[10] Copper (Cu) and iron (Fe) also play vital roles in many of the body's biochemical processes. Thus, deficiencies in Cu and Fe could lead to widespread problems, especially if these deficiencies occur during pregnancy[11] However, cadmium (Cd) and lead (Pb) are nonessential toxic heavy metals that are widespread in the environment and long exposure to them has been documented to disrupt the fetal developmental process and impact the pregnancy outcome.[12] Studies have shown that heavy metals, such as Pb and Cd, are also associated with spontaneous abortion.[13,14] However, this relationship has not been fully clarified to date.

The current study aims to investigate the impact of changes in the levels of serum trace elements and heavy metals in threatened abortion when compared to healthy controls. Although trace

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elements and heavy metals have been studied extensively in normal pregnancies, few studies have investigated how their concentration levels impact pathological pregnancies, such as threatened abortion.

2. METHODS

In this prospective study, we examined 45 pregnant women with a diagnosis of threatened abortion (with a gestational age of 6 to 14 weeks), who were patients at the Van Gynecology and Obstetrics Clinic. Threatened abortion was defined by history of vaginal bleeding with closed cervix and clinical examination in the first trimester. The control group included 40 apparently healthy nonpregnant women. The pregnant women were monitored during their pregnancy period. Pregnant women who ignored their pregnancy protocol were called, and information about their pregnancy was obtained and recorded. The pregnancy period of all the pregnant patients were recorded, and patients with a preterm delivery (<37 weeks of gestation) or an abortion (<24 weeks of gestation) were accepted into the study.

The gestational age was estimated from the patient's last menstrual period and the first trimester ultrasound. If the self-reported last menstrual period was >7 days from the calculated last menstrual period based on ultrasonography, then the ultrasonography result was used to assign the gestational age. The exclusion criteria were as follows: multiple pregnancies, patients who smoked and patients who had uterine and placental abnormalities, diabetes mellitus, hypertension, polyps, cervicitis, or cervical myomas. Women who had taken any vitamin supplements were excluded. All patients were informed about the details of the study, and written consent was obtained. All procedures were performed in accordance with the ethical standards of the Declaration of Helsinki. Permission was obtained from the Van Regional Training and Research Hospital Ethics Committee for Non-interventional Clinical Researches (12.02: 28.02.2017).

Biochemical analyses were performed at the Central Research Laboratory of Yuzuncu Yil University and the Biochemistry Laboratory of the Chemistry Department, Faculty of Science at Yuzuncu Yil University. Blood samples from all participants were taken at the same time. Overnight fasting blood samples (5 mL) of the pregnant patients and the nonpregnant healthy controls were aseptically taken from the antecubital fossa vein using new, disposable pyrogen-free needles. The puncture site was cleaned with disinfectant to prevent contamination when the blood was taken. After the blood samples were obtained, they were placed in a plain tube and kept for 1 to 2 hours on a desk to clot and centrifuged at 5000 rpm for 10 minutes. Then serum was separated and stored at –20°C for determination of the biochemical parameters. The serum levels of the trace elements and heavy metals such as manganese (Mn), magnesium (Mg), Zn, Fe, Cu, cobalt (Co), Pb, and Cd were determined by using UNICAM-929 atomic absorption spectrophotometer (AAS) (Unicam Ltd, Cambridge, UK).

2.1. Statistical analysis

Descriptive statistical data for the continuous variables were expressed as the mean ± SD. In the two group comparisons, the Mann–Whitney *U* test was used in situations where the normality condition was not satisfied, and a *t*-test was used in cases where a normal distribution was expected. The results were considered to be statistically significant when $p < 0.05$. The data were analyzed by using the SPSS 20 software (SPSS Inc., Chicago, IL, USA).

