LEAD, CADMIUM AND ZINC IN INFERTILE WOMEN COMPARED TO FERTILE WOMEN

BY

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ABSTRACT

Blood lead and cadmium levels and serum zinc concentration of 50 clinically unexplained infertile women were determined using Perkin- Elmer 2380 Atomic Absorption Spectrophotometer and compared with 20 fertile women. Blood lead and cadmium levels of infertile women (14.22 ± 2.34 & 3.05 ± 0.63 µg /dl) were higher than those of the fertile women (9.76 ± 2.34 & 1.40 ± 0.41 µg /dl) whereas serum zinc were significantly lower (68.64 ± 15.54 versus 97.84 ±13.43 µg %). Urban infertile had a highly significant elevation of blood lead and cadmium levels when compared to that of urban fertile women. Urban infertile women had higher blood lead and cadmium levels and lower zinc concentrations when compared with rural infertile women. Infertile women who were exposed to heavy smoking. Infertile women who exposed to heavy smoking" passive smokers" had a higher blood lead (15.61 ± 1.42 µg /dl) when compared to that of infertile women who were not exposed to smoking (13.10 ± 0.42 µg /dl). Infertile women whose husband occupation were painters; solders and drivers had higher blood lead levels than other occupations. Concerning cadmium risk factors, infertile women who consumed excess canned food and exposed to smoking had higher blood cadmium levels (3.87 ± 0.05 and 3.63 ± 0.28 µg /dl) than infertile women who did not consume canned food or not exposed to smoking (2.91 ± 0.85 and 2.5 ± 0.26 µg /dl respectively). There was highly significant positive correlations between the ;duration of infertility and both blood lead (r = 0.831) & cadmium levels (r = 0.808) and highly significant negative correlation between duration of infertility and serum zinc levels of infertile women (r = - 0.79). In conclusion , estimation of blood lead and cadmium levels may have an important role in unexplained infertility. Zinc supplementation may reduce absorption of lead and cadmium .

INTRODUCTION

Heavy metals can influence female fertility at every phase of reproduction. They may induce hormonal disorders, preventing ovulations and pregnancies (Gerhard and Run nebaum, 1992 & Gerhard et al., 1998). Lead is a widely spread environ-

mental pollutant, found in many homes e.g. in fixtures and fittings, past use of paints, in alloys, food kept in lead - glazed ceramics, fungicides, petroleum products, agricultural soil, herbal medicine, cigarettes etc.. It is known to affect both male and female reproductive systems in human (Harbison, 1998) and experimental animals (Junaid et al., 1997). Lead pretreatment for rats enhanced some parameters of estrogen stimulation and inhibited other estrogenic responses (Tchernitchin et al., 1998).

Cadmium is a highly toxic cumulative heavy metal . Human exposure to it has been and continues to be a major concern . It is estimated to have a biological half life in humans of 20 years. Cadmium usage has increased dramatically during the past 40 to 50 years and continues, gradually, to increase (Michael et al., 2001). So, it poses a significant threat to the human population and environment. Cadmium is able to decrease preovulatory luteinizing hormone (LH) levels in blood and inhibit ovulation in rats (Waalkes, 1995). The mechanism of the negative action of cadmium in the organism is most probably due to its competition with the vitally important trace element - zinc. Absorption of both lead and cadmium is increased in presence of zinc deficiency (Drbohlave, et al., 1998).

Zinc is a nutritionally essential trace metal. Sea foods, meats, whole grains, dairy products, nuts, and legumes are high in zinc. More than 200 metalloenzymes belonging to six major categories-including oxidoreductases, ransferases, Hydrolases, lyases, isomerases,and ligases-require zinc as a cofactor. It stabilizes membranes by binding ligands in membranes that maintain the normal structural geometry of the protein and lipid components (Drbohlave, et al., 1998).

Only a few modern epidemiologic studies have addressed lead and cadmium's effects on reproductive outcome in women, so that the present work was designed to estimate lead , cadmium and zinc in infertile women compared to fertile women.

