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Study on the Correlation Between Heavy Metals and Sex Hormone Levels in Serum of E-Waste Dismantling Area Males

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An E-waste dismantling area in southern China was selected as the exposure area and a green plantation with no E-waste dismantling was chosen as a control area. Sex hormone levels were measured in the serum of 187 males in the exposure area employed in E-waste dismantling, 74 males in the exposure area not employed in E-waste dismantling and 92 males in the green plantation. Information was collected on the ages of study subjects, E-waste dismantling techniques and processes, length of time employed in E-waste dismantling, eating habits and living habits. Serum results from the three treatment groups were analyzed for correlations between the concentration of heavy metals and Follicle stimulating hormone and Luteinizing hormone levels. We found that males with occupational exposure to E-waste have higher levels of Follicle stimulating hormone in their serum than males who do not have occupational exposure to E-waste. The average value of which was 10.94 mLU/mL. The serum Luteinizing hormone levels of the non-occupational exposure group was higher (average value of 5.80 mLU/mL) than serum Luteinizing hormone levels of the green plantation group. The B-Pb ad B-Cd concentrations of occupation group males are similar to the concentrations of non-occupation group males, while they are higher than the B-Pb ad B-Cd concentrations of green plantation group males. A correlation analysis suggests that there are significant correlations between the serum Follicle stimulating hormone levels and B-Cu, B-Pb, B-Cd of occupational group males. The serum Follicle stimulating hormone levels have significant correlations with B-Pb and B-Cd of non-occupational group males. There was no correlation between Luteinizing hormone levels and the heavy metal concentrations of occupation group and non-occupational group males. There were significant correlations between serum Follicle stimulating hormone levels and the pickling dismantling method, consumption of external food and alcohol consumption ($P < 0.05$). There was no significant correlation between the other confounding factors and serum Follicle stimulating hormone or Luteinizing hormone levels in males employed in E-waste disassembling.

Keywords: E-waste, Follicle stimulating hormone, Luteinizing hormone, Heavy metal.

INTRODUCTION

With the rapid development of economy throughout the world, the production of solid waste, especially E-waste like abandoned household electronic appliances, telecommunications devices and electronic meters and instruments, has increased sharply, which has already constituted the major threat on the environment. In developed countries, E-waste is growing at the rate of 16 to 28 % per 5 years, which is 3 to 5 times of the growing speed of urban living wastes. Similarly, E-waste also presents a rapidly growing trend in China. According to the incomplete statistics, in the first half of the year 2010, the growth rate of mobile phones, notebook computers, integrated circuits (IC), *etc*. was more than 30 % and even close to 50 %. More than 36 million ton E-waste is produced every year all around the world. 2.3 million ton E-waste is produced in China per year, second only to the production of the United States, which is 3 million tons in total a year. At present, China has strictly banned the import of E-waste and most of the developed countries have taken effective measures to control it. However, the cost of dealing with E-waste in developed countries is far higher than that in developing countries, so the majority of world massive E-wastes have mostly been transferred to Asia, 90 % of which are transferred into China. People in several areas of China still follow the original means of manual dismantling, burning and acid pickling to extract precious metals from E-waste, which has brought in serious environmental problems to China. Heavy metals from E-waste such as lead, cadmium and persistent organic compounds like polychlorinated biphenyls (PCBs), polybrominated diphenyl (PBDEs) *etc*., are released into the surroundings, which severely endanger the health of local residents and organisms.

Secretion of sex hormone is directly controlled by the pituitary and hypothalamus, while the sex hormone secreted by gonadal can affect the activity of the pituitary and the hypothalamus by means of positive and negative feedbacks. The three tissues interrelate and restrict each other, which constitute the hypothalamic-pituitary-gonadal axis. The pituitary can secrete follicle stimulating hormone (FSH) and luteinizing generate hormone (LH). Follicle stimulating hormone has a role in the promotion of sperm development, while LH has a function of stimulating Leydig cells to generate the male hormone. It has been reported that E-waste dismantling releases pollutants which affect sex hormone through various exposure routes. Thoreux's study found that Pb could reduce the reproductive capacity and human sperm activity, affecting the level of male sex hormones[1]. Kuriyama *et al.* found that exposing male mice to the low-dose BDE-99 through their skin could affect the process of spermatogenesis and led to the decline of the number of the sperms and the sperm cells[2]. The researchers in various countries have studied E-waste pollution in various environmental media and the effects on human health by various exposure routes[3-5], but there are few reports about the effects of E-waste dismantling on human sex hormone levels and its mechanism exploring.

