Annals of Medical Research
DOI: 10.5455/annalsmedres.2019.12.889 2020;(27)2:454-60

Original Article

Evaluation of specific heavy metal levels of pregnant women complicated by neural tube defects

Talip Karacor[1], Pinar Kirici[1], Mehmet Can Nacar[1], Mehmet Bulbul[1], Muhittin Onderci[2], Nurullah Peker[3]

[1]Adiyaman University, Faculty of Medicine, Department of Obstetrics and Gynecology, Adiyaman, Turkey
[2]Adiyaman University, Faculty of Medicine, Department of Biochemistry, Adiyaman, Turkey
[3]Dicle University, Faculty of Medicine, Department of Obstetrics and Gynecology, Diyarbakir, Turkey

Abstract

Aim: The etiology of neural tube defect (NTD) is still not clear enough. In our study, we aimed to evaluate plasma heavy metal levels of pregnant women with NTD and to determine whether there is a relationship between heavy metal levels and NTD severity.

Material and Methods: TThis study was conducted in Adıyaman University, Department of Gynaecology and Obstetrics. The study included 38 pregnant women with NTD and 42 pregnant women with healthy infants. Pregnant women who have NTD were divided into two groups as NTD Type 1 and NTD Type 2 according to the anomaly type. Levels of heavy metals such as Mercury (Hg), Cadmium (Cd), Cobalt (Co), Lead (Pb), Manganese (Mn) and Arsenic (As) were compared between groups.

Results: Plasma Hg, Co, Cd and Pb levels were higher in NTD group than control group. (p values, respectively; $p < 0.001$, $p = 0.001$, $p < 0.001$, $p < 0.001$). As and Mn levels were not statistically different between the two groups (p values; $p = .519$, $p = .819$, respectively). In the NTD group, Hg was found to be higher in NTD Type 1 than NTD Type 2 ($p < 0.001$).

Conclusion: It is obvious that some histomorphological changes are formed in the cardinal ligaments of patients with uterine prolapse due to pressure on the uterus. We believe that the increase in the number of extravasated erythrocytes and the thickness of the vascular wall and peripheral nerve should be supported by further studies.

Keywords: Anencephaly; heavy metal; neural tube defect; pregnancy; spina bifida

INTRODUCTION

Neural tube defect (NTD) is a congenital anomaly caused by the central nervous system (CNS) not being completely closed during embryogenic development. Its incidence varies between 0.5 and 6.5 per thousand worldwide (1,2). NTD types are anencephaly, encephalocele, meningomyelocele, meningocele, and spina bifida (3,4). Anencephaly cases where calvarium and brain development are significantly affected are fatal (5). Infants born with NTD at the level of medulla spinalis may survive after surgical treatment. However, the majority of infants surviving after surgical treatment require lifelong medical support (6). It is thought that NTD has many etiological causes such as environmental factors, genetic and nutritional deficiencies. The most accepted and supplemented etiological cause in worldwide is folic acid deficiency (7,8). However, it is known that folic acid supplementation does not completely eliminate NTD. Therefore, it is accepted that the etiological factors for NTD are still not fully explained. Some trace elements and some vitamin deficiencies have been shown to cause NTD (9-11). In addition, many studies have shown that exposure to radiation and toxic agents increase the incidence of NTD (12). While the deficiency of vitamins and trace elements is more prominent among the etiologic causes in underdeveloped countries (11), exposure to toxic agents such as heavy metal is more prepotent in developed countries (13). The dose and duration of exposure to these toxic agents determine the severity of fetal damage (14). In our current study, we compared the levels of heavy metals such as mercury (Hg), cobalt (Co), cadmium (Cd), arsenic (As), manganese (Mn) and lead (Pb) which are considered toxic to human health in the blood of pregnant women with NTD in their infants and pregnant women with healthy infants We also compared the levels of these heavy metals among themselves according to NTD types. Thus, we aimed to investigate whether heavy

Received: 26.12.2019 **Accepted:** 05.02.2020 **Available online:** 06.03.2020
Corresponding Author: Talip Karacor, Adiyaman University, Faculty of Medicine, Department of Obstetrics and Gynecology, Adiyaman, Turkey **E-mail:** talipkaracor@gmail.com

metal levels in maternal blood have an effect on fetal NTD development and whether there is a relationship between heavy metal levels and NTD severity.

