Levels of heavy metals in pregnant women with feta central nervous system anomalies using ICP-OES

Safa Y Abdo, Inga Zinicovscaia, Yushin N. and Mostafa Seleem

Presented by

Safa Y Abdo

Introduction

- Central nervous system (CNS) is one of the most frequent sites for diagnosed congenital abnormalities (10 per 1000 live births).
- Ultrasound screening for fetal brain malformations is usually performed at 19–22 weeks' gestation (Ceausescu et al., 2018)

Why?

- Environmental pollution and industrialization expose humans to heavy metals in every period of their lives
- Exposure to heavy metals during pregnancy carries a great risk to the mother and the fetus.

Heavy metals effects

- Heavy metals like cadmium (Cd), lead (Pb), and mercury (Hg) have been found to affect reproductive outcomes ***(Flora S. J. and Agrawal, 2017)***.
- In animal experiments, it was determined that exposure to As, Hg, Pb, and Cd during the prenatal period could result in the development of neural tube defect (NTD) which is one of the most common types of central nervous system anomalies (Chow et al,2008).
- These heavy metals have also been suggested as a potential risk in the development of NTD in clinical trials (Jin et al,2013, Carrillo-Ponce et al,2004)).

Why hair samples?

- Hair sampling is a noninvasive and accessible technique that can be used to monitor internally accumulated levels of metals (Pereira et al., 2004; Wang et al., 2009).
- Hair sections can be used to indicate population exposure characteristics during specific time windows by assuming a consistent growth rate of hair (average 1 cm per month) (Srinivas et al., 2001).

Aim

- This study aimed to assess the possible association between heavy metals and fetal central nervous system anomalies

Procedure

Study subjects and groups: This study is an observational case-control study

- It included 40 pregnant women with gestational age (18- 40week) who were divided into two groups (each of 20 participants) as follows:
  - **Group A (control group):**
    - Included pregnant women with normal fetal anatomy scan
  - **Group B (study group):**
    - Included pregnant women with sonographically confirmed fetal central nervous system anomalies.

Patients consent:

- A written informed consent was obtained from all participants before inclusion in the study, explaining the value of the study, plus the procedures that were commenced.

Study Setting and duration:

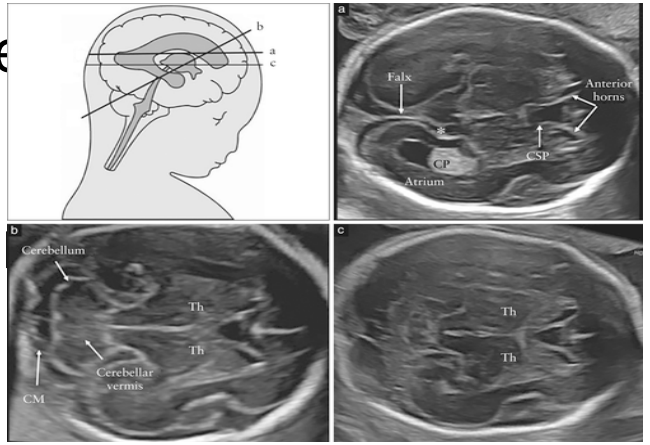
- The study was performed starting from March 2021 till October 2022
- At Obstetrics and Gynecology Department of Kasr Al-Ainy Hospitals , Cairo University, Egypt.
- Sample preparation and measurements were conducted at Frank Laboratory, Joint institute for nuclear research, Dubna, Russia.

Exclusion criteria:

1. Pregnant women with fetal central nervous system anomalies of known genetic background , positive consanguinity, women over 40 years or with history of ionizing radiation in the first two trimesters of pregnancy, intrauterine infections, drugs and other chemicals which are known to cause fetal congenital anomalies.
2. Diabetes Miletus.

Methodology

- **For all women, the followin done :**
  - Complete history taking including:
    - Demographic data (age, Parity …etc).
    - General medical, and obstetric history
    - Occupation , type of drinking water (tap water or filter) and type of kitchen utensil (aluminum , ceramic ,tefal).
    - Address, house brick and the prese
  - Clinical examination
  - Obstetric ultrasound (trans-abdo



Hair samples:

- At least 4 gm hair samples from all the women were collected using stainless steel scissors, and were cut from the back of the head, as near as possible to the scalp.
- We observed that hair samples of all subjects were not dyed with naked eyes.
- The hair samples were sealed separately in labeled polyethylene zip-lock bags, and transferred to Frank laboratory for analysis.

