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Research Article / Araştırma Makalesi

The Role of Serum Cadmium Levels in the Etiology of Premature Ovarian Failure: A Case-Control Study

Prematürovaryan Yetmezlik Etyolojisinde Serum Kadmiyum Düzeyinin Rolü:Vaka Kontrol Çalışması

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Abstract

Premature ovarian failure (POF) is a clinical syndrome known to affect approximately 1% of women of reproductive age. A number of genetic, autoimmune, metabolic, infectious and environmental causes have been identified in the etiology of POF. Cadmium is a toxic metal which can accumulate in ovaries and causes a significant decrease in the binding of gonadotropins that regulate steroidogenic enzyme activity in granulosa cells. We aimed to investigate the role of cadmium in the etiology of POF. Thirty-five patients with POF were enrolled in group 1 which was the study group. Two control groups were formed from healthy participants. Thirty-fiveparticipants who were age-matched young women with regular menstruation were included in group 2.Thirty-fivehealthy postmenopausal women were included in group 3. Patients' demographic data were recorded. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4) and cadmium levels were measured. There was a statistically significant difference between the groups for gravida, parity and living children ($p<0.001$), but no difference was found for abortion ($p=0.430$). While there was a statistically significant difference between the groups for serum FSH, LH and E2 levels ($p<0.001$), no statistically significant difference was found for T3, T4 and TSH. Serum cadmium levels were found to be statistically significantly decreased in group 1 compared to g roups 2 and 3 ($p<0.001$). This study revealed that cadmium had no direct effect on the development of POF, but it should be kept in mind that the synergistic and antagonistic effects of metals may affect this result.

Keywords: Premature ovarian failure, Cadmium, Heavy metal exposure, Infertility

Özet

Prematüre over yetmezliği (POF), üreme çağındaki kadınların yaklaşık %1'ini etkilediği bir klinik sendromdur. POF etiyolojisinde bir takım genetik, otoimmün, metabolik, enfeksiyöz ve çevresel nedenler tanımlanmıştır. Kadmiyum, yumurtalıklarda birikebilen ve granüloza hücrelerinde steroidojenik enzim aktivitesini düzenleyen gonadotropinlerin bağlanmasında önemli bir azalmaya neden olabilen toksik bir metaldir. POF etiyolojisinde kadmiyumun rolünü araştırmayı amaçladık. Çalışma grubu olan grup 1'e POF'lu 35 hasta alındı. Katılımcılarla iki sağlıklı kontrol grubu oluşturuldu. Grup 2'ye, kendi yaşlarında düzenli adet gören genç kadınlardan oluşan otuz beş katılımcı, grup 3'e menopoz sonrası sağlıklı otuz beş kadın dahil edildi. Hastaların demografik verileri kaydedildi. Serum folikül uyarıcı hormon (FSH), luteinize edici hormon (LH), östradiol (E2), tiroid uyarıcı hormon (TSH), triiyodotironin (T3), tiroksin (T4) ve kadmiyum düzeyleri ölçüldü. Gruplar arasında gravida, parite ve yaşayan çocuklar açısından istatistiksel olarak anlamlı fark varken ($p<0,001$) abortus için fark bulunmadı ($p=0,430$). Serum FSH, LH ve E2 düzeyleri açısından gruplar arasında istatistiksel olarak anlamlı fark varken ($p<0,001$), T3, T4 ve TSH için istatistiksel olarak anlamlı fark bulunmadı. Serum kadmiyum düzeyleri grup 1'de grup 2 ve 3'e göre istatistiksel olarak anlamlı düzeyde azalmıştı ($p<0,001$). Bu çalışma kadmiyum düzeyinin POF gelişimi üzerinde direkt etkisi olmadığını ortaya koymuştur ancak metallerin sinerjistik ve antagonistik etkilerinin bu sonucu etkileyebileceği de akılda tutulmalıdır.