The correlations between the heavy metals concentrations in the male serum and the sex hormone levels in urinary and the effects of various exposure factors on the occupational male sex hormone levels in the E-waste disassembling area were analyzed in this study to reveal the impact of E-waste in the dismantling process on the human body inner exposure health and to provide reference for the technical improvement of the Chinese E-waste treatment field and the management policy of solid waste resources.

EXPERIMENTAL

Sample: A place in Southern China with the history of E-waste recycling was selected as the exposure area which lies in the southeast coastal area with north latitude 28°22´and east longitude 121°21´, the location of which is shown in Fig 1. One hundred eighty seven male engaged in E-waste dismantling (occupational exposure group) and 74 males didn't engage in E-waste dismantling (non-occupational exposure group)

Fig.1. E-waste exposure location in China

aged from 18 to 80 who have worked in this field more than 1 year were selected. Ninty two males of green plantation without the disassembling in neighboring town which is located in 100 km away were selected as the control group. According to the study purpose, the questionnaire for collecting ages, E-waste dismantling processes, eating habits, life styles and other information was designed. The specific contents were showed in Table-1.

Sample collecting: Subjects in all treatment groups fasted for 12 h and avoided strenuous physical activity for 3 days prior to the collection of 5 mL blood in a coagulant tube. Serum was collected by centrifuge and stored at -20 °C until testing. All treatment subjects were healthy local male residents with normal diets.

Main chemical reagents and instruments: ELISA kits (Wuhan EIAab Science Co, Ltd.) were used to detect human follicle-stimulating hormone (FSH) and human luteotropic hormone (LH). Measuremets were made with a model 680 microplate reader (US Bio-Rad); a TG16-WS (1650D) centrifuge (Shanghai XL Centrifuge Co, Ltd.); and a Z-2000 Zeeman atomic absorption spectrophotometer (HITACHI).

Determination of heavy metals and measurement of sex hormones in serum: Blood samples were digested and serum lead concentration was tested with a Z-2000 Zeeman

TABLE-1
DATA OF POPULATION AND EXPOSURE FACTORS IN E-WASTE DISASSEMBLING AREA MALE

Survey contents		Occupational group (187)	Non-occupational group (74)	Control group (92)
Average residence time (a)		35.45	38.28	40.39
Dismantling data (a)		8.31	-	-
Dismantling ways	Pickling (%)	28.89	-	-
	Chemical solvents (%)	42.76	-	-
	Burning (%)	56.02	-	-
	Wear mask (%)	20.32	-	-
	Wear glove (%)	28.67	-	-
	Manual dismantling (%)	10.57	-	-
Habit of diet and life	Drinking tap water (%)	11.22	9.20	12.52
	Consumption of local rice (%)	90.87	93.28	97.85
	Consumption of external food (%)	76.54	82.12	80.25
	Consumption of external fish (%)	92.04	95.43	89.57
	Smoking (%)	34.54	29.45	39.45
	Drinking (%)	44.28	40.81	40.22

Atomic Absorption Spectrophotometer. The temperatures for drying, ash and atomization were 85-120, 700 and 2300 °C, respectively. Follicle stimulating hormone, Luteinizing hormone were tested by three methods in strict accordance with the instructions of the kits: antigen-antibody reaction, magnetic separation and colour development reaction by enzyme-linked sorbent immunoassay.

Statistical analyses: Statistical analyses were performed with SPSS 15.0 software. Differences in serum heavy metal levels and sex hormone levels of the exposure group and the control group were analyzed with a *t*-test. Correlations between heavy metal levels and sex hormones in the serum with other exposure factors were analyzed with the Spearman method.

RESULTS AND DISCUSSION

Serum sex hormone levels and heavy metal concentrations in males employed in E-waste disassembling: The sex hormone levels and concentrations of Fe, Cu, Pb, Cd in serum (B-Fe, B-Cu, B-Pb, B-Cd) of males in the group employed in E-waste dismantling (occupational group), the group living in the exposure area, but not employed in E-waste dismantling (non-occupational group) and the group living in the green plantation area (control group) are shown in Table-2. Serum FSH levels were the highest (mean 10.94 mLU/mL) in the occupational exposure group. The non-occupational exposure group exhibited the highest serum LH levels (mean 5.80 mLU/mL). The B-Fe, B-Cu, B-Pb concentrations of the non-occupational exposure group were all higher than those of the other groups, whose means were 0.78, 0.22 and 0.21 mg/L, respectively. The B-Pb concentration was far higher in the non-occupational exposure group than the control group by 0.08 mg/L. The B-Cd concentrations of the occupational exposure group were the highest (mean 7.68 μm/L), 5.57 μm/L more than the control group.

