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Blood Cadmium Concentrations in Women with Ectopic Pregnancy

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Abstract The purpose of this study is to investigate the relationship between the blood level of cadmium and the occurrence of ectopic pregnancy. Forty-one (41) case patients with ectopic pregnancy and 41 uncomplicated intrauterine pregnant patients as controls were recruited. The concentrations of cadmium (Cd) were measured from blood samples using atomic absorption spectrometry. The cases and controls were similar in age, body mass index, and smoking habits. The median blood level of Cd was 0.32 μg/l (interquartile range [IQR] 0.00–0.71) in the women with ectopic pregnancies and 0.34 μg/l (IQR 0.09–0.59) in the controls. There was no significant association between blood cadmium levels and ectopic pregnancy.

Keywords Atomic absorption spectroscopy · Cadmium · Ectopic pregnancy · Heavy metals

Introduction

Ectopic pregnancy occurs when a fertilized oocyte implants outside the uterine cavity [1], and it has been estimated that 1–2% of pregnancies are ectopic. Despite advances in diagnosis and treatment, there have been no significant reductions in deaths from ectopic pregnancy, and it is responsible for 4–6% of all maternal deaths (0.50 deaths for every 100,000 live births) [2]. The etiology of ectopic pregnancy is undefined, but it has been well-demonstrated that some factors increase a woman's risk of developing an ectopic pregnancy. The main risk factors are a prior ectopic pregnancy and a previous *Chlamydia trachomatis* infection, which is the main cause of pelvic inflammatory disease [3, 4]. Other risk factors are maternal age, multiple sexual partners, a history of infertility, an induced conception cycle, current intrauterine device usage, and a previous tubal surgery [3, 5]. Moreover, epidemiological studies have shown that cigarette smoking is a major risk factor for tubal ectopic pregnancy [5, 6]. Both animal and human studies have demonstrated the adverse effect of smoking on the function of the fallopian tubes [7], but the underlying mechanism by which cigarette smoking leads to ectopic pregnancy remains unclear.

Cadmium (Cd) is extensively distributed in the environment. Human exposure to Cd is possible through a number of different sources including working in Cd-contaminated occupations (such as mining, smelting, and the manufacturing of batteries, plastic stabilizers, pigments, alloys, and fertilizers [8]), eating contaminated food (especially leafy vegetables, potatoes, seeds, grains, liver, kidney, and crustaceans [9]), and smoking cigarettes [10]. Populations living in urban areas have higher Cd levels than people living in rural areas [11].

Studies have demonstrated that Cd has multiple adverse effects on both male and female reproductive systems, including defective steroidogenesis, diminished semen quality, inhibited folliculogenesis and oocyte maturation, ovulation failure, defective implantation, spontaneous abortion, and birth defects [12]. Apart from occupational exposure, the main source of Cd in humans is inhalation through cigarette smoking [13]. Although smoking is the main preventable risk

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factor for ectopic pregnancy, there have been no studies on the relationship between Cd and ectopic pregnancy.

Current evidence advocates the hypothesis that ectopic pregnancy is caused by a combination of the retention of the embryo in the fallopian tube and an alteration in the tubal microenvironment, leading to early implantation within the fallopian tube [14]. The timing of oocyte pickup and embryo transport is critical because the embryos must reach the uterus during the window when implantation can occur. If the oocyte is not picked up by the fallopian tube or if the embryo moves too rapidly or too slowly through the oviduct, implantation may fail to occur or may be ectopic [7, 15]. The muscles of the fallopian tubes respond to sex steroids. Endogen estrogens stimulate muscle contraction, while progesterone relaxes muscles. Cd functions as a metallo-estrogen. The mechanistic regulatory pathway of Cd-induced effects in smooth muscle varies with the concentration of Cd or the type of smooth muscle (i.e., myometrium, ileum, aorta, or mesenteric artery), as well as among species [7, 16].