Preparation and analysis of hair samples

- 0.2 g of sample were mixed with 5 mL of nitric acid and 2 ml of hydrogen peroxide.
- The digestion was performed at 180 ºC in Mars 6 microwave digestion system (CEM, USA).
- After cooling, the digested samples were quantitatively transferred into 10 ml flasks and made up to the volume with deionized water.
- The content of nine elements (Al, Cd, Co, Cu, Fe, Mn, Pb, Se, Zn) was determined using ICP-OES Plasma Quant 9000 Elite (Analytik Jena, Germany)

Results

Analysis of the epidemiologic data in the two study groups.

	Groups				Test of significance	P value
	Cases group (n = 20)		Control group (n = 20)			
Age (Years)	26.35 ± 6.59		28.85 ± 6.78		t = - 1.183	0.244
Weight (kg)	78.10 ± 8.28		76 ± 9.12		t = 0.763	0.450
Height (cm)	167.60 ± 5.07		169 ± 7.71		t = - 0.678	0.502
BMI (Kg/m2)	27.88 ± 3.39		26.71 ± 3.70		t = 1.039	0.305
Occupation						
House wife	18	90 %	12	60 %	MC = 5.533	0.134
Nurse	1	5 %	2	10 %		
Students	0	0 %	3	15 %		
Teacher	1	5 %	3	15 %		

Analysis of the demographic data in the two study groups.

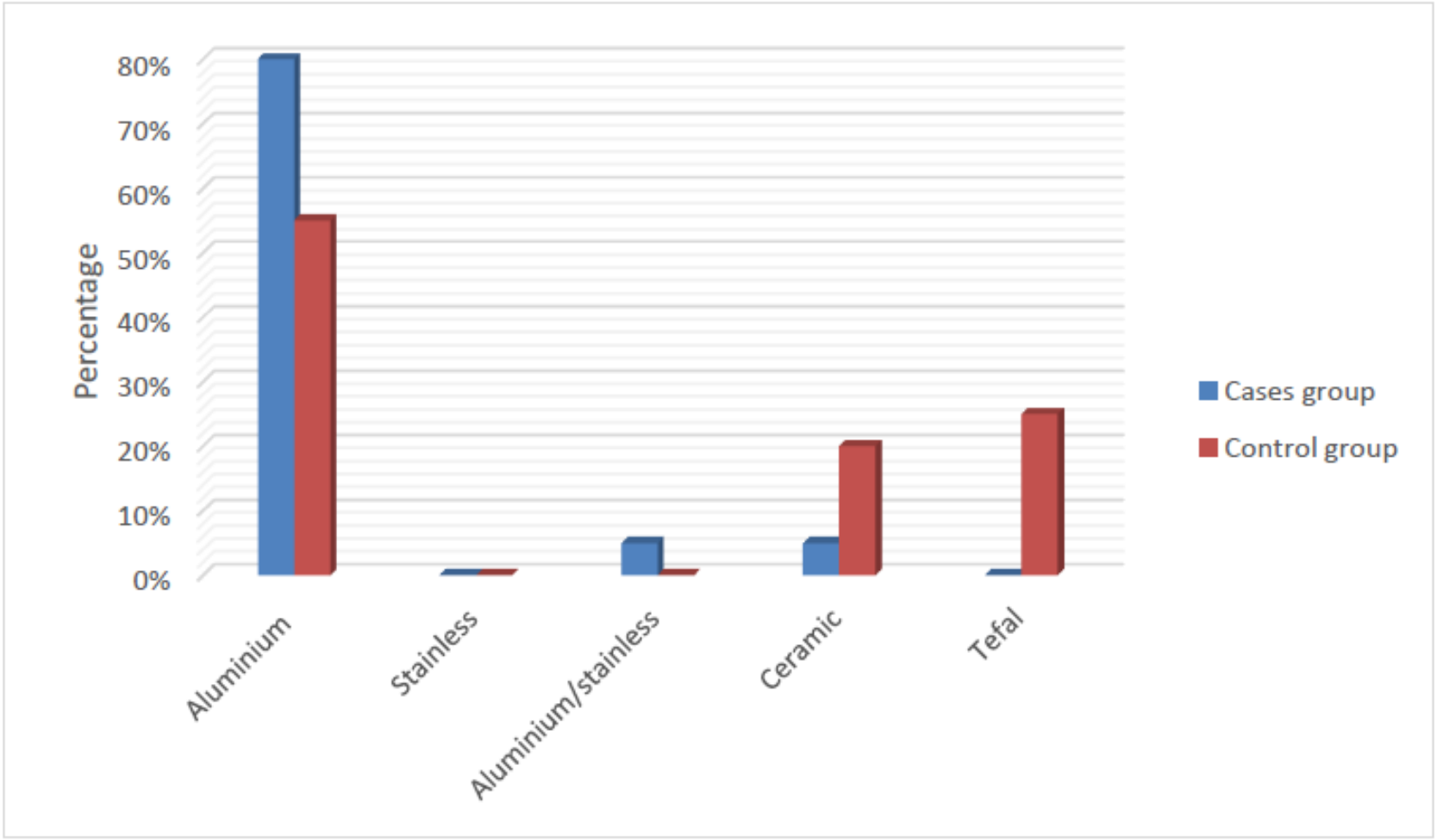
	Groups		Test of significance	P value
	Cases group (n = 20)	Control group (n = 20)		
Gravidity	2 (1-7)	3 (1-5)	z = - 0.138	0.890
Parity	1 (0-4)	2 (0-4)	z = - 0.459	0.646
Abortion	0 (0-3)	0 (0-1)	z = - 1.227	0.220
GA (Weeks)	26.90 ± 5.41	24.70 ± 4.04	t = 1.457	0.153
Method of contraception				
No	6 30 %	5 25 %	MC = 4.485	0.214
COC	5 25 %	1 5 %		
IUDs	9 45 %	13 65 %		
POPs	0 0 %	1 5 %		
Mode of previous deliveries				
Nulli para	6 30 %	5 25 %	$\chi 2 = 1.798$	0.407
Normal vaginal deliveries	7 35 %	11 55 %		
Caesarean section	7 35 %	4 20 %		