3. RESULTS

The descriptive characteristics of the study group are presented in Table 1. There was no difference between the groups in terms of age. None of the pregnant women or the women in the control group were under Fe, mineral, or multivitamin supplementation, and none of the women were consuming alcohol or smoking cigarettes. The control group consisted of volunteers who were apparently healthy nonpregnant women. There were two groups of patients: the first consisted of patients who had live births and the second consisted of patients who experienced spontaneous abortions. Out of the total number of pregnant patients, 10 experienced spontaneous abortions. The remaining 35 pregnant patients had pregnancies that continued beyond 24 weeks of gestation. This study included 24 preterm cases; these patients delivered after 24 to 37 weeks of gestation. Within these preterm cases, 14 patients delivered after 34 to 37 weeks of gestation and 10 patients delivered after 28 to 34 weeks of gestation. In the threatened abortion group, hereditary thrombophilia was found in 10 patients and these patients had used low molecular weight heparin during their pregnancy period. Trisomy 21 was detected via ultrasonography in three patients in the early gestational period and all were terminated from the study. This study does not include detailed diet analysis and lifestyle habits for either group. However, both the study and control group shared similar living conditions and socioeconomic status. All of the study's participants lived in rural areas and their socioeconomic status was classified as low based on their income.

Table 1

Descriptive characteristics of the study group

	Threatened abortion (n = 45)	Healthy control group (n = 40)	*p*
Maternal age, y	28.36 ± 6.37	27.56 ± 5.31	>0.001
Gestational age, wk	8.8 ± 1.4	…	
Gravida	2.5 ± 0.47	…	
Parity	0.54 ± 0.80	…	
Livebirth	0.52 ± 0.66	…	
Abortus	0.62 ± 0.72	…	
Smoking status	None	None	
Alcohol intake	None	None	

*$p < 0.001$ is significant.

The levels of some trace elements and heavy metals in the study groups are shown in Table 2. As shown in the table, serum levels for Zn, Fe, Mg, and Mn levels in the patient group (87.90 ± 9.59 µg/dL and 34.63 ± 14.9 µg/dL and 31.16 ± 5.74 µg/dL and 16.8 ± 5.2 µg/dL) were significantly lower than those of the healthy control group (152.8 ± 3.34 µg/dL and 74.9 ± 3.5 µg/dL and 61.59 ± 4.07 µg/dL and 22.9 ± 1.8 µg/dL) ($p < 0.001$). Conversely, serum levels of Cu (177.9 ± 74.40 µg/dL and 72.2 ± 10.2 µg/dL), Cd (4.59 ± 0.44 µg/dL and 0.20 ± 0.03 µg/dL), and Pb (12.65 ± 0.51 µg/dL and 1.13 ± 0.02 µg/dL) were higher in the

Table 2

Serum trace elements and heavy metal levels in threatened abortion and healthy control group

	Threatened (n = 45) abortion	Healthy (n = 40) control group	*p*
Cu, µg/dL	177.9 ± 74.40*	72.2 ± 10.2	0.001
Zn, µg/dL	87.90 ± 9.59*	152.84 ± 3.34	0.001
Mg, µg/dL	31.16. ± 5.74*	61.59 ± 4.07	0.001
Mn, µg/dL	16.8 ± 5.2*	22.9 ± 1.8	0.001
Fe, µg/dL	34.63 ± 14.9*	74.9 ± 3.5	0.001
Co, µg/dL	1.71 ± 0.14*	1.06 ± 0.02	0.012
Cd, µg/dL	4.59 ± 0.44*	0.20 ± 0.03	0.001
Pb, µg/dL	12.65 ± 0.51*	1.13 ± 0.02	0.001

Values are in mean ± SD; *Statistical significance between patient and control group ($p < 0.05$).

patient group than the control group ($p < 0.001$). Additionally, serum Co levels were not different in the threatened abortion group when compared with the control group (1.71 ± 0.14 μg/dL and 1.06 ± 0.02 μg/dL).