MATERIAL and METHODS

The study was conducted between May 2018 and May 2019 at Adıyaman University, Department of Gynaecology and Obstetrics. Before starting the study, approval was obtained from the local ethics committee on 22.05.2018 with the number 2018 / 4-18. Our study is a prospective case-control study. Thirty-eight pregnant women with NTD anomaly were included in the study. Only folic acid supplemented pregnants were included to the study. They used folic acid before and during pregnancy. Cigarette usage and consanguineous marriage were exclusion criteria for the study. Pregnant women with chronic diseases such as diabetes mellitus, hypertension, hepatitis and taking regular medication were excluded from the study. The diagnosis of NTD was made by ultrasonographic examination. In the US examination, NTD anomalies in fetuses were identified as anencephaly, encephalocele, meningomyelocele and meningocele.

Forty-two pregnant women who applied to the obstetric outpatient clinic for routine examination, who had healthy pregnancy and who were in the same gestational week as the study group were randomly selected and included in the control group. Pregnant women with chronic disease and regular medication were not included in the control group. The NTD and Control groups were informed about the study and written informed consent was obtained. Demographic data of the patients were recorded. Pregnant women in the NTD group were divided into two groups. Pregnant women with anencephaly anomaly were identified as NTD Type 1, and pregnant women with encephalocele, meningocele and meningomyelocele anomaly were identified as NTD Type 2. Heavy metal levels were compared between NTD and control groups. In addition, NTD Type 1 and NTD Type 2 groups were compared in terms of heavy metal levels.

Collection of samples

The NTD group and the Control group were informed about the study and consent was obtained. Then, approximately 3 ml of venous blood was taken into a whole blood tube with EDTA. Samples were stored at -80 degrees until working day without waiting.

Analysis of heavy metals in serum samples

Microwave digestion of serum samples

The samples were analyzed in the central laboratory of Adıyaman University. Blood samples were digested prior to heavy metals analysis using a microwave digestion system. Blood samples were digested in an acid solution using a Berghof MSW-4 model microwave digestion system (Eningen, Germany). Briefly, 2 ml of blood was poored into digestion vessel and 5 ml 65% HNO3 and 2 ml H2O2 were added. Sample vessels were shaked and kept 20 minutes before vessels were closed. Following the digestion, The vessels were cooled for 20 minutes at room temperature and the contents were poured into 15 ml screw cap tubes. The remaining samples were eluted by rinsing the vessels with bidistillated water, and added to the previous sample to complete the volume to 10 ml (15). Temperature program is shown in Table 1.

Table 1. Temperature program for blood digestion

Step	1	2
T (oC)	160	190
P (bar)	40	40
Power (%)	80	90
Time (min.)	5	10

T: Temperature, P: Pressure, Min: Minute

Measurement

Measurements were performed using a NexION 350X ICP-MS device (Perkin Elmer, MA, USA). The instrument parameters are summarized in Table 2.

Table 2. ICP instrumental conditions

Component/Parameter	Type / Value / Mode
Nebulizer	Mainhard (concentric)
Spray Chamber	Glass Cyclonic
Triple Cone Interface Material	Nickel
Plasma Gas Flow	18.0 L/min
Auxiliary Gas Flow	1.2 L/min
Nebulizer Gas Flow	0.80 L/min
Sample Uptake Rate	1 mL/min
RF Power	1500 W
Replicates per Sample	3
Mode of Operation	STD/KED Mode Collision (using He gas)

Statistics

Data were analyzed using SPSS for Windows Version 21.0 (Armonk, NY: IBM Corp.). According to our study results for Haevy metal levels, sample size of the study population was calculated to be 38 patients (α= 0.05 and the study power= 80%). Kolmogorov-Smirnov test was used to analyze the distribution of continuous variables. In the group comparisons, the data which obtained continuous value matching normal distribution were analyzed by Independent Samples T Test / Oneway ANOVA and those who did not comply with normal distribution were analyzed by Mann-Whitney U Test. Kruskal Wallis test was used to compare three groups of variables which have continuous values. Spearman correlation test was used to evaluate

the presence of correlation between heavy metals in NTD development. In this study, the variables that take continuous value are given together with mean ± standard deviation values. Pearson Chi-Square test was used to evaluate categorical variables. Statistical significance level was accepted as p <0.05 and confidence interval as 95%. Receiver operator characteristic curve (ROC) analysis was used to determine cut off levels.