Analysis of the medical, past and family histor the two study groups.

	Groups				Test of significance	P value
	Cases group (n = 20)		Control group (n = 20)			
Smoking						
No	17	85 %	16	80 %	FET = 0.173	0.677
Passive	3	15 %	4	20 %		
Hypertension						
No	19	95 %	17	85 %	FET = 1.111	0.292
Yes	1	5 %	3	15 %		
DM						
No	20	100 %	20	100 %	———	——
Yes	0	0 %	0	0 %		
No	20	100 %	16	80 %	MC = 4.444	0.108
Appendicectomy	0	0 %	3	15 %		
Tonsillectomy	0	0 %	1	5 %		
Family history						
Negative	20	100 %	20	100 %	———	———
Positive	0	0 %	0	0 %		

Analysis of the environmental factors in the two study groups.

	Groups				Test of significance	P value
	Cases group (n = 20)		Control group (n = 20)			
House brick type						
Mud brick	2	10 %	0	0 %	FET = 2.105	0.147
Red brick	18	90 %	20	100 %		
Sanitation						
No	3	15 %	0	0 %	FET = 3.243	0.072
Yes	17	85 %	20	100 %		
Drinking water						
Filter	5	25 %	10	50 %	MC = 3.333	0.189
Tap water	14	70 %	10	50 %		
Filter/tap water	1	5 %	0	0 %		
Utensils						
Aluminium	16	80 %	11	55 %	MC = 10.726	0.030*
Stainless	2	10 %	0	0 %		
Aluminium/stainless	1	5 %	0	0 %		
Ceramic	1	5%	4	20%		
Tefal	0	0%	5	25%		



Utensils' material used in the two study groups

In the current study, the levels of Cd, Co and Se in the control group were 0.16±0.13 mg/kg, 0.26±0.16 mg/kg and 1.34±0.6 mg/kg respectively whereas in the cases group was 0.68±1.5, mg/kg, 0.63±0.9 mg/kg and 1.85±1.0 mg/kg respectively. The Cadmium, Cobalt and Selenium levels was statistically significantly higher in the cases group as compared to the control group

	Al (mg/kg)	Cd (mg/kg)	Co (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Pb (mg/kg)	Se (mg/kg)	Zn (mg/kg)
Control	337.26±323.1	0.16±0.13	0.26±0.16	66.76±69	91.84±66.0	4.42±2.41	5.40±3.3	1.342±0.6	272.714±237
Cases	385.8± 324.4	0.68±1.5	0.63±0.9	50.6±44.3	152.3±153.9	9.21±7.79	7.50±8.80	1.85±1.0	356.4±225.24
P-Value	0.16	0.001	0.001	0.93	0.27	0.11	0.09	0.04	0.07