SUBJECTS AND METHODS

This study was conducted on 70 women; 50 infertile (aged from20-36years) who attended Fertility Unit of Mansoura University Hospital . All had unexplained infertility (40 had primary infertility and 10 had secondary infertility). Thirty eight women were living in Mansoura City and the others were living in rural areas. All women had normal menstrual cycles and regular sexual intercourse two or three times a week; non had relevant past or present medical or surgical features and all were normal on clinical examination. Infertility investigations in these women had revealed no abnormality and semen

analysis of their partners and postcoital tests were normal. Twenty fertile (aged from 20-36years) non pregnant women (control) were randomly selected from Obstetric & Gynecology Department of Mansoura University Hospital. Twelve women were living in Mansoura City and the others were living in rural areas. They were age, weight and height matched. Non of the infertile women or control subjects was receiving medication or suffering from any infection during the study. Informed consent was taken from all subjects to share in this study.

Thorough history was taken from each woman with special emphasis on :

1- Age, residence and occupation.
2- Lead exposure risk factors questionnaires: Usage of kohl and / or lead glazed ceramics; old housing and lead pipes; husband occupation especially workers in painting, solders; drivers; cigarette smoking of the husband (light, moderate or heavy according to Ferris Smoking Index" packs of cigarettes / day x 365 x number of years of smoking.
3- Cadmium exposure risk factors questionnaires : Increased consumption of canned foods, sea foods, coffee and exposure to smoking (passive smokers).
4- History of diabetes, hypertension, hepatic and renal diseases.
5- History of drug intake.

For each participant, ten ml venous blood sample was withdrawn from anticubital vein under complete aseptic condition ; 5 ml were added to EDTA into polypropylene tubes, gently mixed for analysis of lead and cadmium and 5 ml were separated to obtain serum for zinc estimation. They kept at 4°C until assay. Each sample was digested by using wet method of digestion (nitric- perchloric acid) according to the method of Vanloon, 1985. Analysis was done by using Perkin - Elmer 2380 Atomic Absorption Spectrophotometer at Mansoura Faculty of Science. Preparation of standards and samples was carried out under clean conditions using deionized water of Analytic standards of lead, cadmium and zinc were prepared from solutions of metal nitrate (BDH,UK), with concentration of 1000mg/L. All chemicals and reagents used were of ultra pure reagent grade.

All glassware and plasticware were washed three times with deionized water, then soaked in 20% v/v HNO3 overnight. After soaking the glassware was rinsed three times with deionized water and dried.

Data were compared by using student's t-test (to compare two groups), One way ANOVA test (to compare more than two groups). Chi square (x2) was used for

qualitative data (Frequency& proportion) . Correlation co-efficiency was used to test association between variables. These tests were run on an IBM compatible personal computer by using Statistical Package for Social Scientists (SPPS) for windows 11 (SPSS Inc., Chicago, IL ,USA).

RESULTS

Table (1) showed insignificant variation of the age and characteristics of the studied infertile women when compared to fertile (control) women. There was a highly statistical elevation of blood lead and cadmium levels of infertile women (14.22 ± 2.34 & 3.05 ± 0.63 µg/dl) when compared to fertile women (9.76 ± 2.34 & 1.40 ± 0.41 µg/dl). A highly statistical reduction of serum zinc concentration (although within normal range) was found in infertile women when compared to fertile women (68.64 ± 15.54 versus 97.84 ± 13.43 µg%) as shown in table (2). There was a highly statistical elevation of blood lead and cadmium levels of urban infertile when compared to urban fertile women and also in rural infertile women when compared to rural fertile women. Urban infertile had higher blood lead and cadmium levels and lower zinc concentrations when compared with rural infertiles as illustrated in table (3).

