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Is there any association between fetal nervous system anomalies and heavy metal-trace element levels in amniotic fluid?

N. Cim[1], H.E. Tolunay[1], B. Boza[1], M. Bilici[2], E. Karaman[1], O. Cetin[1], R. Yildizhan[1], H.G. Sahin[1]

[1]Department of Obstetrics and Gynaecology, Yuzuncu Yil University Medical Faculty,Van
[2]Yuzuncu Yil University, Van Security Collage, Van (Turkey)

Summary

Aim: In this study the authors aimed to evaluate whether there are any causal relationship between heavy metals-trace elements and fetal malformations of central nervous system (CNS). *Materials and Methods:* The study group consisted of pregnancies with fetal congenital nervous system anomaly (anencephaly, acrania, neural tube defects, etc.) in 16-22 weeks (n=36). Pregnancies with the same weeks of pregnancy who underwent amniocentesis due to high risk in triple test with the result of normal karyotype constituted the control group (n=30). In the both groups the authors analyzed the heavy metals and trace elements in amniotic fluid. Metals and elements were measured by using atomic absorption spectrophotometer technique with a UNICAM-929 spectrophotometer. *Results:* When compared, the groups were similar in terms of age, parity, BMI, and gestational week ($p > 0.05$). In fetal congenital anomaly group the authors detected low levels of copper (Cu) and zinc (Zn) rather than control groups ($p < 0.05$). In fetal congenital anomaly group they detected high levels of lead (Pb) and cadmium (Cd) rather than control groups ($p < 0.05$). Iron (Fe), manganese (Mn), cobalt (Co), nickel (Ni), and Cd levels were similar and there was no significantly difference between the groups ($p > 0.05$). *Conclusion:* This study can contribute benefits to the literature in terms of clarifying the pathogenesis of fetal congenital nervous system anomalies.

Key words: Fetal malformations; Heavy metals; Trace elements; Amniotic fluid.

Introduction

Environmental chemicals, toxic heavy metals, and trace elements are related to spontaneous abortions, premature births, congenital malformations, menstrual cycle disorders, fertility problems, and behavioral problems of offsprings. Early embryogenesis can be affected by these agents with DNA damage. Heavy metals and environmental chemicals can accumulate in organisms and reproductive systems. So that oogenesis, spermatogenesis, fertility, and embryogenesis can be affected by this mechanism [1-3].

While there are still unknown causes of congenital malformations and anomalies, genetic causes, environmental agents, maternal infections, and radiation are accused of fetal malformations. In this study the authors aimed to evaluate whether there any causal relationship between heavy metals-trace elements and fetal malformations of central nervous system (CNS).

Materials and Methods

Patients admitted to a tertiary university hospital (Yuzuncu Yil University) of an eastern region of Turkey in 2017 were included in the study. The study was conducted in accordance with the principles in Declaration of Helsinki. Before commencing the study, the ethics committee approval was included. All patients provided informed consent before enrollment in the study. The study initiated with 66 patients. The study group consisted of pregnancies with fetal congenital nervous system anomaly (anencephaly, acrania, neural tube defects, etc.) in 16-22 weeks (n=36). Pregnancies with the same weeks of gestation who underwent amniocentesis due to high risk in triple test with the result of normal karyotype.

Amniotic fluid (AF) was taken to be sent to the genetic screening via amniocentesis procedure. An amount of AF sample not sent to the genetic screening was stored at -20^{0}C until assays.

Two milliliters of HNO3/H2O2 mixture (2:1) were added to 0.7 grams of the serum samples. The mixture was placed into the water bath at 70°C for30 minutes and stirred occasionally. Then, 1 mL of the same acid mixture was added, and the mixture was transferred into a Teflon vessel bomb for the microwave oven. The bomb was closed, and the solution was placed inside the microwave oven. Radiation was applied for three minutes at 450 W. After addition of 0.5 mL of the same acid mixture, radiation was repeated for three minutes. After cooling for five minutes, 2.0 mL of 0.1 mol/L HNO3 was added, and the solution was transferred to a Pyrex tube. After centrifugation, the clear solution was used to determine manganese (Mn), cadmium (Cd), copper (Cu), lead (Pb), iron (Fe), magnesium (Mg), cobalt (co), and zinc (Zn) levels. They were measured by using atomic absorption spectrophotometer technique with a UNICAM-929 spectrophotometer.