We conducted ROC curve analysis to determine the cutoff level of each element with the highest sensitivity and specificity

sensitivity: ability to diagnose " True positives cases" patients

specificity : ability to exclude " true negatives cases" healthy pregnant

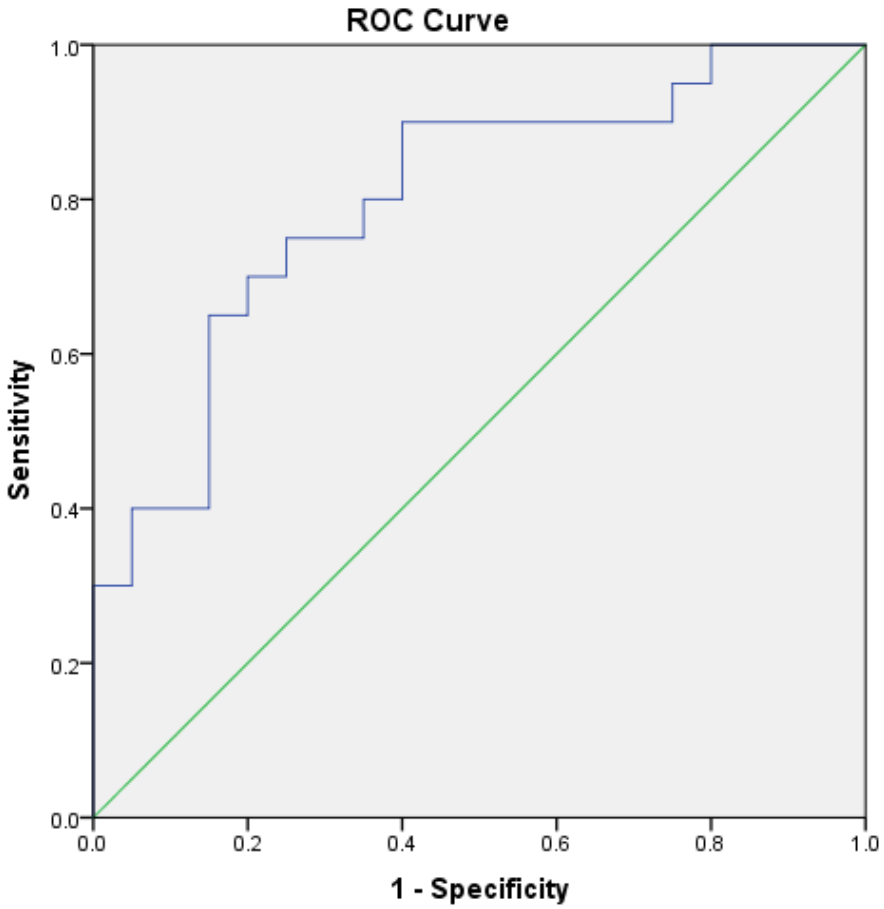
This table shows that:

1. The best cutoff point of cadmium was > 0.21 with 75% sensitivity, 70% specificity.
2. The best cutoff point of cobalt was > 0.32with 80% sensitivity, 70% specificity
3. The best cutoff point of Selenium was > 1.31 with 70% sensitivity, 70% specificity

	Al	Cd	Co	Cu	Fe	Mn	Pb	Se	Zn
AUC	0.63	0.80	0.80	0.51	0.61	0.65	0.66	0.69	0.67
Cut off point	>260.60	>0.21	>0.32	>22.74	>68.44	>3.70	>4.26	>1.31	>231.7
Sensitivity	70%	75%	80%	70%	70%	70%	80%	70%	65%
Specificity	60%	70%	70%	65%	45%	60%	60%	70%	60%