The aim of this study was to investigate the relationship between blood levels of Cd and the occurrence of ectopic pregnancy.

Materials and Methods

Study Population and Study Design

This case-control study was conducted between February 2014 and December 2015 at Malatya State Hospital in Malatya, Turkey; the study was approved by the Ethical Review Board at Malatya and was designed and carried out in accordance with the Declaration of Helsinki. All the subjects entered the study only after informed consent was obtained.

The diagnosis of ectopic pregnancy was made using combinations of serial measurements of serum β-human chorionic gonadotropin (hCG) and transvaginal ultrasonography [17]. We considered the women to have an ectopic pregnancy if they had a subnormal increase (< 50%, whether rising, falling, or plateauing) in the levels of serum β-hCG over 2 days. Then, we evaluated these women with transvaginal ultrasonography. If an ultrasound scan showed an empty uterus with an adnexal mass, such as a tubal ring or a gestational sac with a fetal pole, with or without a fetal heartbeat or an adnexal mass other than a simple cyst, we diagnosed ectopic pregnancy. We excluded women who had conceived by assisted reproductive technology procedures (n = 3) or with a history of recurrent ectopic pregnancy (n = 2). Consequently, a total of 41 women meeting the inclusion criteria were enrolled in this study.

The controls were selected from the same time period and urban area as the patients with ectopic pregnancies. The control group consisted of 41 women with uncomplicated intrauterine pregnancies at 10 weeks' gestation or less. All of the subjects were living in Malatya, Turkey, and had no history of occupational exposure to Cd. The information collected from each woman by means of a face-to-face interview included sociodemographic characteristics, smoking status, and reproductive and medical histories. In addition, the age and smoking status of the male partner was recorded.

Measurements of Metal Concentrations

From each patient, 10 ml of peripheral blood was drawn by venipuncture. We used thermally convective wet-acid digestion methods, which have been described previously by many investigators [18–20]. Following this method, a 1-ml blood sample was placed in a propylene tube and a 4:1 nitric acid (HNO_3):perchloric acid ($HClO_4$) mixture was added. The mixture was digested in a water bath at 80 °C for 2 h. The digestion was continued until the samples became colorless. The samples were then diluted to 10 ml with deionized water. Graphite furnace atomic absorption spectrometry with an atomic absorption spectrometer (PerkinElmer AAnalyst 800, PerkinElmer Inc., Waltham, MA, USA) was used to determine the blood concentrations of Cd. The detection limit was 0.01 μg/l for Cd. The correlation coefficient for the Cd calibration curve was 0.994. The laboratory was blinded to the subjects' data, and three replicates were performed per sample.

Statistical Analysis

The data were stored and analyzed using the Statistical Package for Social Science Software, release 11.0 (SPSS,

Table 1 Sociodemographic features and clinical characteristics of cases (women with ectopic pregnancies) and controls

Variables	Cases (n = 41)	Controls (n = 41)	p values
Age (years)	29.9 ± 4.8	28.6 ± 4.9	0.22
Male age (years)	34.0 ± 4.3	31.4 ± 4.7	0.10
Height (m^2)	163 ± 6.1	164 ± 5.5	0.64
Weight (kg)	68.2 ± 10.3	65.2 ± 8.4	0.35
Gravidity	2 (1–3)	2 (1–3)	0.64
Parity	1 (0–2)	1 (0–2)	0.36
Livebirths	1 (0–2)	1 (0–2)	0.38
Spontaneous abortions	1 (0–1)	0 (0–1)	0.008
Employed	13 (31.8%)	7 (17.1%)	0.12
Education (years of schooling)			
≤ 8	12 (29.3%)	14 (34.1%)	0.63
> 8	29 (70.7%)	27 (65.9%)	
Smoking			
Active	6 (14.6%)	4 (9.8%)	0.72
Passive	16 (39.0%)	16 (39.0%)	1.00
None	19 (46.4%)	21 (51.2%)	