RESULTS

In our study, the pregnants in the NTD and control groups were between 12 weeks + 0 days and 20 weeks + 0 days. There was no statistically significant difference between the two groups in terms of maternal age and gestational age. The mean Hg, Co, Cd and Pb levels were found statistically higher in the NTD group compared to the control group (p values; p <0.001, p = 0.001, p <0.001, p <0.001, respectively). However, there was no statistically significant difference between the two groups in terms of As and Mn levels (p values; p = .519, p = .819, respectively).

Table 3. Demographic data and heavy metal levels of NTD and Control group in pregnant women

	NTD Group n: 42	Control Group n: 38	p
Maternal age	27.8±5.6	29.1±4.2	.071
Pregnancy week	15.1±1.0	15.6±1.9	.088
Hg (µg/L)	0.96±0.39	0.44±0.37	<0.001
Co (µg/L)	0.82±0.33	0.55±0.37	0.001
Cd (µg/L)	3.04±1.37	0.54±0.48	<0.001
As (µg/L)	3.07±1.78	2.92±1.05	.519
Mn (µg/L)	6.78±4.54	5.44±1.48	.819
Pb (µg/L)	23.22±7.24	15.14±3.18	<0.001

Hg: mercury, Co: cobalt, Cd: cadmium, Pb: lead As: arsenic, Mn: manganese

Table 4. Heavy metal levels between control, NTD Type 1 and NTD Type 2 groups

	Control group (n:40)	NTD Type 1 (n:16)	NTD Type 2 (n:20)		
				I-II	<0.001
Hg (µg/L)	0.44+0.37	1.31+0.28	0.68+0.16	I-III	0.021
				II-III	<0.001
				I-II	0.037
Co (µg/L)	0.55+0.37	0.82+0.36	0.82+0.31	I-III	0.019
				II-III	1.000
				I-II	<0.001
Cd (µg/L)	0.54+0.48	3.15+1.09	2.96+1.59	I-III	<0.001
				II-III	1.000
As (µg/L)	2.92+1.05	3.16+1.89	3.00+1.73		.974
				I-II	1.000
Mn (µg/L)	5.44+1.48	5.62+4.32	7.693+4.61	I-III	0.039
				II-III	.180
				I-II	<0.001
Pb (µg/L)	15.14+3.18	24.24+8.74	22.41+5.89	I-III	<0.001
				II-III	.974

I: Control group, II: NTD Type 1, III: NTD Type 2, Hg: mercury, Co: cobalt, Cd: cadmium, Pb: lead As: arsenic, Mn: manganese

Table 5. Cut off values and sensitivity-specificity for Hg, CO, Cd and Pb levels

Variables	AUC	SE	95%CI	Cut off value	Sensitivity	Specificity
Hg (mg/L)	0.844	0.046	0.755-0.934	0.569	81%	75%
Co	0.810	0.055	0.703-0.917	0.553	83%	80%
Cd	0.936	0.033	0.871-1.000	1.447	89%	95%
Pb	0.888	0.036	0.816-0.959	17.193	81%	80%