The Statistical Package for the Social Sciences (SPSS) version 20.0 was used for all statistical analyses. The Shapiro-Wilk test was used to test distribution of normality. Mann Whitney U test is performed to determine the differences between the groups. A p value < 0.05 was considered statistically significant.

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Table 1. — *Demographic variables of the anomaly and normal groups.*

	Anomaly group Median (Min–Max) n=36	Control group Median (Min-Max) n = 30	*p* value
Age (years)	27,7 (20-40)	27,3 (20-40)	0.846
Parity	2,02 (1-4)	2,03 (1-4)	1.000
Hemoglobin (mg/dl)	11,88 (9-15)	11,83 (9-15)	0.791
Body mass index (kg/m^2)	22,9 (19-28)	22,7 (19-28)	0.461

Table 2. — *The comparison of heavy metals and trace elements of the groups.*

	Anomaly group Median (Min–max) n=36	Control group Median (Min-max) n = 30	*p* value
Fe (μg/100 ml)	0.111 (0.005-0.301)	0.072 (0.004-0.216)	0.075
Mn (μg/100 ml)	0.009 (0.003-0.016)	0.012 (0.003-0.026)	0.054
Cu (μg/100 ml)	0.006 (0.003-0.021)	0.011 (0.001-0.028)	0.005
Pb (μg/100 ml)	0.036 (0,010-0.064)	0.019 (0.002-0.040)	0.000
Zn (μg/100 ml)	0.569 (0.040-2.191)	2.916 (0.026-6.976)	0.001
Co (μg/100 ml)	0.026 (0.012-0.052)	0.029 (0.022-0.045)	0.079
Ni (μg/100 ml)	0.011 (0.003-0.025)	0.012 (0.002-0.025)	0.918
Cd (μg/100 ml)	0.007 (0.004-0.012)	0.005 (0.001-0.009)	0.020

Results

When compared, the groups were similar in terms of age, parity, BMI, and gestational week ($p > 0.05$) (Table 1).

In the fetal congenital anomaly group the authors detected low levels of Cu and Zn rather than control groups (Table 2). Cu levels were 0.006 μg/100 ml and 0.011 μg/100 ml in the fetal anomaly and control groups, respectively ($p = 0.005$). Zn levels were 0.569 μg/100 ml and 2.916 μg/100 ml in the fetal anomaly and control groups, respectively ($p = 0.001$).

In the fetal congenital anomaly group the authors detected high levels of Pb and Cd rather than control groups (Table 2). Pb levels were 0.036 μg/100 ml and 0.019 μg/100 ml in the fetal anomaly and control groups, respectively ($p < 0.001$). Cd levels were 0.007 μg/100 ml and 0.005 μg/100 ml in fetal anomaly and control groups, respectively ($p = 0.005$).

Fe, Mn, Co, Ni, and Cd levels were similar and there was no significantly difference between the groups ($p = 0.020$) (Table 2).

Discussion

There are few studies investigating heavy metals and trace elements in AF in pregnancy with fetal congenital nervous system anomalies to the present authors' knowledge. In this study they detected low levels of Zn and Cu in AF in pregnancies with anomaly compared to the healthy group. Interestingly the finding that Zn is reduced in AF of the pregnancies with fetal congenital nervous system anomalies has not been reported previously. Also Pb levels are increased in AF of pregnancies with fetal congenital anomaly group when compared the normal group.