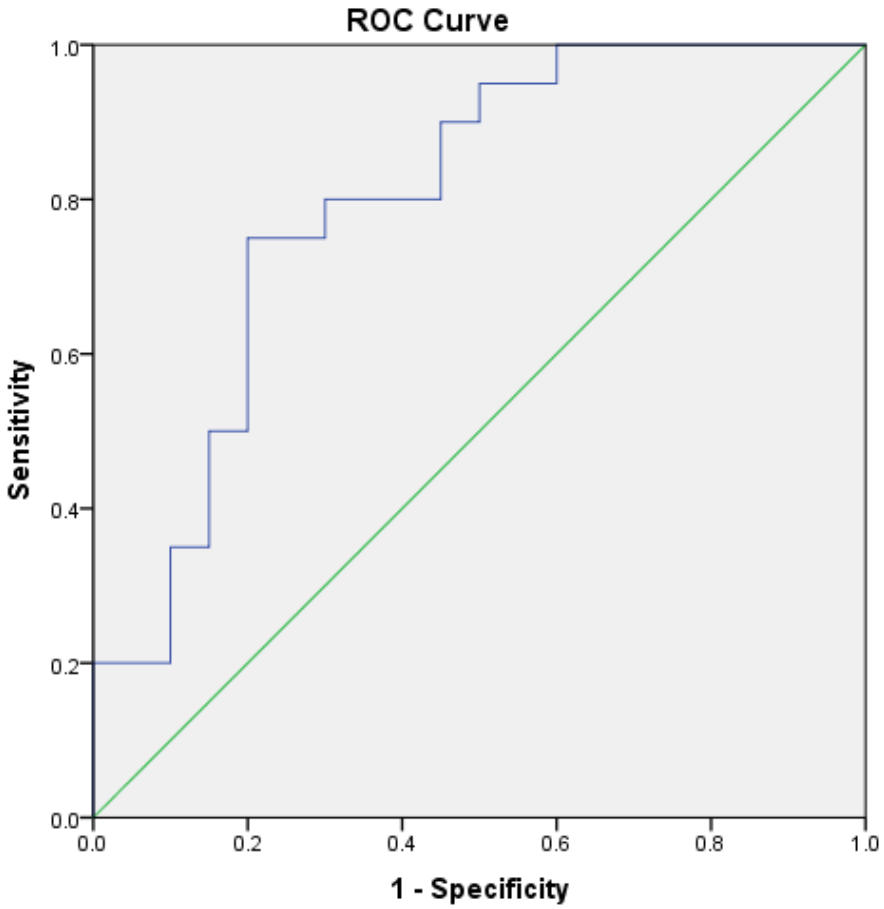
Cd

	Cd
AUC	0.80
Cut off point	<0.126
Sensitivity	90%
Specificity	60%



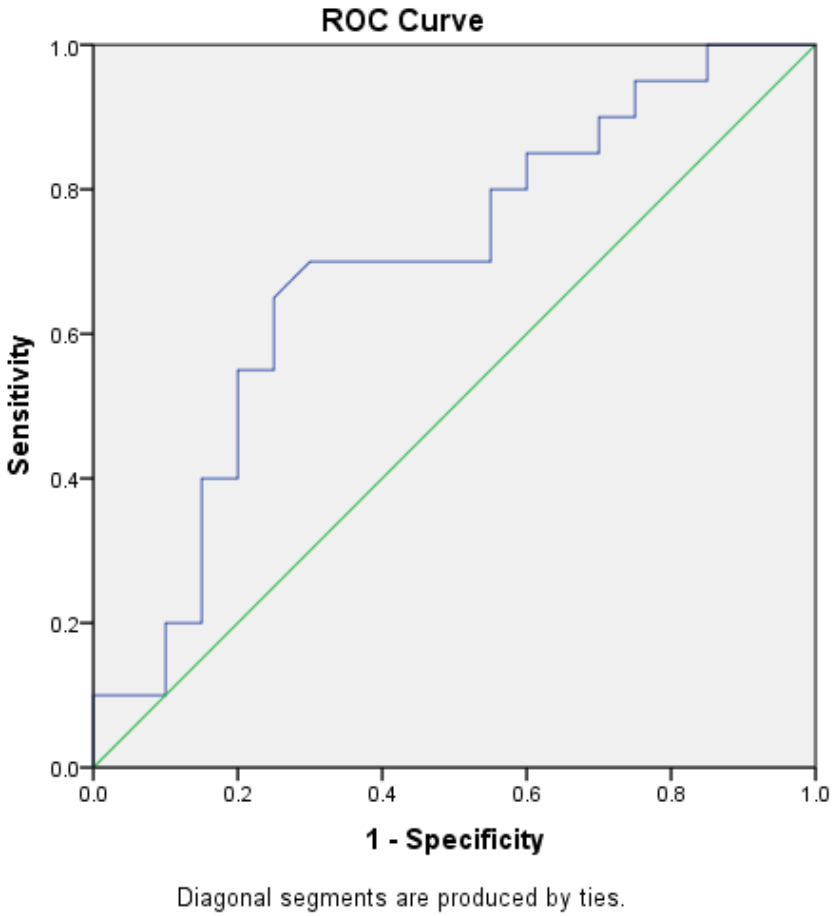
Co

	Co
AUC	0.80
Cut off point	<0.322
Sensitivity	80%
Specificity	70%



Se

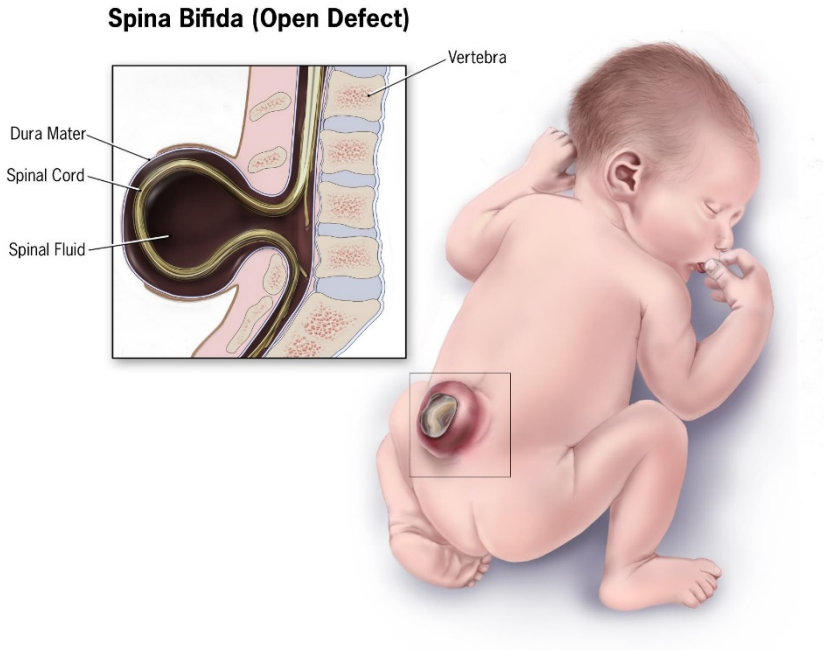
	Se
AUC	0.69
Cut off point	<1.31
Sensitivity	70%
Specificity	70%



Cd

- Our findings of Cd is in accordance with Liu et al. who reported that the median concentration of Cd in maternal serum was significantly higher in cases with Neural tube defects NTD cases (1.30 ng/g) than in controls (0.93 ng/g) (Liu M. et al., 2021).
- This also agreed with the results of a Turkish study reported that the levels of Cd was significantly higher in NTD cases than in control subjects, as detected either in maternal or infant plasma (Demir et al., 2019).

