

Association of Between Heavy Metal and Trace Element Levels in Blood and Cervical Mucus with Female Infertility

Kan ve Servikal Mukusta Ağır Metal ve Eser Element Düzeyleri ile Kadın İnfertilitesi İlişkisi

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ABSTRACT Objective: Infertility affects 10-15% of couples during the reproductive period. Ovulatory disorders are more common in younger women whereas unexplained infertility occurs more commonly in older women. Recently, many environmental agents, such as cigarettes, alcohol, heat, electromagnetic energy and radiation have been shown to have negative effects on the reproductive system. Environmental factors and exposure to heavy metals change fertility patterns in adults by affecting germ cell maturation, fertilization and the endocrine system. We aimed to investigate the effect of trace elements and heavy metals in blood and cervical mucus on female infertility. **Material and Methods:** Study was performed with 50 women: Infertile (n=35) and controls (n=15). Blood and cervical mucus samples were collected to measure Zn, Cu, Cd, and Pb levels. **Results:** Mean blood plasma and cervical mucus Zn, Cd, and Cu levels in the infertile group were significantly lower than those in the control group. Whole blood Pb level was higher in the infertile group compared to the control group. Although blood Cd level tended to be higher in smokers and cervical mucus Cd level tended to be lower in controls, neither of these difference were statistically significant. **Conclusion:** Lower levels of plasma and cervical mucus Zn and Cu were thought to result from higher levels of Cd and Pb in blood and cervical mucus. Abnormal levels of heavy metals in blood and cervical mucus may have adverse effects on the female reproductive function.

Key Words: Cervix mucus; metals, heavy; trace elements; infertility, female

ÖZET Amaç: İnfertilite, üreme çağındaki çiftlerin %10-15' ini etkilemektedir. İleri yaşlarda daha yaygın olarak açıklanamayan infertilite, genç kadınlarda ise ovulatuvar rahatsızlık gözlenmektedir. Son zamanlarda, sigara, alkol, ısı, elektromanyetik enerji ve radyasyon gibi çok sayıda çevresel ajanın üreme sistemine olumsuz etkisi olduğu gösterilmiştir. Çevresel faktörler ve ağır metallerle maruz kalma, germ hücre olgunlaşmasını, döllenmeyi ve endokrin sistemi etkileyerek erişkinlerde doğurganlık durumunu etkilemektedir. Bu çalışmada kan ve servikal mukusta eser elementlerin ve ağır metallerin kadın infertilitesi üzerine etkisini araştırmayı amaçladık. **Gereç ve Yöntemler:** Çalışma 35 infertil ve 15 kontrol, toplam 50 kadın ile gerçekleştirildi. Zn, Cu, Cd ve Pb düzeylerini ölçmek için kan ve servikal mucus örnekleri toplandı. **Bulgular:** Ortalama kan plazması ve servikal mucus Zn, Cd, ve Cu düzeyleri infertil grupta kontrol grubuna göre anlamlı olarak düşüktü. Tam kan kurşun düzeyi infertil grupta kontrol grubundan daha yüksekti. Sigara içenlerde kan Cd düzeyi daha yüksek ve kontrol grubunda servikal mucus Cd düzeyi daha düşük olma eğiliminde olmasına rağmen, her iki farklılık da istatistiksel olarak anlamlı değildi. **Sonuç:** Plazma ve servikal mucus Zn ve Cu düzeylerinin daha düşük olması, kan ve servikal mukusta Cd ve Pb'un daha yüksek olmasından kaynaklandığını düşündürmektedir. Kan ve servikal mukusta ağır metallerin anormal düzeyinin kadın üreme fonksiyonu üzerine olumsuz etkisi olabilir.