Correlation analyses between serum heavy metal levels and sex hormone concentrations: Results on the significance of correlations between serum heavy metal levels and hormone concentrations in the occupational exposure group are shown in Table-3. There were no correlations between the FSH and LH levels and the lg B-Fe, lg B-Cu, lg B-Pb and lg B-Cd concentrations ($P > 0.05$) in the serum of the occupational and non-occupational exposure groups. The serum FSH level in the occupational exposure group was not significantly correlated with the lg B-Fe concentration ($P > 0.05$), but was significantly correlated with the lg B-Cu ($r = 0.591$, $P < 0.05$), lg B-Pb ($r = 0.622$, $P < 0.05$) and lg B-Cd ($r = 0.593$, $P < 0.05$) concentrations. In the non-occupational exposure group there was no significant correlation between the FSH level and the lg B-Fe, lg B-Cu concentrations ($P > 0.05$), but there was a significant correlation between FSH level and lg B-Pb ($r = 0.521$, $P < 0.05$) and lg B-Cd concentrations ($r = 0.515$, $P < 0.05$). In the control group there was no correlation between FSH and LH levels and lg B-Fe, lgB-Cu, lg B-Cd concentrations ($P > 0.05$). However, there was a significant correlation between FSH levels and lg B-Pb concentration ($r = 0.507$, $P < 0.05$).

Correlation analyses between dismantling methods and serum sex hormone levels in males employed in E-waste disassembling: The main methods of dismantling E-waste in our study area are pickling, chemical solvents, burning and manual dismantling. We explored the potential that different dismantling methods may affect heavy metals exposure for males employed in E-waste disassembling and therefore affect the sex hormone levels of these males. The dismantling method

TABLE-2
SEX HORMONE LEVELS AND HEAVY METAL CONCENTRATIONS IN SERUM OF MALES EMPLOYED IN E-WASTE DISASSEMBLING

	Occupational group		Non-occupational group		Control group	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Sex hormone levels in serum						
FSH (mLU/mL)	187	10.94 ± 11.21	67	10.58 ± 10.29	92	8.44 ± 7.18
LH(mLU/mL)	187	5.74 ± 5.94	67	5.80 ± 6.01	92	4.69 ± 4.20
Heavy metal concentrations in serum						
B-Fe (mg/L)	187	0.78 ± 0.42	67	0.76 ± 0.44	92	0.77 ± 0.53
B-Cu (mg/L)	187	0.19 ± 0.09	67	0.22 ± 0.11	92	0.20 ± 0.10
B-Pb (mg/L)	187	0.18 ± 0.96	67	0.21 ± 1.20	92	0.12 ± 1.34
B-Cd (μg/L)	187	7.68 ± 4.79	67	7.59 ± 4.86	92	2.11 ± 2.53

TABLE-3
CORRELATION BETWEEN HEAVY METAL LEVELS AND SEX HORMONE LEVELS IN SERUM OF E-WASTE DISASSEMBLING AREA MALES

Correlation		Occupational exposure group		Non-occupational exposure group		Control group	
		lgFSH	lgLH	lgFSH	lgLH	lgFSH	lgLH
lg B-Fe	Correlation	0.032	-0.212	0.107	0.135	0.034	0.116
	Significance	0.857	0.228	0.545	0.447	0.848	0.512
lg B-Cu	Correlation	-0.596	-0.124	-0.001	0.143	0.144	0.064
	Significance	0.000**	0.486	0.997	0.42	0.119	0.718
lg B-Pb	Correlation	-0.622	-0.115	-0.521	-0.133	0.507	0.111
	Significance	0.000**	0.581	0.000**	0.454	0.000**	0.228
lg B-Cd	Correlation	0.593	0.024	-0.515	-0.122	-0.226	0.253
	Significance	0.000**	0.892	0.000**	0.618	0.200	0.070

**In.the 0.01 level (bilateral) was the significant correlation and dismantling time were the major factors affecting heavy metal exposure. Our results suggest that there is a significant correlation ($P < 0.05$) between dismantling method and sex hormone levels of males.

Correlation analyses between confounding factors and serum sex hormones levels in males employed in E-waste disassembling: We quantified confounding factors to heavy metal exposure such as using protective tools (gloves and masks), oral exposure (consumption of local rice or seafood) and living habits (smoking and alcohol consumption). Correlations between the quantified values of serum sex hormone concentrations in males exposed to heavy metals in the E-waste disassembling area and confounding factors were analyzed (Tables 4 and 5). There were significant correlations between serum FSH levels and the pickling dismantling method, consumption of external food and alcohol consumption ($P < 0.05$). There was no significant correlation between the other confounding factors and serum FSH or LH levels in males employed in E-waste disassembling.