Blood lead levels were higher in infertile women who were high coffee consumers (more than 3 cups/day) and exposed to heavy smoking from husbands "passive smokers" (14.56 ± 1.46 µg/dl and 15.61 ± 1.42 µg/dl) when compared to that of infertile women who were not coffee consumers and not exposed to smoking (13.78 ± 1.50 and 13.10 ± 2.00 µg/dl). Infertile women who's husband occupation were workers (painters; solders; drivers) had higher blood lead levels than other occupations (Table 4).

Concerning cadmium risk factors, infertile women who consumed excess coffee and / or canned food and exposed to smoking had higher blood cadmium levels (3.09 ± 0.45 ; 3.87 ± 0.05 and 3.63 ± 0.28 µg/dl) than infertile women who were not coffee or canned food consumers or not exposed to smoking (2.59 ± 0.55; 2.91 ± 0.85 and 2.5 ± 0.26 respectively) as shown in table (5). Table (6) showed highly significant positive correlations between the duration of infertility and both blood lead (r = 0.831) & cadmium levels (r = 0.808) and a highly significant negative correlation between duration of infertility and serum zinc levels of infertile women (r = -0.979).

DISCUSSION

Lead is an environmental pollutant known to affect reproductive organs. It may produce abnormal reproductive function. Reported effects in women include infertility, miscarriage, pre-

eclampsia, pregnancy hypertension and premature delivery (Villagra et al.,1997). Previous researches indicated that lead may be toxic at levels previously thought to have no effect (Winder,1993). Cadmium is an abundant non essential element that is present in food, water, and environment. It accumulates in human tissues and thus also in reproductive tract and may have an impact on fertility (Drbohlav et al.,1998).

The results of the present study showed high blood lead levels in infertile women when compared to fertile. This was in accordance with the result of Zenz, et al. (1994) who reported decreased fertility in exposed female workers.

The observed high blood lead levels of the studied infertile women may be attributed to variable environmental sources for exposure with absence of general preventive strategies for controlling these sources. Contaminated soils from airborne pollution, use of leaded pesticides or from paints of the exterior of nearby building , use of leaded water pipe lines and living near heavy automobile traffic using leaded gasoline (urban areas) are all factors contributing to lead exposure among women (Klaassen, 2006) .

The result of the present study revealed a statistically significant elevation of lead levels in women using kohl. Kohl is composed of lead sulphide so, it is considered a continuous source of lead exposure with possible absorption from skin.

In experimental animals, lead affect female reproductive organs through different mechanisms. It may interact at the enzyme level ; may interfere with the action of reproductive hormones at the target organ, modifying the activity of estrogen receptors in the pregnant uterus and inhibiting responses where estrogens play a role. Lead may induce imprinting mechanisms, causing persistent changes in uterine estrogen receptors and ovarian Leutinizing Hormones receptors following perinatal exposure. Also, it may interfere at the level of hypothalamus-pituitary, decreasing pituitary response to growth hormone releasing estrogens in the uterus (Junaid et al., 1997). Lead is a potent reproductive toxicant in humans and experimental animals, it seems to affect the follicular development and maturation (Junaid et al., 1997). This targeting can result in loss of ovarian steroid hormones, eventual ovarian failure, and ultimate disruption of neuroendocrine feedback causing increased levels of Follicle Stimulating Hormones and Leutinizing Hormones (Heidelberg, 2005).

Elevated lead levels observed among urban than rural infertile women may be explained by exposure of women to more air pollution and heavy traffic areas or

thought possibly to be related to sedentary urban lifestyle in contrast to rural life. Low calcium intake ; high lactose or fat diet (which can enhance lead accumulation) may play a role (Barclay,2003).

The present work showed that infertile women had elevated cadmium levels when compared with fertile women. This is in agreement with a study of 400 infertile women , the cadmium excretion was positively associated with a history of miscarriages. It was found to be correlated inversely with follicular oestradiol concentrations. The cadmium and lead excretion were negatively associated with prolactin , thus heavy metals seem to have a negative impact on ovarian, especially luteal, as well as pituitary function , which may be important in the pathogenesis of infertility (Gerhard et al., 1998). Tobacco smoking may significantly increase exposure to cadmium., motor vehicle exhausts contributes to air pollution. Waste disposal (incineration) and the use of sewage sludge as a fertilizer result in large input to land (Klaassen, 2006) .