Anahtar Kelimeler: Prematüre overyan yetmezlik, Kadmiyum, Ağır metal maruziyeti, İnfertilite

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1. Introduction

Premature ovarian failure (POF) affects approximately 1% of women of reproductive age. It is a clinical syndrome characterized by cessation of ovarian function before age 40, menstrual irregularity, high gonadotropins, and low estrogen levels (1). Although the most notableclinical effect of POF on women's health is subfertility, many problems such as osteoporosis, increased risk of cardiovascular disease, and sexual dysfunction may occur in long term due to estrogen deficiency. A number of genetic, autoimmune, metabolic, infectious and environmental causes have been identified in the etiology of POF. However, in most of the patients, the underlying cause cannot be revealed despite all diagnostic tests. The mechanisms suggested in the etiopathogenesis are a decrease in the initial ovarian reserve and an increase in the rate of ovarian follicle atresia. It has been suggested that the reproductive aging in humans is part of the same biological process based on oxidative stress-induced DNA damage and apoptosis (2,3).

Heavy metal definition is used for metals with a density of more than 5 g/cm^3 in terms of physical properties. This group includes more than 60 metals such as cadmium, iron, chromium, lead, mercury, copper, nickel, cobalt, arsenic, and zinc. Cadmium is a toxic metal. The two most important sources of exposure are diet and tobacco smoking (4). Cadmium is one of the most important environmentally damaging metals in industrialized areas. In addition to renal damage, it has been shown in animal studies that cadmium can cause harmful effects in many tissues such as lung, liver, bone and ovary (5). It has been shown that cadmium and lead cause a significant decrease in the binding of gonadotropins that regulate steroidogenic enzyme activity in granulosa cells (6).

In the light of the studies, it can be hypothesized that any defect in biosynthesis pathways of ovarian steroidogenic hormones may result in POF. Therefore, we aimed to investigate the role of cadmium, which has been shown to impair the biosynthesis of steroidogenic hormones, in the etiology of POF.

2. Materials and Methods

This comparative study which was approved by the local ethics committeewas conducted with the participation of patients who applied to our tertiary university hospital between 2014 and 2015.Thirty-five patients with POF were enrolled in group 1 which was the study group. Two healthy control groups were formed with participants. Thirty-five participants who were age-matched young women with regular menstruation were included in group 2. Thirty-five healthy postmenopausal women were included in group 3.

The diagnosis of POF was made before the age of 40 when two serum FSH values measured at least one month apart were above 40 IU/L (7). Inclusion criteria for the POF group were determined as having FSH levels of 40 IU/L and above in two separate cycles and being between the ages of 18-39. Exclusion criteria for all groups were determined as the presence of hypothalamic and pituitary tumors, ovarian enlargement of unknown etiology, presence of ovarian cyst, polycystic ovary appearance, previous ovarian surgery, chemotherapy or radiotherapy history, chromosomal abnormality, presence of a medical condition that could be clinically significant and affect the study results such as thyroid disease or diabetes mellitus, history of autoimmune or metabolic disease, presence of ovarian, breast and uterine cancer.

Patients' age, body mass index (BMI), obstetric history, age at menarche, employment status, income level, place of residence (urban/rural), family history, smoking or alcohol status, combined oral contraceptive (COC) use were recorded. Serumfollicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), thyroid-stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) were measured by chemiluminescence method (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA). Cadmium analysis was performed with 2 cc venous

blood taken in the morning after 8 hours of fasting and measurements were made with Atomic Absorption Spectrometer (Perkin Elmer AAnalyst 800).

Statistical Package for the Social Sciences (SPSS) version 17.0 software was used for the statistical evaluation of the research data. Quantitative variables were defined as mean, standard deviation, and qualitative variables were defined as numbers and percentages. Whether the quantitative variables showed normal distribution was tested with the Shapiro Wilk test. The comparison of the groups according to the results of normality test was made with ANOVA and Kruskal-Wallis analysis of variance. Bonferroni and Mann-Whitney U tests were used for posthoc analysis. $p<0.05$ was considered statistically significant.

The study was conducted in line with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki guidelines and its amendments (ethics committee approval IRB no: 2015-06). Informed consent was obtained from all patients.

3. Results

The characteristic of participants were summarized in Table 1. The mean ages of the participants in group 1, 2 and 3 were determined as 31.7±4.78, 31.7±4.91,54±4.5, respectively.