In humans sex hormone secretion is regulated by the hypothalamus-pituitary-testicular axis. The hypothalamus secretes a gonadotropin-releasing hormone (GnRH), which then circulates through the hypophysial portal vein. Gonadotropin-releasing hormone promotes the pituitary gland to secrete FSH and LH to regulate the function of the reproductive system. Meanwhile, the reproductive organs produce sex hormones to regulate the function of the hypothalamus and the pituitary by means of a negative feedback loop. In this way the endocrine activities of sex hormones are maintained at a stable level. Testosterone has an inhibitive effect on GnRH synthesis in the hypothalamus and LH secretion by the pituitary gland. Negative feedback regulation of FSH is realized by an inhibitor secreted by Sertoli cells that can specifically inhibit the FSH secretion of the pituitary to inhibit the process of spermatogenesis[6].

The results of this study showed that there was a correlation between serum FSH levels and B-Pb and B-Cd levels in the occupational, non-occupational exposure groups and the control group. There was no correlation between serum LH levels and B-Pb and B-Cd levels in those same groups. Presumably, changes in FSH levels could be the result of Pb and Cd acting on the stromal testosterone cells, causing testosterone concentration to decline and leading to a change in serum FSH concentration *via* the negative feedback of the hypothalamus-pituitary-testicular axis.

Batra *et al.*[7] showed that lead exposure in people and some animals could lead to a reduction in total number of sperm, increased sperm aberration rate, weakened sperm motility, lowered serum androgen levels and lowered reproductive capacity in general. Bizarro *et al.*[8] reported that continuous exposure to lead could result in abnormality and apoptosis of the stromal cells, eventually blocking the synthesis of testosterone and feedback of the serum FSH levels. Jia *et al.*[9] found that when a dark-spotted frog (Rana nigromaculata) was infected with low lead levels (0.1-1.6 mg/L) for 30 d, the number and activity rate of sperm was reduced as the lead exposure dose increased. The sperm deformity rate increased significantly as the lead exposure concentration rose and took on a dose-effect relationship. This indicates that the lead exposure caused significant toxic effects on the testis of Rana nigromaculata. Yu Min *et al.*[10] found low blood FSH levels (6.45 mU/mL) in males that had been exposed to lead, medium FSH levels (7.99 mU/mL) in males that had been exposed to a medium concentration of Pb and the highest FSH levels (8.41 mU/mL) in a high Pb exposure group of males. The differences between these FSH levels were all statistically significant. Gunnarsson *et al.*[11] reported that when rodents were injected with a Cd solution of a certain dose, the reproductive system was damaged by Cd and sex hormone levels were changed. Zeng *et al.*[12] survey of 263 volunteers on mainland China found

TABLE-4
CORRELATION ANALYSES BETWEEN DISMANTLING METHOD AND SERUM SEX HORMONE LEVELS IN MALES EMPLOYED IN E-WASTE DISASSEMBLING

Impact factors	lg-FSH		lg-LH	
	Correlation	P-value	Correlation	P-value
Pickling	0.706	0.000**	0.279	0.11
Chemical solvents	0.285	0.102	0.023	0.895
Burning	0.353	0.04	0.699	0.005*
Manual dismantling	0.226	0.200	0.031	0.863
Dismantling time	0.567	0.007**	0.612	0.013*

* In the 0.05 level (bilateral) was the significant correlation, ** In the 0.01 level (bilateral) was the significant correlation

TABLE-5
CORRELATION BETWEEN SEX HORMONE LEVELS IN MALE SERUM AND THE CONFOUNDING FACTORS

Confounding factors	FSH		LH	
	Correlation	P-value	Correlation	P-value
Wear safety mask	-0.291	0.095	0.093	0.095
Wear safety gloves	-0.211	0.231	-0.137	0.441
Drink tap water	0.064	0.718	-0.100	0.572
Consumption of local rice	0.517	0.010*	0.319	0.066
Consumption of external food	-0.123	0.488	0.196	0.267
Consumption of external fish	0.09	0.613	-0.263	0.133
Alcohol consumption	0.119	0.410	0.095	0.988
Tobacco smoking	0.578	0.000**	-0.040	0.824