The possible causes of fertility disorders in conjunction with cadmium is probably the damage of granulosa cells and thus dysfunction as regards production of steroid hormones with full impact on female fertility (hormone disruptors) (Drbohlave et al., 1998). It is able to decrease preovulatory leutinizing hormone (LH) levels in blood and inhibit ovulation so, it exerted a direct effect on both granulosa cell morphology and on steroid biosynthesis (Pasky et al.,1997).

The observed association between exposure to smoking and increased lead and cadmium levels with females infertility is consistent with that reported by Olsen who mentioned that cigarette smoking (either active or passive) reduces fertility. This association was found to be dose dependent. Female smoking has been associated with infertility as well as sub fecundity (Olsen, 1991). He observed that partner's smoking habits related to sub fecundity in a dose- response manner in women who smoked as well as in women who did not. Hruska, et al. (2000) found an association between cigarette smoking and female infertility. A recent study by Nas et al. (2007) reported that smoking has a harmful effects on tubal function and menstrual cycle resulting in subsequent increased uterine contractility and failure of quiescence of the uterus.

Reduction of an increased heavy metal body load improved the spontaneous conception chance of infertile women. The sodium 2,3- dimercaptopropane sulfonate (DMPS) test was a useful and complementary diagnostic method. Adequate treatment provided successful alternatives to conventional hormonal therapy (Gerhard

and Run nebaum,1992).

The reduction of serum zinc of the studied infertile women may be attributed to interaction with the elevated cadmium levels. This support the hypothesis of Jameson (1980) but not support that of Soltan & Jenkins (1983) and Sing et al (1989) who found no significant differences between fertile and infertile women. Cadmium replaces zinc in its cellular linkages (Gerhard et al.,1998). Zinc deficiency resulted in a four fold increase of intestinal cadmium absorption from food. Furthermore, zinc promotes the renal elimination of cadmium (Sowa and Stebert,1985). Zinc deficiency in female can lead to impaired synthesis , secretion of (FSH) and (LH) , abnormal ovarian development , disruption of estrous cycle (Bedwal, and Bahuguna, 1994).

As lead contamination in Egypt constitutes a public health problem, vigorous and community - based health education program should be implemented in an attempt to reduce lead exposure. A community based lead and cadmium poisoning prevention programs are also required. A national environmental lead hazard reduction efforts should be done as reducing lead in industrial emission, gasoline, and cans, lead contaminated soil, lead paints and old lead pipes.

In conclusion, estimation of lead and cadmium may be important in cases of unexplained females infertility. Each woman with unexplained infertility should be subjected to heavy metals estimation prior to hormonal treatment. Women should be advised to be pregnant after reduction of the high levels, as reduction of an increased heavy metal body load may improve the conception chance of infertile women. This is done by using chelating agents. Chelation therapy should be done cautiously in a hospital to choose the proper agent, suitable route and to avoid the possible side effects. Females should be advised to optimize caloric, iron and calcium intake specifically to reduce lead absorption. Supplementation of zinc to infertile women may reduce absorption of heavy metals reducing its deleterious effect.

Table (1): The characteristics of the studied women (n=70) (infertile and fertile).