NTD



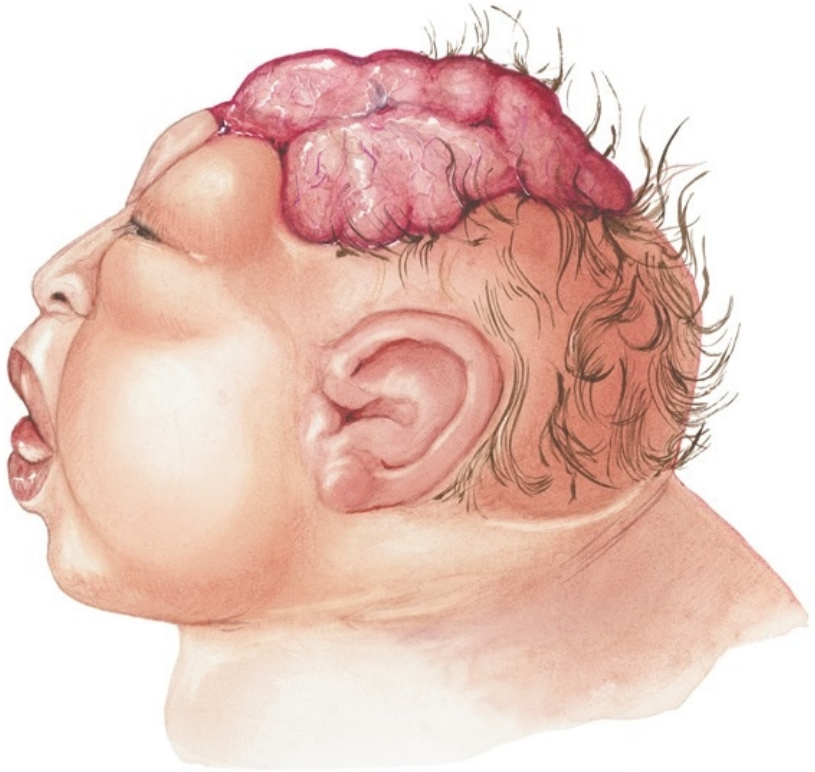
- Cd is a non-essential element which is toxic at very low doses, and it is non-biodegradable with a very long biological half-life ***(Genchi et al., 2020)***.
- Cadmium exerts toxic effects on the kidney, the skeletal and the respiratory systems, and is classified as a human carcinogen.
- Human exposure occurs mainly from consumption of contaminated food, active and passive inhalation of tobacco smoke, fossil fuel combustion, incineration of municipal waste (especially cadmium containing batteries and plastics), fertilizer, electronic waste and inhalation by workers in the non-ferrous metal industry ***(Aliyu et al., 2016).***