Data shown as mean ± standard deviation or median (IQR)

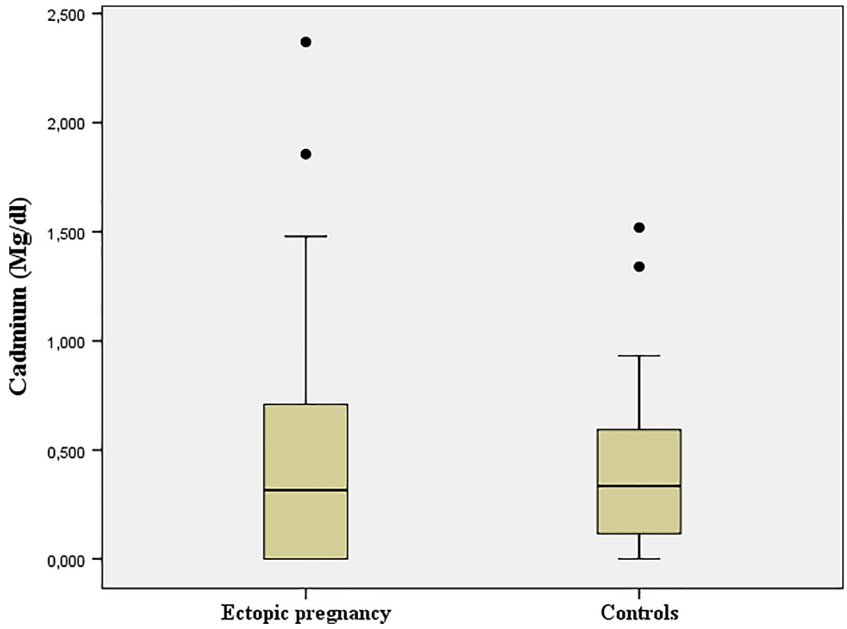

Fig. 1 Cadmium concentrations in the blood of cases (women with ectopic pregnancies) and controls

Inc., Chicago, IL, USA) for Windows package software. The Kolmogorov-Smirnov test was used to evaluate normality. Variables with a normal distribution were shown as the mean + standard deviation (SD), and variables with a skewed distribution were shown as the median and interquartile range (IQR). The groups were compared using Student's *t* test for parametric data and the Mann-Whitney *U* test for nonparametric data. The odds ratio (OR) and 95% confidence intervals (CI) were used describe the association between ectopic pregnancy and Cd. Multivariable modeling was employed to determine which characteristics were associated with ectopic pregnancies. A *p* value less than 0.05 was considered to be statistically significant.

Results

The sociodemographic features, clinical characteristics, and smoking habits of the women with ectopic pregnancies and of the controls are presented in Table 1. The cases and controls were similar in age and body mass index. The cases were more likely than the controls to have spontaneous abortions. Apart from this, the sociodemographic characteristics and smoking habits were similar in both groups. There was no statistically significant difference between the two groups in terms of the age of the male partner.

The median blood Cd concentration was 0.32 μg/l (IQR 0.00–0.71) in the women with ectopic pregnancies, compared with 0.34 μg/l (IQR 0.09–0.59) in the controls. There was no statistically significant difference between the cases and controls in the blood Cd levels (Fig. 1). After adjustment to a logistic model (adjusting for age, parity, spontaneous abortions, smoking, and blood Cd concentrations), there were no statistically significant associations between any variables and ectopic pregnancies (Table 2).

There was no statistically significant difference in the blood Cd levels of nonsmoking women in either group with regard to passive smoke exposure for the women with ectopic pregnancies and controls. The median blood Cd level was 0.33 μg/l (IQR 0.06–0.76) in nonsmoking women with ectopic pregnancies whose partners did smoke, compared with 0.39 μg/l (IQR 0.00–0.67) in nonsmoking women with ectopic pregnancies whose partners did not smoke ($p = 0.33$). The median blood Cd level was 0.35 μg/l (IQR 0.11–0.61) in the controls whose partners did smoke, compared with 0.37 μg/l (IQR 0.07–0.57) in the controls whose partners did not smoke ($p = 0.38$).