* In the 0.05 level (bilateral) was the significant correlation, ** In the 0.01 level (bilateral) was the significant correlation

a significant correlation between B-FSH level and B-Cd level, which indicated that Cd exposure might suppress FSH levels. Zeng's result is consistent with that of this study. Li *et al.*[13] injected rat enterocoelia with 0, 0.25, 0.5 and 1 mg/(kg bw) of cadmium over 7 days. Their results showed that the number of epididymal sperm was significantly reduced compared to the control group, indicating that cadmium weakens the spermatogenic function of rats. Xu *et al*.[14] reported similar results. Zeng *et al.*[15] exposed rats to cadmium through drinking water (0.50, 100 and 200 mg/kg). They found that LH levels (2.53 ng/mL) were significantly lower in the cadmium-infected group exposed to a concentration of 200 mg/kg over six months than LH levels of the control group (4.52 ng/mL). These LH levels are significantly different, but Zeng *et al.*[15] did not find any obvious differences in FSH levels which was inconsistent with the results of this study. These results suggest that there may exist a certain dose-effect relationship between Pb and Cd concentrations in human blood and sex hormone levels in the human body. The nature of this dose-effect relationship is currently being tested by this study group. Studies on the effect of Cu-Zn interactions on the reproductive system and sex hormone levels in the human body have been carried out in China and elsewhere[16,17], but the correlation between Cu serum levels and sex hormone levels is rarely reported. In general little is known about the effect of Cu on reproductive systems. This study found a correlation between serum FSH levels and B-Cu levels in the occupational exposure group, but no correlation between serum FSH levels and B-Cu levels in the non-occupational exposure group. Therefore, the effect of Cu on human sex hormone levels requires further study.

This study found a correlation between serum FSH levels and the acid pickling method of E-waste dismantling, as well as a correlation between serum LH levels and the directly-burning method of E-waste dismantling. However, there was no correlation between sex hormone levels and the other dismantling methods. This might be because the various pollutants released by the E-waste dismantling process enter into the human body through different exposure routes and caused different levels of damage to the reproductive system. We found that the dismantling time is a significant confounding factor to determining sex hormone levels. The correlation between serum FSH and LH levels in occupational males and the dismantling time was shown to be significantly correlated. This may be because the exposure doses of Pb and Cd in occupational males investigated in this study increased along with an increase in dismantling time.

This study found a correlation between serum FSH levels and the consumption of local rice in the occupational exposure male group. But there was no correlation between sex hormone levels and other oral exposure routes. This may be because the enrichment concentration of pollutants in the human body and corresponding health effects are different when exposure is through different oral intake media. The metabolic and enrichment mechanisms of pollutant exposure from local rice have not been determined so far[18].

This study also found that long-term alcohol consumption caused some damage to the human reproductive system. Shi *et al.*[19] demonstrated that ethanol and its metabolites produced lipid peroxidation in testicular cells, which, as a result, damage spermatogenic cells, but support cells and stromal cells. The effects of alcohol on serum LH and FSH levels are rarely reported. This study did not find any correlation between alcohol consumption and serum sex hormone levels.

Ochedalski *et al.*[20] found that smoking could change the sex hormone levels in the male body. Compared with males who have never smoked, levels of 17-β-estradiol, corticosteroids and estriol levels in male smokers increased significantly, while the FSH, LH, PRL and testosterone levels were obviously reduced. Sofikitis *et al*.[21] found that serum testosterone, LH and FSH levels of regular male smokers was not obviously reduced compared with those of non-smokers. Guang and Weng[22] found that smoking could change male hormone levels. The levels of FSH and estradiol in male smokers increased significantly compared to male non-smokers. The same conclusion was drawn in this study, because carbon monoxide, nicotine, tar, cadmium, lead and other hazardous substances from burning cigarettes entered into blood circulation through breathing, interfered with the testicular microcirculation through a long-term accumulation and eventually changed the sex hormone levels.

The serum FSH and LH levels of occupational group males are respectively 10.94 and 5.74 mLU/mL. The serum FSH and LH levels of non-occupational group males are respectively 10.58 and 5.80 mLU/mL. The serum FSH and LH levels of control group males are relatively lower which are 8.44 and 4.69 mLU/mL. The B-Pb ad B-Cd concentrations of occupation group males are similar to the concentrations of non-occupation group males, while they are higher than the B-Pb ad B-Cd concentrations of control group males.

There are significant correlations between the serum FSH levels and B-Cu, B-Pb, B-Cd of occupational group males. The serum FSH levels have significant correlations with B-Pb and B-Cd of non-occupational group males. There was no correlation between LH levels and the heavy metal concentrations of occupation group and non-occupational group males. There were significant correlations between serum FSH levels and the pickling dismantling method, consumption of external food and alcohol consumption ($P < 0.05$). There was no significant correlation between the other confounding factors and serum FSH or LH levels in males employed in E-waste disassembling.

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