While there was a statistically significant difference between the groups in terms of gravida, parity and living children ($p<0.001$), no difference was found for abortion ($p=0.430$). There was no statistically significant difference between the groups for age at menarche, employment status, and income level, place of residence, family history, smoking, alcohol orCOC use. There was no participant working in any occupation that could cause cadmium exposure.

The hormone profile and blood cadmium levels of the participants were summarized in Table 2. While there was a statistically significant difference between the groups for serum FSH, LH and E2 levels ($p<0.001$), no statistically significant difference was found for T3, T4 and TSH. Serum cadmium levels were found to be statistically significantly decreased in group 1 compared to groups 2 and 3 ($p<0.001$).

Table 1. Demographic data of the participants

	Group 1 *Patients with POF*	Group 2 *Age-matched control*	Group 3 *Postmenopausal control*	p value
Age (years)	31.7 ± 4.78	31.7 ± 4.91	54 ± 4.5	0.958
BMI (kg/m2)	24.1 ± 4.07	25.3 ± 5.05	27.2 ± 4.31	0.434
Gravida	0.86 ± 1.38	1.81 ± 1.70	3.80 ± 2.15	**<0.01***
Parity	0.63 ± 1.24	1.49 ± 1.56	3.11 ± 1.69	**<0.01***
Live	0.66 ± 1.24	1.56 ± 1.54	3.11 ± 1.64	**<0.01***
Spontaneous abortion	0.25 ± 0.74	0.23 ± 0.60	0.51 ± 1.17	0.430
Age of menarche	12.7 ± 1.5	12.9±1.3	12.9±1.8	0.745
Employment status				
Working	2 (5.7)	10 (28.6)	4 (11.4)	0.095
Unemployed	33 (94.3)	25 (71.4)	31 (88.6)	0.095
Family income				
Below minimum wage	27 (77.1)	23 (65.7)	22 (68.5)	0.214
Above minimum wage	8 (22.9)	12 (34.3)	13 (31.4)	0.214
Residence				
Urban	30 (85.7)	30 (85.7)	30 (85.7)	1.000
Rural	5 (14.3)	5 (14.3)	5 (14.3)	1.000
Family history	6 (17.1)	7 (20)	1 (2.9)	0.078
Smoking (pack-years)	11.2 ± 13.10	6.12 ± 4.99	10.0 ± 4.08	0.470
Alcohol	0 (0)	1 (2.9)	0 (0)	1.000
COC use	3 (8.6)	4 (11.4)	0 (0)	0.137

** $p<0.05$*

Data are given as mean ± standard deviation and number (percentage)

POF: Premature ovarian failure, BMI: Body mass index, COC: Combined oral contraceptive

Table 2. Hormone profiles and serum cadmium levels of the participants

	Group 1 *Patients with POF*	Group 2 *Age-matched control*	Group 3 *Postmenopausal control*	p value
FSH (mIU/mL)	93.15 ± 38.2	6.37±1.65	69.2±33.8	**<0.01***
LH(mIU/mL)	50.31±21.3	4.37±1.80	32.2±21.3	**<0.01***
Estradiol (pg/mL)	20.08±15.8	79.2±32.8	20.2±12.6	**<0.01***
TSH (uIU/mL)	2.21±1.44	1.92±0.93	2.10±2.04	0.690
T3 (pg/mL)	3.37±0.65	3.06±0.86	2.80±0.98	0.028
T4 (ng/mL)	1.04±0.37	1.12±0.67	1.14±0.65	0.579
Cadmium (mg/L)	0.45±2.44	0.51±0.42	0.78±2.02	**<0.01***

** $p<0.05$*
Data are given as mean ± standard deviation
POF: Premature ovarian failure, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid-stimulating hormone, T3: Triiodothyronine, T4: Thyroxine

4. Discussion

In this study, we found that serum cadmium levels in the POF group were statistically significantly lower than in the controls.