Parameters	Infertile n=(50)	Fertile n=(20)	Statistical analysis Test	P
Age(years): Mean ± S.D	26.4 ± 4.79	28.15± 4.43	t =1.41	0.163
Residence: Urban n (%) Rural n (%)	38(76) 12(24)	12(60) 8(40)	X^2 = 1.79	0.181
Type of infertility: Primary Secondary n (%)	40(80) 10(20)			
Occupation: Housewives n (%) Employees Teachers Farmers	37(74) 6(12) 6(12) 1(2)	11(55) 5(25) 3(15) 1(5)	X^2 = 3.188	0.527
Use of kohl n (%)	27(54)	6(30)	X^2 = 3.352	0.069
Use of lead glazed ceramics	12(24)	4(20)	X^2 = 0.130	o.719
Coffee consumption: High Absent	13(26) 33(66)	4(20) 16(80)	X^2 =2.212	0.530
Old houses & lead pipes n (%)	50(100)	20(100)		
Consumption of canned food n (%)	7(14)	5 (25)	X^2 = 1.217	0.270
Husband occupation: n (%) employees Teacher& engineer & doctor Farmer	 41(82) 8(14) 2(4)	 16(80) 3(15) 1(5)	X^2 =3.096	0.797
Husband smoking habit: n (%) Non smokers. Smokers: Heavy Moderate Mild	 26(52) 18(36) 5(10) 1(2)	 9(45) 5(25) 3(15) 3(15)	X^2 = 0.280	0.597

Insignificant at P>0.05

Table (2) : lead; cadmium and zinc levels among the studied women (n =70).

Parameters	Infertile Mean ±S.D	Fertile Mean ±S.D	Statistical analysis Student t- test	P value
Blood lead levels (µg/dl)	14.22 ± 2.34	9.76 ± 2.34	9.441	0.0001**
Blood cadmium levels (µg/dl)	3.05 ± 0.63	1.40 ± 0.41	10.733	0.0001**
Serum zinc conc.(µg.%)	68.64 ± 15.54	97.84 ±13.43	- 7.224	0.0001**

** highly significant at p<0.0001

Table (3) : Statistical comparison between infertile and fertile women as regard blood lead, cadmium and zinc levels (n=70).

Parameters	Infertile women Mean ± SD	Fertile women Mean ± SD	Statistical analysis t test	P
Blood lead levels (µg /dl):				
Urban	14.55 ± 1.46	9.58 ± 2.62	7.44	<0.0001**
Rural	13.82 ± 1.52	10.25 ± 1.95	5.77	<0.0001
Blood cadmium levels :				
Urban	3.01 ± 0.66	1.33 ± 0.42	8.19	<0.0001**
Rural	3.16 ± 0.51	1.51 ± 0.40	7.67	<0.0001
Serum zinc conc. :				
Urban	69.89 ±15.88	98.27 ± 15.48	-5.25	<0.0001**
Rural	64.67 ±14.30	97.25 ±10.99	10.24	<0.0001

** highly significant at p< 0.001

Table (4) : Statistical comparison between different categories of the studied parameters (infertile women characteristics and lead risk factors) as regard blood lead levels.

Parameters		Blood lead levels of Infertile Mean ± SD	Statistical analysis Test	P
Residence:	Urban	14.56 ± 1.46	t = 0.09	0.01*
	Rural	13.82 ± 1.51		
Occupation:	Housewives n (%)	14.09 ±1.47	One way	>0.05
	Employees	15.20 ±1.75	Anova	
	Teachers	15.22 ±1.68	F= 0.904	
	Farmers	13.90		
Use of kohl:	+ ve	14.56 ± 1.46	t = 1.76	0.001**
	-ve	13.82 ±1.51		
Use of lead glazed ceramics:	+ve	15.37 ± 1.51	t = 3.15	<0.001**
	-ve	13.87 ± 1.31		
Coffee consumption:	High	14.34 ± 1.14		
	Absent	13.78 ± 1.50	t = 1.43	>0.05
Husband occupation:	Workers	15.09 ± 1.57		
	Employees	13.26 ± 3.54	F = 3.90*	0.0001**
Teachers& engineers& doctors		13.67 ± 3.75		
	Farmers	13.93 ± 2.54		
Husband smoking habit:				
	Non smokers	13.10 ± 2.00	F = 21.05**	0.0001**
	Smokers Heavy	15.61 ± 1.42		
	Moderate	14.76 ± 2.36		
	Mild	13.95 ± 1.51		

Insignificant at P>0.05 * significant at P<0.01 ** highly significant at p<0.001

Table (5) : Statistical comparison between different categories of the studied parameters (infertile women characteristics and lead risk factors) as regard blood cadmium levels.