Anahtar Kelimeler: Servikal mukus; metaller, ağır; eser elementler; infertilite, kadın

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Infertility affects 10-15% of couples during the reproductive period.¹ Ovulatory disorders are more common in younger women, whereas unexplained infertility occurs more commonly in older women. Ovulatory (15%), tuboperitoneal (35%), male factors (35%), unexplained (10%), and both male and female factors (35%) are causes of infertility.² Unexplained infertility is a diagnosis introduced by excluding other causes, and the prevalence ranges from 5 to 37% in different populations.³ Recently, many environmental agents, such as cigarettes, alcohol, heat, electromagnetic energy and radiation have been shown to have negative effects on the reproductive system. Environmental factors and exposure to heavy metals change fertility patterns in adults by affecting germ cell maturation, fertilization and the endocrine system.^{4,5} Not only the type of material, but also the exposure time determines the effects of these factors on reproductive health. Heavy metals may affect ovulation and cause abnormal sperm production based on their type, exposure duration and severity.^{6,7}

Animal studies indicate a strong relationship between fertility and a lack of Cu and Zn. Selenium and Zn prevent damage from toxic metals such Cd and Co.^{8,9} Cadmium and Zn can replace other metals in many vital enzymatic reactions and prevent function.¹⁰ Lead and Cd cause glutathione exhaustion and deterioration in the balance of free radicals. Zinc provides protection against Pb and Cd with its antioxidant properties.

Although numerous studies have demonstrated effects of trace elements and heavy metals on human health, their effects on female fertility have not been fully explained. In contrast to genetic causes, environmental and lifestyle factors causing poor fertility are treatable and preventable. Cervical and mucus factors should be considered when investigating causes of infertility. In this study, we measured the levels of Zn, Cu, Cd, and Pb in whole blood (WB), blood plasma (BP), and cervical mucus (CM) of infertile women and compared with those of normal subjects.

MATERIAL AND METHODS

This study was performed at Eskisehir Osmangazi University School of Medicine, Department of Obstetrics and Gynecology, Center for Reproductive Health. Fifty cases (infertile group, n=35; control group, n=15) were included after obtaining the permission of the Faculty Ethics Board. Exclusion criteria were impaired sperm parameters, users of hormonal contraceptives and intrauterine devices.

Gynecological history was reviewed, and physical and pelvic examinations were performed in all cases. Patients were evaluated by measuring follicle stimulating hormone (FSH), luteinizing hormone (LH), 17 β -estradiol, prolactin and thyroid stimulating hormone levels and obtaining a transvaginal ultrasound on days 2-5 of menstrual bleeding. Zinc, Cu, Cd and Pb levels were measured in WB, BP, and CM in all cases. Blood samples were obtained from patients for WB (into EDTA-containing tubes) and BP [into heparin-containing tubes, followed by centrifugation (2000 \times g, 5 min), and the supernatants were obtained].

Cervical mucous samples (1 mL) were collected with an intrauterine insemination apparatus (#4502-B; Gynetics Medical, Hamont Achel, Belgium) connected to a tuberculin syringe before a gynecological examination on days 14-21 of the menstrual cycle. Samples were kept at -20°C until analysis.

Heavy-metal analytical solutions were prepared by dilution in distilled water. Organics were removed by burning and applying acid. Cervical mucus samples were prepared by adding acid (100 μ L concentrated HNO₃; Merck, Whitehouse Station, NJ, USA) then diluting to a particular volume with distilled water. The burning procedure was applied initially to the WB samples at 600°C to constant weight. After the burning procedure, 0.5 mL of concentrated HNO₃ and 0.5 mL of an acid mixture (2% v/v HClO₄/HNO₃) were added. After this procedure, the samples were incubated in a water bath at 60°C, and the analytical solutions were prepared. The final elemental analysis was performed

with a polarized Zeeman atomic absorption spectrophotometer (Hitachi 180-70; Mountain View, CA, USA).

STATISTICAL ANALYSES

The SPSS software (SPSS 13, Chicago, IL, USA) was used for analyses. Descriptive parameters are presented as means±standard deviation, median (25th-75th percentiles) or as percentages. Two-sided t-tests was used to analyze the differences in means of continuous variables in two groups when the variables were normally distributed. If variables were non-normally distributed, Mann-Whitney U-test was used. When there were more than two groups, one way ANOVA and Kruskal Wallis tests were used, respectively and Bonferroni correction was used for multiple comparisons ($\alpha^*=0.05/3=0.017$). Fisher's exact test (two-sided) and chi-square test were used when comparing the proportions between groups. Pearson correlation analysis was conducted to determine the association be-

tween infertility parameters, heavy metals and trace elements. A p-value of <0.05 was considered significant.

RESULTS

Thirty (85.71%) and 12 (80%) housewives were in the infertile and control groups, respectively. The mean infertility period, weekly intercourse, weight, length, body mass index, and baseline FSH and LH levels were similar in patients and control subjects (Table 1).