Discussion

In the present study, there was no significant association between blood Cd levels and ectopic pregnancy.

An important limitation of this study was its observational nature, because of which the causality cannot be inferred. In

Table 2 Risk factors for ectopic pregnancies by final logistic regression analysis

Variables	Adjusted OR	95% CI	*p* value
Maternal age	0.95	0.84–1.07	0.43
Spontaneous abortions	0.51	0.20–1.32	0.16
Cigarette smoking	1.28	0.25–6.51	0.76
Cadmium levels	0.88	0.26–2.97	0.84

OR odds ratio, *CI* confidence interval

The final logistic model included all the listed variables

addition, blood Cd reflects only recent Cd exposure; we did not evaluate Cd in urine, which is a marker of chronic exposure. On the other hand, to the authors' knowledge, this is the first study to investigate the existence of a relationship between the blood concentrations of Cd and ectopic pregnancy.

Epidemiological studies have demonstrated that tobacco smoking is associated with ectopic pregnancy [5, 6]. Cigarette smoke is a complex suspension that contains 4000 chemicals including nicotine, carbon monoxide, arsenic, lead, and Cd [21]. Cd is ubiquitous in the environment, and women have higher Cd levels than men, probably as a result of a higher gastrointestinal absorption of Cd in women with low-iron deposits or owing to hereditary factors [22]. Moreover, Cd accumulates more in pregnant women than nonpregnant women [23]. In this study, it seems that the women were probably exposed to heavy metals from air, water, soil, or vegetables [24–26].

Cd is a highly toxic metal ranked among the top 7 on the 2017 Agency for Toxic Substances and Disease Registry Priority List of Hazardous Substances [27]. It can act as an endocrine disrupting chemical, and studies have shown Cd-altered steroid hormone production in the ovaries [28, 29]. Cadmium does reach and collect in the follicular fluid of smokers [30], and Cd levels in the ovaries are higher in smokers than in nonsmokers [31]. Moreover, an in vitro study demonstrated that the exposure of oocyte cumulus complexes to Cd suppressed FSH-induced expansion of the cumulus cells and decreased the synthesis and accumulation of hyaluronic acid in the cumulus matrix [32]. These studies show that the matrix of the oocyte cumulus complex is also a target of Cd, and damage to the matrix can affect the pickup of complexes by the oviduct. However, the effects of Cd on the preimplantation embryo in the fallopian tube are not known.

In this study, we did not find any significant association between blood Cd levels and ectopic pregnancy. The effect of Cd on embryo transport through the oviduct and on ovarian progesterone secretion has been studied in an animal model [33]. In agreement with this study, those authors showed that Cd accumulates dose- and time-dependently in the oviducts, but Cd does not interfere with embryo transport through the oviduct [33]. In this study, we did not find any statistically significant difference regarding the blood Cd levels in non-smoking women in either group with regard to passive smoke exposure. In agreement with this study, a study by Willers et al. [34] found that the blood Cd levels of children were not affected by parental smoking. In contrast, another study showed that exposure to cigarette smoke via active and passive smoking increases blood Cd by an average of 0.01 μg% over the background [35]. However, apart from home exposure, the main sources of exposure to environmental cigarette smoke are worksites and public places [36].

In conclusion, this study showed that low-environmental levels of Cd exposure do not constitute a significant risk factor for ectopic pregnancies. Although smoking is a risk factor for ectopic pregnancy, we did not find any statistically significant difference in smoking habits between the two groups. Further research in a large series is needed to confirm these results and to elucidate the mechanism of the interaction between heavy metals and ectopic pregnancy.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflicts of interest.

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