Air, water and foods are polluted with the effect of increasing energy use, industrial development and harmful chemicals released by urbanization with rapid increase in the world population. Cadmium is a heavy metal emitted into the environment due to pollution from many sources. Cadmium has a biological half-life of 15-30 years and basically has a low rate of elimination from the body. Physiological concentrations of cadmium in humans have been determined as 2.9±2.5 µg/l in blood, 0.19±0.2 µg/l in seminal plasma and 6.73±0.31 µg/l in follicular fluid (8). It accumulates for a long time in the blood, liver, kidney, placenta, testis and ovary. Long-term exposure to cadmium has been shown to have toxic effects in both humans and animals(9). Exposure to cadmium increases the incidence of renal disease, hypertension, osteoporosis, leukemia, and cancers of the lung, liver, bladder, pancreas, breast, and prostate(10).

Although many studies have been conducted on the effects of heavy metals such as cadmium on human health, their effects on female reproductive health have not been entirely explained. It has been reported that impairments in female fertility that reduce fecundity and fertility rates were associated with increased environmental toxins and industrial processes such as metal production and fuel residues (11-13).

Recently, Da Costa CS et al. revealed that subacute cadmium exposure can cause polycystic ovarian syndrome and premature ovarian failure through its effect on the hypothalamus-pituitary gonadal axis. However, the most important difference of this study from our study was that the effect of cadmium on premature ovarian failure was also evaluated in female rats. It was not human work(14). In another study, the effects of cadmium chloride on follicle development, oocyte formation and toxicity on oocytes at different developmental stages were investigated. This study showed that cadmium chloride toxicity increased as the exposure dose to cadmium increased. However, this study is methodologically different from our study. In this study, rat pre-antral follicle culture method was used to examine basic female reproductive functions in terms of follicular development, hormone synthesis and oocyte formation(15).Another study reported that subacute oral exposure of female rats to cadmium may cause long-term disorders in the reproductive system(16).Because it affects the reproductive system, cadmium exposure may be a cause of estrogen-dependent diseases such as breast cancer, endometrial cancer, endometriosis, and spontaneous abortions(17).

In studies examining the effect of cadmium on gonadotropin levels; studies have shown that cadmium exposure reduces gonadotropin secretion in females and has a toxic effect on granulosa cells(18). In vitro exposure to cadmium or lead has been found to reduce gonadotropin binding and steroid production(19). In a study, it was reported that gonadotropin binding decreased in female rats exposed to cadmium and lead in vivo and in vitro, and this decrease was due to the change in membrane structure.(6) In the same study, it was revealed that there was an increase in free radicals, a decrease in glutathione levels, and a slight increase in lipid peroxidation of the membranes in the granulosa cells of female rats given cadmium. They suggested that there was a decrease in gonadotropin binding after membrane damage with an increase in free radicals and this was the major mechanism that causes dysfunction in the reproductive system. Also it has been shown that the number of follicles and the granulosa cell population in developing follicles decreased as a result of decreased ovarian steroid level. As a result, they determined that these changes could cause infertility. Similarly, in a study conducted by Paksy et al., it was reported that 40% and 87% infertility were observed in rats exposed to low dose and high dose cadmium chloride, respectively (20).

Cadmium disrupts the oxidative balance by forming free radicals in the organism. Depletion of glutathione, which clears free radicals from the organism, is important for decreased ovarian function. For this reason, it has been hypothesized that heavy metals may also play a role in POF. However, we found that serum cadmium level in the POF group was statistically significantly lower compared to the healthy control groups.

To our knowledge, this is the first study in the literature which was investigated the role of cadmium in the etiology of POF. This study was the most important limitation as it was not an in vitro or animal study. On the other hand, there are also some limitations. The number of patients in our study was limited. In addition, human exposure to heavy metals often involves more than one metal, although studies investigating the effects of heavy metals on the reproductive system have generally examined the effect of a single metal. The combined of metals can have synergistic or antagonistic effects. For this reason, it is difficult to research the effects of metals on human health. Thus, this is another limitation of our study.

5. Conclusion

The relationship of cadmium with POF could not be demonstrated in our study. The role of cadmium in the etiology of POF will be clarified with further studiesin larger populations.

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Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of the Malatya (Decision no:2015/06, Date: 11.02.2015).

Informed Consent: The authors declared that it was not considered necessary to get consent from the patients because the study was a retrospective data analysis.

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