AUC: Area under curve, SE: Standard error

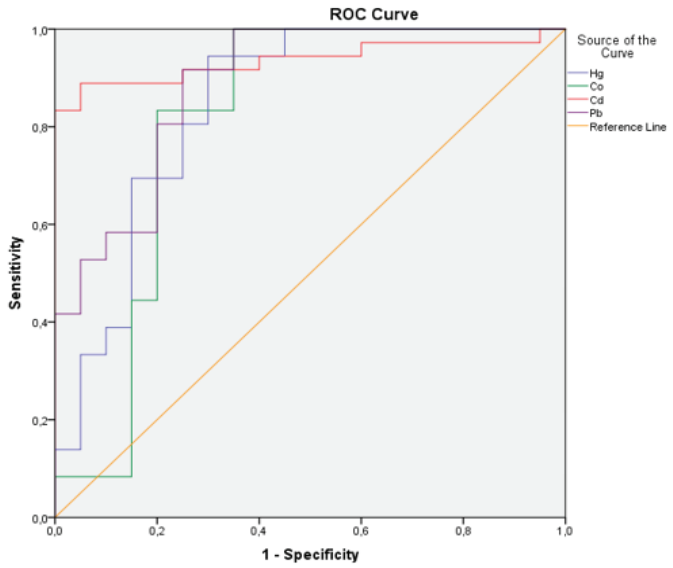
Demographic data and heavy metal levels of NTD and control group are shown in Table 3. When the NTD Type 1 and NTD Type 2 groups were compared with the control group separately, Hg, Co, Cd and Pb levels in both groups were statistically higher than the control group (p value; p = 0.037, p = 0.019, p <0.001, respectively). p <0.001, p <0.001, p <0.001). When the NTD Type 1 and NTD Type 2 groups were compared in terms of heavy metal levels, Co, Cd, Pb, Mn and As levels were not statistically different between the two groups (p = 1.000, p = 1.000, p = .974, p = .974). (p = 180). However, Hg levels were statistically different in all three groups (p values; p <0.001, p = .021, p <0.001, respectively). Hg level was highest in NTD Type 1 group and lowest in control group. The results of the comparison of the control group, NTD Type 1 and NTD Type 2 groups in terms of heavy metal levels are shown in Table 4.

In the study, we found a positive correlation between Hg vs Co, Hg vs Cd, Hg vs Pb, Co vs Cd, Co vs Pb, Cd vs Pb and As vs Mn. The correlation relationship between heavy metals is summarized in Table 6. ROC analysis performed to determine the Hg, Co, Cd and Pb cut-off values which were found statistically higher in the NTD group than the Control group was shown in Figure 1. The specificity and sensitivity values of the cut-off values are shown in Table 5.

Table 6. The relationship among heavy metals

	r	p
Hg vs Co	0.298	**0.009**
Hg vs Cd	0.473	**<0.001**
Hg vs As	0.189	.102
Hg vs Mn	0.037	.754
Hg vs Pb	0.429	**<0.001**
Co vs Cd	0.311	**0.006**
Co vs As	0.081	.488
Co vs Mn	0.104	.369
Co vs Pb	0.240	**0.037**
Cd vs As	-0.044	.703
Cd vs Mn	0.000	1.000
Cd vs Pb	0.548	**<0.001**
As vs Mn	0.560	**<0.001**
As vs Pb	-0.192	.097
Mn vs Pb	0.025	.828

Hg: mercury, Co: cobalt, Cd: cadmium, Pb: lead As: arsenic, Mn: manganese



Hg: mercury, Co: cobalt, Cd: cadmium, Pb: lead As: arsenic, Mn: manganese

Figure 1. Receiver operator characteristic (ROC) curve analysis for Hg, Co, Cd and Pb

DISCUSSION

How toxic substances pass through the placental barrier and affect fetal development depends on maternal serum level, gestational week, and exposure time. The maternal blood level of toxic substances may also affect the severity of the occured fetal anomaly (16). In our current study, we compared pregnant women with NTD and pregnant women in the control group in terms of heavy metal levels. In addition, we compared statistically highly detected heavy metals in NTD Type 1 and NTD Type 2 groups. Thus, we aimed to investigate whether there is a relationship between the concentration of highly detected heavy metals and the severity of NTD anomaly. In our study, Hg, Co, Cd and Pb concentrations in the NTD group were statistically higher than the control group. When we examined the literature, we found that there was no consensus on this issue. While some studies reported that a group of heavy metals were associated with NTD (11), another studies reported the opposite result (17,18). In experimental animal studies conducted to evaluate the effect of Hg exposure in offspring during pregnancy, it has been observed that Hg may trigger NTD development or columna vertebral anomalies (19,20). In human studies, Jin et al reported in their study that the placental methyl mercury level in the group with NTD was higher than the control group (21). In another study similar to our study, Ozel et al. compared plasma Hg levels of pregnant women with NTD and control group and they reported that Hg levels were statistically similar between the two groups (18). In our current study, Hg level was statistically higher in NTD group.