AF surrounds the fetus and protects it from external conditions during pregnancy. AF is very important for fetal and cellular growth, proliferation, and embryonic development [4]. AF reflects fetal status and plasma. AF is mainly produced by the fetus and mostly consists of fetal urine. That is why ingredients of AF can reflect fetal serum status [5, 6]. It is known that abnormalities of AF can be associated with poor obstetrical outcomes [7, 8].

Some studies have determined the levels of trace elements and heavy metals in AF. It was shown that these heavy metals and trace elements may accumulate in AF from the early stages of pregnancy. However, how and what effects these elements have on fetal development or what the long-term effects of this early exposure are not fully known [9].

Prenatal period is regarded as the most sensitive time in human development due to the many differences in fetal cellular division. At the same time, many febrile biochemical pathways differ from adults, therefore, teratogens may exhibit pathotypic sensitivity at low exposure levels, which are not particularly harmful to the mother [10].

Heavy metals are transferred to the fetus by trans-placental transfer. The placenta acts as a barrier to the transport of toxic compounds, allowing oxygen and nutrients to pass through during pregnancy; the barrier function occurs via metallothionine [11, 12]. Several in vitro and in vivo studies have been performed to determine the effects of environmental exposure on fetal development [13].

Umbilical cord and maternal blood samples were generally used to investigate the effect of environmental pollutants on uterus [14, 15]. It is reported that placental tissues could be used as a biomarker in the investigation of the effects of toxic metals on maternal and fetal health. The present study showed that heavy metals in the AF are associated with CNS anomalies, and especially during pregnancy, the effect of toxic metals may be evident.

Cu is an essential trace element which plays very important roles in different metabolic process and enzymatic reactions. Cu involves in cytochrome oxidase enzyme systems. Cell proliferation is very active in embryogenesis and so cytochrome oxidase take part in a very important point in embryogenesis. Cu plays important role in embryogenesis due to being a part of cytochrome oxidase [16]. In the present study the authors found low levels of Cu in AF in fetal anomaly group. This finding supports the beneficial effect of Cu on fetal neurogenesis. On the other hand, some controversial effects in obstetrical outcomes

and reproductive effect of Cu have been shown in a few studies [3, 17].

Zn is essential for normal fetal growth and development. It is required for transcription, cell division, growth, and differentiation. Heavy Zn deficiency is embryotoxic and teratogenic, and is a cause of abnormal fetal development [18]. In animal and human studies, maternal Zn deficiency is determined as a major risk factor for neural tube defects [19]. The efficacy of periconceptional folic acid supplementation in reducing neural tube defect occurrence and recurrence has been proven [20]. In a study, researchers showed that Zn deficiency in pregnant rats decreases folate bioavailability of folinic acid [21]. On the other hand there are still controversial findings about AF Zn levels in neural tube defects and high-risk pregnancies. There are also few studies on this subject [22]. The present authors found decreased levels of Zn in AF in the fetal nervous system anomaly group. Use of Zn and supplementation could be discussed and should be kept in mind with the help of further studies regarding this subject.

Pb is especially neurotoxic. Disruption of key molecules during neuronal migration and differentiation is probably the mechanism of this neurotoxicity. Possibly increased Pb levels could play a role in the pathogenesis of diseases due to the damage in other trace elements metabolism [23]. In this study the authors found higher levels of Pb in AF in the anomaly group.

In conclusion, this finding can explain a part of pathogenesis of neural system anomalies in this study. This study can contribute benefits to the literature in terms of clarifying the pathogenesis of fetal congenital nervous system anomalies. We can contribute to decrease the perinatal mortality and morbidity with advanced diagnosis and treatment attempts by detection of congenital malformations in early gestational weeks. Other studies are needed to expand knowledge on the developmental effects of most heavy metals and trace elements in humans.

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Corresponding Author:
N. CIM, MD
Department of Obstetrics and Gynaecology
Faculty of Medicine, Yuzuncu Yil University
Van (Turkey)
e-mail: numancim@yahoo.com