Zinc and Cu levels in the BP and CM of the infertile group were lower than those in the control group. However, Pb levels in the WB were higher in the infertile group than those in control subjects. Lead levels in the CM and Cd levels in the WB were not significantly different between the groups. However, mean Cd level in the CM was higher in the infertile group compared to the control group (Table 1).

TABLE 1: Comparison of duration of infertility, weekly intercourse, weight, height, body mass index, basal FSH and LH level, Zn, Cu, Pb and Cd levels of WB, BP and CM of infertile patients with control group.

		Infertile (n=35) (Average±SD) M(25-75 percentiles)	Control (n=15) (Average±SD) M(25-75 percentiles)	p
Age(year)		29.23±4.39	28.67±4.97	0.704
Duration of infertility (year)		7.71±4.73	---	
Weekly intercourse (n)		3±1.05	2.86±0.64	0.562
Housewife (n)		(30) 85%	(12) 80%	0.683
Smoker (n)		(4) 11.4%	(4) 26.66%	0.220
Weight (kg)		65.85±11.02	67.66±11.77	0.614
Height (cm)		160.57±5.95	161.46±4.95	0.582
BMI (kg/cm ²)		25.93±4.84	25.52±4.99	0.795
FSH(mIU/mL)		6.82±3.33	8.34±7.44	0.453
LH(mIU/mL)		5.55±3.47	4.96±2.44	0.490
Zn	BP µg/L	790.00 (632-947)	1560.00 (862-1720)	0.001
	CM mg/L	10.90 (7.12-13.82)	32.40 (15.15-34.05)	<0.001
Cu	BP µg/L	1408±455	1785±537	<0.001
	CM mg/L	0.72±0.30	1.32±0.49	<0.001
Pb	WB µg/L	39.77±14.05	29.17±16.05	0.02
	CM µg/L	17.26±5.05	16.11±4.35	0.420
Cd	WB µg/L	1.1 (0.93-1.25)	0.76 (0.60-1.38)	0.127
	CM µg/L	14.10±3.04	11.47±3.22	0.008

BMI: Body mass index; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; Zn: Zinc; Cu: Copper; Pb: Lead; Cd: Cadmium; BP: Blood plasma; CM: Cervical mucus; WB: Whole blood; SD: Standart Deviation; M: Median.

TABLE 2: Comparison of Zn, Cu, Pb, Cd levels of WB, BP and CM of infertile subgroup patients with the control group.

Trace elements		Types of Infertility				General p	Multiple Comparison p
		Ovulatory factor (n=12) (Average±SD) M(25-75 percentiles)	Tubal factor (n=10) (Average±SD) M(25-75 percentiles)	Unexplained (n=13) (Average±SD) M(25-75 percentiles)	Control (n=15) (Average±SD) M(25-75 percentiles)		
		Age (year)	30±5.1	31.1±3.58	27.08±3.55		
Zn	BP µg/L	880 (715-910)	840 (690-980)	640 (607-952)	1560 (862-1720)	0.009	p ₁ =0.253 p ₂ =0.186 p ₃ =0.008
	CM mg/L	11.2±3.59	12.99±7.61	10.07±4.34	26.43±10.56	0.008	p ₁ <0.001 p ₂ =0.004 p ₃ =0.001
Cu	BP µg/L	1602±496	1578±537	1099±281	1785±537	0.002	p ₁ =0.573 p ₂ =0.452 p ₃ =0.001
	CM mg/L	0.64±0.26	0.77±0.25	0.75±0.37	1.32±0.49	0.001	p ₁ <0.001 p ₂ =0.004 p ₃ =0.001
Pb	WB µg/L	38±13	40±10.4	41±17	29±16	0.245	
	CM µg/L	19±5.3	17±5.1	15.4±4.3	16.1±4.3	0.362	
Cd	WB µg/L	1.08±0.34	1.13±0.43	1.15±0.31	1±0.54	0.144	
	CM µg/L	14.5±3.2	14.5±3.2	13.3±2.5	11.4±3.2	0.001	p ₁ =0.012 p ₂ =0.021 p ₃ =0.124

Zn: Zinc; Cu: Copper; Pb: Lead; Cd: Cadmium; BP: Blood plasma; CM: Cervical mucus; WB: Whole blood; SD: Standart Deviation; M: Median; p₁: Between ovulatory factor and control groups, p₂: between tubal factor and control groups, p₃: between unexplained and control groups.