It has been shown in studies that another heavy metal Cd accumulates in the placenta, impairs placental function

and consequently leads to fetal development retardation. In the experimental study of Zhang et al., pregnant rats were injected intraperitoenal Cd on the 8th day of pregnancy. They reported that extradactily, tail deformity and NTD frequency were increased in the group injected with CD (22). In addition, Webster et al in their experimental study reported that when pregnant rats were given intraperitoneal Cd early in embryological development, the incidence of NTD increased in progeny (23).

In our study, Cd was statistically higher in NTD group according to the control group. We think that this high Cd value contributes to the formation of fetal NTD.

Although Co is widely used in industry, it is also frequently used in mobile phone batteries that people are in contact with, dental prosthesis and bone prosthesis in the field of health (24-26).

Many studies have shown that Co can cause neurological symptoms and diseases in humans. However, toxicity mechanisms are still not known clearly (25-27). Co has the capacity to produce reactive oxygen species (ROS), which are extremely harmful to DNA and other biomolecules. These ROS can cause DNA breaks in cross-links (28-30). ROS and DNA damage negatively affect the development of CNS, as well as the development of many organs. Also, various studies have shown that Co nanoparticles can migrate along neuron axons and dendrites and eventually cause neurotoxic effects (31). In a recent study, Zheng et al reported that neurotoxicity was higher in the rat group they gave intraperitoneal Co (32). Although the effect mechanism of Co on NTD development is not known clearly, we found Co level statistically higher in NTD group than Control group in our study. With this result, we think that high maternal Co level contributes to NTD development.

In our study, the other heavy metal was Pb which we found statistically high in NTD group. Pb has become more important as a toxic agent in occupational poisoning (33). In addition, there have been recent studies in both experimental studies and human epidemiological studies that reported that lead was also toxic on the offspring (34, 35). Lead exerts its teratogenic effect on offspring by disrupting the metabolism of trace elements such as folic acid and zinc (36).

In the ROC analysis of Hg, Co, Cb, Pb values that were found statistically higher in the NTD group than the control group, minimum sensitivity was 81%, minimum specificity was 75%, maximum sensitivity was 89% and maximum specificity was 95% for these four parameters's specifited cut-off values. With these results, we think that high Hg, Co, Cb, Pb can be caused to NTD. In another study similar to our study, Özel et al. found 76.2% sensitivity and 67% specificity for the cut-off values they specified for Pb. In addition, in this study, they reported the sensitivity as 76.2% and the specificity as 62% for the cut-off values they specified for Mn (17). In our study, we found that more numbers heavy metals may etiological reason for NTD when compared with this mentioned study. In our study, we found a positive correlation between Hg vs Co, Hg vs Cd, Hg vs Pb, Co vs Cd, Co vs Pb, Cd vs Pb and As vs Mn. This result denoted the conclusion that more than one heavy metal can have a synergistic effect for NTD development. New studies with the combination of these heavy metals will be useful in explaining the role of heavy metals in NTD development.

In the NTD group, we compared the higher concentrations of heavy metals between NTD Type 1 and Type 2 groups. Cd, Co and Pb levels were statistically similar between the two groups. However, Hg level in NTD Type 1 group was statistically higher than NTD Type 2 group. Anencephaly, which constitutes the NTD Type 1 group, is the heaviest type of NTD. Encephalocel, meningocele and meningomyelocele, which make up the NTD Type 2 group, are the lighter type (4). According to this result, we think that as Hg level increases, the severity of NTD increases. In their study, Lei et al found that the level of Hg differs from NTD types similar to our study (37). However, in their study, Lei et al. reported that Hg level was higher in the spina bifida group than in the anencephali group. In their epidemiological study, Bound et al investigated the reason for the difference in NTD incidence in adjacent regions. They found that the population in the regions with higher NTD had higher lead levels in drinking water. They reported that drinking water lead level was at the highest concentration, especially in the region where anencephali type NTD was more numerous (38).

The limitations and strengths of study

The incidence of NTD in the community is 0.1-0.6%. For this reason, the number of cases is limited in NTD related prospective studies. The low number of cases in our study was an important limitation of our study (2). Congenital abnormalities such as NTD show multifactorial inheritance. For this reason, it is difficult to investigate a single etiological cause (9). It is difficult to minimize other factors in studies comparing a single environmental factor while investigating the etiology of NTD. In our study, the professional status of the pregnant women, their places of residence and their eating habits were not known. Duration of exposure to toxic agents in NTD development is also an important factor for the development of anomaly (16). It was not known when the heavy metal loads of NTD and control group pregnant women increased. For this reason, we could not calculate the exposure times of pregnant women in the NTD group to heavy metals. Along with environmental factors, genetic factors are also blamed for NTD development (11,12). But in our study, genetic analysis of NTD group's fetus and Control group's new borns were not performed. This was another limitation of our study.