4. DISCUSSION

Trace elements play an essential role in the maintenance of health, and either excessive accumulation or deficiency can result in various diseases. The concentrations of these elements are altered during pregnancy because of the requirements of the growing fetus and changes in the mother's physiology.[15,16]

Hereditary and acquired thrombophilias are cases where the risk of venous thrombosis increases. Thrombophilia may occur in pregnancy due to Factor V Leiden mutation; prothrombin G20210A mutation; *MTHFR* gene mutations (677 and 1298 mutations); and protein C, protein S, and antithrombin deficiencies.[17,18] The association between thrombophilia and pregnancy loss has been identified but the underlying mechanism remains unclear. However, it is estimated that pregnancy loss might be caused by thrombosis in decidual vessels.[19] In our study, the number of patients with thrombophilia in the abortion group was significantly higher. It is possible that this can be attributed to the higher marriage rates among close relatives in the region and similarities of the economic and sociocultural levels of the participants.

Fe is an important trace element, which is a component of hemoglobin and myoglobin. It is also involved in the transport, storage, and use of oxygen.[20] Increased oxidative stress, hypoxia in the placenta and fetus, and reduced immunity have been suggested to be associated with Fe deficiency during pregnancy.[21] In developing countries, Fe deficiency is highly prevalent in women (especially pregnant women) and it is an important public health problem.[22] Our study found that serum Fe levels were lower in cases of threatened abortion when compared with the control group. A previous study found that serum levels of Fe were lower in patients who had miscarriages when compared with control group of healthy pregnancies.[8] The consequences of maternal Fe deficiency are serious, both for the mother and the developing fetus; these include increased risk of mother mortality and morbidity and increased complications in the prenatal and postnatal period for the fetus. Therefore, pregnant women should use Fe supplements during pregnancy, as recommended by a health specialist, to prevent adverse pregnancy outcomes and to meet the needs of the fetus.

Cu is another essential trace element, which is required for the formation of many enzymes that play important roles in the human body. An increase in maternal serum Cu concentration during pregnancy is attributed to the increase of ceruloplasmin as a result of elevated levels of oestrogen.[23] We found that serum Cu level was higher in cases of threatened abortion when compared with healthy control group. Our results were contrary to the work of Alebic-Juretic et al.,[24] who found that Cu concentrations were lower in pathological pregnancies such as spontaneous abortion and threatened abortion in the first trimester. However, Borella et al.[25] found that Cu concentrations were higher in cases of threatened and spontaneous abortion. During pregnancy, the metabolism of Cu and Fe is tightly interlinked, and the deficiency of one has marked effects on the metabolism of the other metal. In the mother, Fe deficiency results in an increase in liver Cu levels. This is associated with an increase in serum Cu levels in the mother and in the activity of maternal serum ceruloplasmin.[26]

Zn plays an important role in a variety of enzymes such as DNA and RNA polymerase and participates in important biochemical metabolic processes.[27] Low serum Zn levels have been suggested to influence embryonic and fetal development through various mechanisms, including reduced cell proliferation, reduced protein synthesis, increased rate of cellular oxidative damage, and increased rate of apoptosis.[28] In this current study, there were significant decreases in the serum levels of Zn in threatened abortion cases when compared with the controls. Some previous studies have shown that Zn deficiency during pregnancy in the early weeks may lead to abortion and congenital malformations.[29] For this reason, monitoring of serum Zn levels in pregnant women is important to ensure proper fetal development.

Mg and Mn are additional essential trace elements necessary for metabolic regulation and bone formation. Mg also acts as a cofactor for many enzymes and plays a significant role in peripheral vasodilation and neurochemical transmission.[30,31] Mg deficiency in pregnancy has been associated with some pregnancy complications such as hypertension, preeclampsia, placental dysfunction, and premature labor.[32] We found that serum Mg and Mn levels were lower in the threatened abortion group when compared with the healthy control group. Previous studies have shown no differences between pathological pregnancies and healthy controls with respect to Mg levels.[32,25] It has been reported that Mg supplementation during pregnancy may reduce many pregnancy complications (such as preterm births and intrauterine growth retardation). However, Omeljaniuk et al.[33] found in their study that the Mn concentration in the serum of women who have had a miscarriage was significantly higher when compared with the control group. Although Ajayi et al.[32] also found that levels of Mn were higher in cases of recurrent abortion, this difference was not statistically significant.