Parameters		Blood cd levels of Infertile Mean ± SD	Statistical analysis Test	P
Residence:	Urban	3.01 ± 0.67	t = 0.47	>0.10
	Rural	3.16 ± 0.51		
Occupation:	Housewife	3.00 ± 0.613	One way Anova F= 0.77	>0.05
	Employee	3.68 ± 0.70		
	Farmer	3.16		
Coffee consumption	High	3.09 ± 0.45	F = 1.15	>0.05
	Moderate	2.98 ± 0.37		
	Absent	2.95 ± 0.55		
Increased consumption of canned food:	+ve	3.87 ± 0.05	t = 4.36	<0.0001**
	-ve	2.91 ± 0.85		
Husband	Workers	3.18 ± 0.66	F = 1.81	>0.05
	Employees	2.85 ± 0.58		
	Farmers	2.71 ± 0.02		
Husband smoking habit:				
	Non smokers	2.50 ± 0.26	t = 14.7	<0.0001**
	Smokers	3.63 ± 0.28		

Insignificant at P>0.05 **Highly significant at P<0.001

Table (6) : Correlation between Duration of infertility with blood lead & cadmium levels and serum zinc conc.

Parameters	Correlation co-efficiency	Significance (2- tailed)
Blood lead level	0.831	0.0001**
Blood cadmium level	0.808	0.0001**
Serum zinc conc.	- 0.979	0.0001**

** correlation is highly significant at the 0.01 level

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الرصاص والكادميوم والزنك فى النساء العقيمات مقارنة بالنساء الغير عقيمات

المشتركون فى البحث

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نظراً لانتشار العقم بين النساء وعدم معرفة أسبابه فى نسبة كبيرة منهم وزيادة الملوثات البيئية التى من الممكن أن تؤدى إلى العقم أجرى هذا البحث على سبعين سيدة وتم أخذ عينات دم من خمسين سيدة تعانى من العقم (غير معروف أسبابه) لقياس عناصر الرصاص والكادميوم والزنك وتم مقارنتهم بعشرين سيدة لاتعانى من العقم (مجموعة ضابطة).

وقد أظهرت الدراسة مايلى :

١- زيادة نسبة كل من الرصاص والكادميوم ونقص تركيز الزنك فى النساء العقيمات عند مقارنتهم بالمجموعة الضابطة وكانت تلك الزيادة أكبر فى النساء اللاتى تقطن فى المدن عن هؤلاء اللاتى تقطن فى القرى.

٢- كما كانت نسبة الرصاص أعلى فى النساء اللاتى تتعرض للتدخين واللاتى يعمل أزواجهن فى مهن الدهان واللحام وقيادات السيارات.

٣- كانت نسبة الكادميوم أعلى النساء اللاتى تتعرض للتدخين واللاتى تستهلكن الأطعمة المحفوظة.

٤- لوحظ وجود إرتباط معنوى إيجابى بين فترة العقم ونسبة كل من الرصاص والكادميوم فى الدم وارتباط معنوى سلبى بين فترة العقم وتركيز الزنك فى النساء العقيمات.

ومن هذه النتائج يتضح أن قياس نسبة الرصاص والزنك أصبحت ضرورية فى حالات عقم النساء الغير واضح أسبابه فإذا كانت مرتفعة يجب إقلالها مما قد يحسن كفاءة الجهاز التناسلى للإنجاب، كما أوضحت تلك الدراسة أهمية إعطاء تلك الحالات عنصر الزنك عند بدء علاجهم لأن لديه القدرة على التقليل من إمتصاص تلك العناصر الضارة كما يجب إعطاؤه كوقاية من الآثار الضارة لتلك العناصر.