The strengths of our study are; the strongest aspect of our study is that it is a prospective case-controlled study. All cases in the study were selected from the same region, therefore ethnic and geographical factors affecting NTD incidence were minimized.

CONCLUSION

There is no clear information about which heavy metals cause specific fetal anomalies. We think that high maternal plasma Hg, Cd, Co and Pb concentrations may pose a risk for NTD development. Especially as plasma Hg concentration increases, the severity of NTD anomaly may increase. Serum heavy metal concentration analysis may be recommended before considering pregnancy in women who work in the risky profession or who are at risk in terms of exposure to heavy metals. Studies with large case series will help to explain the effects of heavy metals on pregnancy and offspring more clearly.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.

Talip Karacor ORCID: 0000-0003-1349-1569
Pinar Kirici ORCID:0000-0001-7616-4181
Mehmet Can Nacar ORCID: 0000-0001-5463-4197
Mehmet Bulbul ORCID:0000-0001-5695-2586
Muhittin Onderci ORCID: 0000-0002-8100-0242
Nurullah Peker ORCID: 0000-0002-3285-9990

REFERENCES

1. Wang ZP, Shang XX, Zhao ZT. Low maternal vitamin B(12) is a risk factor for neural tube defects: a metaanalysis. J Matern Fetal Neonatal Med 2012;25:389-94.
2. Gu X, Lin LM, Zheng XY, et al. High prevalence of NTDs in Shanxi Province: a combined epidemiological approach. Birth Defects Res A Clin Mol Teratol 2007;79:702-7.
3. Cherian A, Seena S, Bullock RK, et al. Incidence of neural tube defects in the least-developed area of India: a population-based study. The Lancet 2005;366:930-1.
4. Padmanabhan R. Etiology, pathogenesis and prevention of neural tube defects. Congenit Anom 2006;46:55-67.
5. Rajab A, Vaishnav A, Freeman NV, et al. Neural tube defects and congenital hydrocephalus in the Sultanate of Oman. J Tropical Pediatrics 1998;44:300-3.
6. Zaganjor I, Sekkarie A, Tsang BL, et al. Describing the prevalence of neural tube defects worldwide: a systematic literature review. PloS one 2016;11:0151586.
7. Heseker HB, Mason JB, Selhub J, et al. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. Br J Nutr 2009;102:173-80.
8. Bell KN, Oakley GP. Tracking the prevention of folic acid–preventable spina bifida and anencephaly. Birth Defect Res A 2006;76:654-7.
9. Demir N, Başaranoğlu M, Huyut Z, et al. The relationship between mother and infant plasma trace element and heavy metal levels and the risk of neural tube defect in infants. J Matern Fetal Neonatal Med 2019;32:1433-40.
10. Liu J, Jin L, Zhang L, et al. Placental concentrations of manganese and the risk of fetal neural tube defects. Journal of Trace Elements in Medicine and Biology 2013;27:322-5.
11. Detrait ER, George TM, Etchevers HC, et al. Human neural tube defects: developmental biology, epidemiology, and genetics. Neurotoxicology and teratology 2005;27:515-24.
12. Wertelecki W, Yevtushok L, Kuznietsov I, et al. Chornobyl, radiation, neural tube defects, and microcephaly. European journal of medical genetics. 2018;61:556-63.
13. Brender JD, Suarez L, Felkner M, et al. Maternal exposure to arsenic, cadmium, lead, and mercury and neural tube defects in offspring. Environ Res 2006;101:132-9.
14. Wang B, Zhu Y, Yan L, et al. Association of maternal chronic arsenic exposure with the risk of neural tube defects in Northern China. Environment international. 2019;126:222-7.
15. Özdabak HN, Karaoğlanoğlu S, Akgül N, et al. The effects of amalgam restorations on plasma mercury levels and total antioxidant activity. Archives of oral biology 2008;53:1101-6.
16. Hamlin HJ, Guillette LJ. Embryos as targets of endocrine disrupting contaminants in wildlife. Birth Defects Res C Embryo Today 2011;93:19-33.
17. Özel Ş, Ozyer S, Aykut O, et al. Maternal second trimester blood levels of selected heavy metals in pregnancies complicated with neural tube defects. J Matern Fetal Neonatal Med 2019;32:2547-53.
18. Spyker JM, Smithberg M. Effects of methylmercury on prenatal development in mice. Teratology 1972;5:181-90.
19. Hassan SA, Moussa EA, Abbott LC. The effect of methylmercury exposure on early central nervous system development in the zebrafish (Danio rerio) embryo. Journal of Applied Toxicology 2012;32:707-13.
20. Jin L, Liu M, Zhang L et al. Exposure of methyl mercury in utero and the risk of neural tube defects in a Chinese population. Reproductive Toxicology 2016;61:131-5.
21. Zhang GB, Wang H, Hu J et al. Cadmium-induced neural tube defects and fetal growth restriction: Association with disturbance of placental folate transport. Toxicology and Applied Pharmacology 2016;306:79-85.
22. Webster WS, Messerle K. Changes in the mouse neuroepithelium associated with cadmium-induced neural tube defects. Teratology 1980;21:79-88.
23. Jiang H, Liu F, Yang H et al. Effects of cobalt nanoparticles on human T cells in vitro. Biological Trace Element Research 2012;146:23-9.