The infertile cases were divided into three subgroups: Ovulatory, tubal, and unexplained infertility. A comparison according to infertility type revealed that mean Zn and Cu levels in the BP of the unexplained infertility group were lower than those in the controls (Table 2). Furthermore, mean Zn and Cu levels in the CM were lower in all three infertile subgroups compared to the controls. In contrast, Pb levels in BP and CM were not significantly different among the groups. Cd level in CM was significantly higher in cases of ovulatory and tubal disorders compared to the control group.

Eight smokers (16%) and 42 non-smokers (84%) were also evaluated for Zn, Cu, Cd and Pb levels in blood and CM. Cd level in WB was higher in smokers, whereas it was lower in CM of smokers. No significant difference was observed when the Zn, Cu, Cd, Pb levels in WB and CM were compared according to smoking status, infertility (primary vs. secondary) or age (20-29 vs. 30-42 years).

The correlation analysis showed that Zn level in CM was significantly positively correlated with Cu level in CM (p=0.002, r=0.42), whereas a negative correlation was observed between the Pb level in WB and the Zn level in BP and CM (p=0.042, r=-0.29; p=0.008, r=-0.37, respectively). Negative correlations were detected between the Zn level in BP and the Cd level in WB and CM (p=0.001, r=-0.47; p=0.001, r=-0.55, respectively; Figures 1, 2) and between the Zn level in CM and the Cd level in WB (p=0.005, r=-0.39).

DISCUSSION

This is the first study to show that Cd and Pb levels in CM and BP differ in infertile and fertile women. The results appear valid; most of the patients were housewives and had no history of direct exposure to chemicals or heavy metals.

Rückgauer et al. studied plasma trace element reference values in different age groups, and the plasma Zn level was 16.6 mmol/L (1085 mg/L).¹¹ In

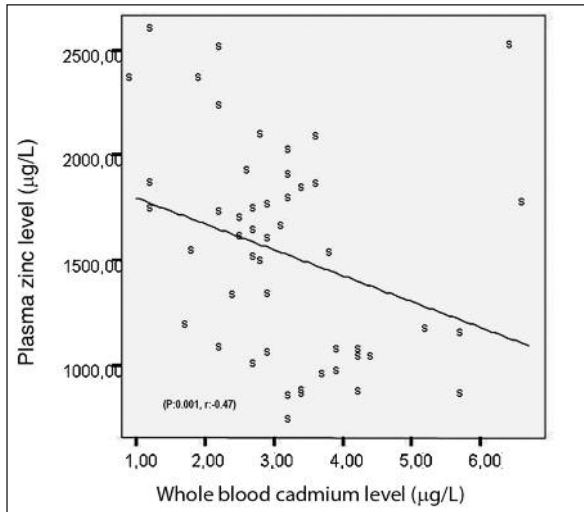


FIGURE 1: The correlation graphic between the zinc level of blood plasma and cadmium level of whole blood ($p=0.001$, $r=-0.47$).

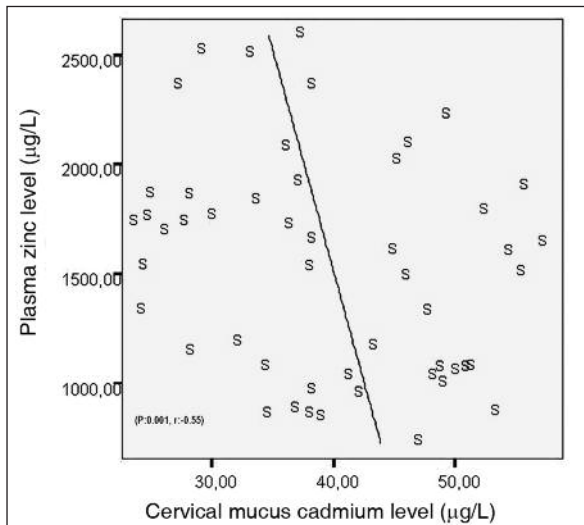


FIGURE 2: The correlation graphic between the zinc level of blood plasma and cadmium level of cervical mucus ($p=0.001$, $r=-0.55$).