- Cadmium is used in cigarette paper to make the paper burn slower.
- These metals are either released into the air by tobacco smoke or are retained in the cigarette ash.
- According to (WHO 1992), a cigarette's tobacco contains about 0.5 - 2.0 μg of cadmium and about 10% of the cadmium content is inhaled when the cigarette is smoked.
- The non-smoker may passively inhale significant amount of cadmium along with inhaled tobacco smoke **(Organization, 2011)**
- Several animal studies have shown a considerable embryo fetotoxic activity of Cadmium compounds.

Co

- Cobalt are naturally found in most rocks, soil, water, plants, and animals, typically in small amounts.
- A biochemically important cobalt compound is vitamin B12 .
- Cobalt compounds are used as colorants in glass, ceramics, and paints, as catalysts, and as paint driers
- Co is utilized in the manufacture of metal alloys with high melting point, strength and resistance to oxidation, as fertilizers and in some dental amalgams.

- Few studies studied the association between Co toxicity and fetal anomalies.
- Yan et al, 2016 studied the association of essential trace metals in maternal hair with the risk of NTD in the Offspring found that Co concentration in the total NTDs group was higher than in the control group.
- In another study plasma Co levels were determined to be similar in NTD group and control group (Nihat et al.2017)



- For most people, food is the largest source of cobalt intake.
- Studies in animals suggest that intake high amounts of cobalt during pregnancy might affect the health of the developing fetus.

Se

- Selenium (Se), an essential micronutrient that maintains physiological functions (Janz, 2012).
- Selenium (Se) releases into water from agricultural soils and from storage sites for coal fly ash.
- The selenium content of grains and vegetables generally depends on the selenium content in the corresponding soils.

- The presence of selenium is related to human activities like burning fossil fuels and incineration of garbage, tires and paper.
- low plasma levels of trace elements Zn and Se were also found to be risk factors for NTD (Nihat et al.2017).
- However, several studies have shown that the effects of selenium on human health may be both beneficial and harmful with a very narrow safety range.

- To date, there are no reliable data on teratogenic effects of selenium.
- Animal experimental data are not conclusive: the selenium salts have been found to cross the placenta in hamsters (Willhite, Ferm, & Zeise, 1990) and rats (Bou-Resli, Mathew, Dashti, & AlZaid, 2002).
- However, in various animal species, including primates, the placenta seems to protect fetuses from selenium toxicity in case of maternal selenium poisoning (Hawkes et al., 1994).
- In mice, chronic exposure to selenium during pregnancy and lactation caused low birth weight and poor neonatal survival (Schroeder & Mitchener, 1971).
- In some species, high chronic dose levels of selenium have been associated with birth defects, (Ferm, Hanlon, Willhite, Choy, & Book, 1990; Ridgway & Karnofsky, 1952).

Conclusion

- Although our study can not prove the causal-effect of Cd, Co and Se on CNS anomalies of human fetuses, it highlights the possible association between them and fetal CNS anomalies.
- There is a need to assess heavy metals and trace elements levels in the hair of women who are welling to be pregnant and live in areas with possible environmental pollution.
- Our study should be considered as a **red flag** and should be followed by further studies.

Thank You

safaabdo@gstd.sci.cu.edu.eg