24. Olivieri G, Hess C, Savaskan E et al. Melatonin protects SHSY5Y neuroblastoma cells from cobalt-induced oxidative stress, neurotoxicity and increased β-amyloid secretion. Journal of pineal research. 2001;31:320-5.
25. Oldenburg M, Wegner R, Baur X. Severe cobalt intoxication due to prosthesis wear in repeated total hip arthroplasty. The J Arthroplasty 2009;24:825-15.
26. Jiang H, Liu F, Yang H et al. Effects of cobalt nanoparticles on human T cells in vitro. Biol Trace Elem Res 2012;146:23-9.
27. Jordan CM, Whitman RD, Harbut M. Memory deficits and industrial toxicant exposure: a comparative study of hard metal, solvent and asbestos workers. Int. J. Neurosci 1997;90:113-28.
28. Karovic O, Tonazzini I, Rebola N, et al. Toxic effects of cobalt in primary cultures of mouse astrocytes. Similarities with hypoxia and role of HIF-1alpha. Biochem. Pharmacol 2007;73:694-708.
29. Yang SJ, Pyen J, Lee I, et al. Cobalt chloride-induced apoptosis and extracellular signal-regulated protein kinase 1/2 activation in rat C6 glioma cells. J Biochem Mol Biol 2004;37:480-6.
30. Oberdörster G. Oberdörster. E. Oberdörster. J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113:823-39.
31. Zheng F, Luo Z, Zheng C et al. Comparison of the neurotoxicity associated with cobalt nanoparticles and cobalt chloride in Wistar rats. Toxicol Appl Pharmacol 2019;369:90-9.
32. Tang N, Zhu ZQ. Adverse reproductive effects in female workers of lead battery plants. International J Occupational Med and Environmental Health 2003;16:359-61.
33. Dawson EB, Evans DR, Harris WA, et al. Amniotic fluid B12, calcium, and lead levels associated with neural tube defects. American J Perinatology 1999;16:373-8.
34. Mousa AM, Al-Fadhli AS, Rao MS, et al. Gestational lead exposure induces developmental abnormalities and up-regulates apoptosis of fetal cerebellar cells in rats. Drug and chemical toxicology 2015;38:73-83.
35. Vinceti M, Rovesti S, Bergomi M, et al. Risk of birth defects in a population exposed to environmental lead pollution. Science of the Total Environment 2001;278:23-30.
36. Jin L, Zhang L, Li Z, et al. Placental concentrations of mercury, lead, cadmium, and arsenic and the risk of neural tube defects in a Chinese population. Reproductive toxicology 2013;35:25-31.
37. Bound JP, Harvey PW, Francis BJ, et al. Involvement of deprivation and environmental lead in neural tube defects: a matched case-control study. Archives Of Disease In Childhood 1997;76:107-12.