our cohort, mean Zn levels in BP were 790 mg/L in infertile group and 1560 mg/L in the control group. Furthermore, mean plasma Zn levels were lower in the infertility subgroups than those in the control group, and a significant decrease was observed in the unexplained infertility group. Although a limited number of studies have reported Zn levels in BP, our findings are consistent with the literature.^{9,11-15} In previous studies, Zn levels were measured as the dry weight of CM.^{12,16} The

only study on this topic was conducted by Chuang et al. in fertile patients, and Zn levels were 10.9 mg/L and 32.4 mg/L in the CM of infertile and control groups, respectively.¹⁷ In that study, CM Zn levels were 11.2 ± 3.59 mg/L in patients with ovulatory disorders, 10.0 ± 4.3 mg/L in patients with unexplained infertility, and 12.99 ± 7.6 mg/L in patients with tubal disorders. Again, all three groups had significantly lower CM Zn levels than those in a control group. In our study, the Zn level in the CM was lower (<60 mg/L) than that reported by Chuang et al.¹⁷

The Cu levels in BP tended to be lower in all three infertility subgroups than the control group, but a significant difference was observed only in the unexplained infertility group. The Cu levels in BP were consistent with the literature.^{11-13,15,18} In previous studies that examined Cu in CM, the CM Cu levels were similar with those in the present study.^{17,19}

Chang et al. found that average Pb levels in blood were 35.5 ± 14 µg/L in infertile and 27.8 ± 21 µg/L in fertile women.²⁰ In the present study, the Pb levels in WB were 39.8 ± 14 µg/L and 29.2 ± 16 µg/L in the infertile and control groups, respectively. Lead levels in CM were similar in the infertile (17.3 ± 5 µg/L) and control (16 ± 4.4 µg/L) groups. Our findings were also similar to the blood Pb levels previously reported for infertile cases.^{20,21} Cervical mucus Pb levels were 19.3 ± 5.3 µg/L in those with ovulatory disorders, 17.2 ± 5.1 µg/L in those with tubal factors and 15.4 ± 4.3 µg/L in those with unexplained infertility.

Cadmium levels in WB were 1.1 (0.9-1.2) µg/L and 0.76 (0.60-1.38) µg/L in the infertile and control groups, respectively. Whole blood Cd levels were 1.08 ± 0.34 µg/L in patients with ovulatory dysfunction, 1.13 ± 0.43 µg/L in patients with tubal factors and 1.15 ± 0.3 µg/L in patients with unexplained infertility. McKelvey et al. reported that the mean blood Cd level was 0.79 µg/L in women.²¹ In present study, the CM Cd levels were 14.1 ± 3 µg/L and 11.47 ± 3.2 µg/L in the infertile and control groups, respectively. Cervical mucus Cd levels were 14.5 ± 3.2 µg/L, 14.5 ± 3.2 µg/L and

13.3±2.5 µg/L in women with ovulatory disorders, in women with tubal factor and in women with unexplained infertility, respectively. In a study performed in Taiwan, the CM-Cd level was 29.7±40.43 µg/L, and no change in Cd levels was found according to age.¹⁷ Also in this study, no difference was detected for Cd levels in WB and CM according to age.

Previous reports showed no correlation between Zn and Cu levels in BP and CM or between Zn levels in BP and CM in reproductive women.^{12,22} A positive correlation was detected between blood levels of elements other than Pb and a transition of them into CM. BP and CM were significant in terms of Zn level, whereas Cu and Cd were not significant. The levels of Zn and Cu in BP reflected the level of these elements in CM. Zinc and Cu are required by organisms, and a positive correlation between these elements occurs. However, the harm caused by Cd and Pb is well known, and a negative correlation exists between Zn and Cu, and Cd and Pb. It may be concluded that while Zn and Cu have positive effects on fertility, Cd and Pb have negative effects. Cd and Pb levels were higher in

infertile woman when compared to the control women; however, it is unclear what effect this had on infertility. Thus, larger prospective studies are needed in infertile women.

CONCLUSION

Zinc and Cu levels were lower and Cd levels were higher in infertile women compared to the control subjects. In particular, patients in the unexplained infertility subgroup had a significant association between Zn and Cu levels. Cadmium in CM was higher in infertile women compared to the controls, particularly in the ovulatory and tubal infertility subgroups. The effects of these elements must be considered in the etiology of infertility. More studies are needed to validate these novel findings.

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