Cobalt is a transition metal and a component of vitamin B_{12}.[34] The maternal concentrations of metals including cobalt may change along pregnancy. Women accumulate more cobalt than men at similar exposure levels, which may be related to higher metabolic Fe loss.[35] Measurements of trace metal changes during pregnancy have been considered in some cases but these studies did not include cobalt. In this current study, we found no difference between the patient group and the healthy control group in terms of cobalt levels.

Cd is a toxic heavy metal considered to be a metalloestrogen because it can bind to the estrogen receptor subtypes, alpha and beta, and stimulates them. Therefore, Cd is considered to be an important factor in the etiology of breast and endometrial carcinoma, endometriosis, and spontaneous abortions.[36] In addition, studies have demonstrated that women tend to have higher concentrations of Cd in blood, urine, and kidneys than men. One possible reason for elevated Cd levels in women could be that the absorption of Cd increases significantly with Fe deficiency;[37] it is worth noting that the toxic effects of Cd increases during pregnancy for both the mother and the fetus. In the general nonsmoking population, diet is the most important source of Cd, and it can be found in contaminated drinking water, paints, welding by-products, and some shellfish.[38] In this study, serum Cd levels were found to be significantly higher in the threatened abortion group (4.59 μg/dL ± 0.44 μg/dL) than in the control group (0.20 μg/dL ± 0.03 μg/dL). Similarly, Ajayi et al.[32] found higher serum Cd levels in cases of recurrent spontaneous abortions (4.58 ± 0.77 μg/dL) compared with the control group (2.49 ± 0.09 μg/dL). Otebhi and Osadolor[39] also observed a significant increase in Cd levels in the blood of pregnant women with a history of pregnancy complications when compared with pregnant women who had no history of complications.

Pb is a toxic metal and widely distributed in the environment. Levels of Pb of approximately 10 μg/dL in women have been linked to increased risks of pregnancy hypertension, spontaneous abortion, and reduced fetal growth.[40] It was reported that Pb can be highly toxic even at low doses, although there is no clear evidence that at such levels it can affect the reproduction system.[41] It was also found that its level increases with age, which may be associated with the release of Pb previously accumulated

in bones.[14] We found that serum Pb levels were higher in the threatened abortion group (12.65 ± 0.51 μg/dL) when compared with the control group (1.13 ± 0.02 μg/dL). Similarly, Ajayi et al.[32] found significantly higher concentrations of Pb in cases of recurrent spontaneous abortion (85.96 ± 1.09 μg/dL) when compared with the control group (60.70 ± 1.40 μg/dL). However, Vigeh et al.[42] found that mean Pb concentrations in blood did not differ significantly between spontaneous abortion cases and ongoing pregnancies (3.51 ± 1.42 μg/dL and 3.83 ± 1.99 μg/dL, respectively). In Eastern Turkey region, exposure to various environmental toxic substances (such as contaminated tap water and air pollution) and the low education and socioeconomic status of people may explain the high serum levels of heavy metals.

There are some limitations to this study. First, the study population was small. It would be appropriate to use a larger sample size in order to verify the relationship between threatened abortion and trace element levels in future research efforts. Second, we have not included a detailed analysis of diet and lifestyle habits for either group. However, we believe that our study contributes to the body of prior research on cases of threatened abortion.

In conclusion, changes in the levels of essential trace elements (especially the reduction of Fe and Zn) and elevation of the levels of some toxic heavy metals in blood may be important for threatened abortion etiology, treatment, or prevention. Threatened abortions occur frequently, especially in developing countries, and cause economic and psychological problems (such as depression and anxiety). Therefore, the concentrations of these elements in the early stages of pregnancies may be important diagnostic and prognostic parameters for threatened abortions.

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