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Original Research

Semen Quality and Lead Concentrations of Men in an Electronic Waste Environmental Pollution Site

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Abstract

The aim in this study was to assess relationships between men exposure to lead and semen-quality parameters in an environmental pollution site. We recruited 95 men through the pollution area and two controls. We measured semen quality using computer-aided semen quality analysis, and lead levels in seminal plasma using graphite gurnace atomic absorption spectroscopy. The results showed that the semen quality of men from the pollution area was lower than those of other control districts. The mean of seminal plasma lead value in the pollution area were higher than reference values for controls. Seminal plasma lead values displayed a significant negative correlation with norm morph sperm rates. Male reproductive health may be threatened by environmental pollution, and it may be influenced by local population diathesis.

Keywords: environmental pollution, male reproductive health, lead

Introduction

The male reproductive system is very sensitive to the environment, and environmental pollution is one factor contributing to a decrease of sperm quality for human beings [1, 2]. Evidence for declining sperm counts and quality, especially in young men in recent decades, indicates that environmental impact on spermatogenesis is becoming an important health issue [3-5]. Some researchers have reported that lead may affect sperm chromatin by altering sperm Zn availability [1]. Sperm chromatin structure is affected at lead level in blood higher than 45 μg/dL [6]. Acharya has reported an increasing number of atypical spermatozoon and a decrease in sperm counts in mice after intraperitoneal injection of 100 mg lead acetate/kg of body weight [7]. Some scholars reported steroidogenic effects of lead in male Wistar rats [8].

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Rapid development of modern science and technology has lead to the current period of electronic products, thus large quantities of electrical and electronic waste equipment (e-waste) are generated. The dismantling and disposal of e-waste in China has caused concern because of its impact on the environment and risks to human health. E-waste recycling in China is often performed by family-run workshops using uncontrolled methods that damage the environment and threaten local people's health [9-11]. There are more than 1000 different substances in e-waste, many of which are highly toxic. A main material in e-waste is lead. Every computer contains between 1-2 kg of lead. Lead is used in PC applications; it makes up to 37% of the tin-lead solder that connects computer chips to the printed wiring boards, 20% of the weight of the monitor is lead, and it is used as a plastic stabilizer in PVC cabling [12]. Several studies have reported residents living in e-waste recycling site with soaring levels of lead [13-17] and lead concentrations in the surface soils. Surface water and sediments of e-waste recycling region have been monitored [18, 19].

So we presume that male reproductive health may be influenced by local e-waste environmental pollution. We investigated lead levels and sperm quality of men in an e-waste environmental pollution site to explore the effects of e-waste environmental pollution on male reproductive health.

Materials and Methods

Study Population

Thirty volunteers were recruited from an e-waste environmentally polluted area (exposure group). Volunteers are workers and other staff in a factory engaged in dealing with e-waste. For control groups volunteers were recruited from towns 100 km away (control group one, 32 individuals) and 200 km away (control group two, 33 individuals) from the polluted area. The population, traffic density, cultural background, lifestyle, and socioeconomic status were very similar to each other in these towns. The selection criteria were age (20-45 years), comparable lifestyle and socioeconomic status, etc. This study excluded adult men that have congenital diseases, hypertension, diabetes mellitus, infection symptoms, and male reproductive disease. The study was approved by the Human Ethics Committee of the Medical School of Ningbo University.

Sample Collection and Storage

Sperm were collected from the 30 individuals from the environmentally polluted area, and 32 and 33 individuals from control sites of control group 1 and control group 2. Participants had been directed to abstain from ejaculation for 3 to 5 days before providing the semen. Semen was collected via masturbation into separate sterile glass beakers, and incubated at 37º for 30 min, allowing the sperms to stay active. Then semen was diluted to obtain a concentration for computer-aided sperm analysis. After that, the samples were subjected to 1,000 g centrifugation for 25 minutes to break the seminal plasma. Seminal plasma was stored at -20º for detecting lead concentrations.

Computer-aided Semen Quality Analysis

After liquefaction and within 1 hour of ejaculation, the samples were analyzed for semen volume and pH. Each specimen was diluted at least 1:1 with phosphate buffered saline and loaded into one chamber of a 20-micron-deep chamber, placed on a stage warmer set to 37ºC, and observed. The sperm count and motility were determined by using an eyepiece reticule and bright-field light microscopy at×400 total magnification (sperm analytical system MX7.5). Sperm motility was defined as WHO motility grade A (rapidly progressive motility), grade B (progressive motility), grade C (non-progressive motility), or grade D (immotile) by systematic visual scanning of the microscopic field. Each analysis was conducted in duplicate for each specimen on the same microcell by the same technician. Sperm concentration values were measured using the improved Neubauer hemocytometer method according to WHO 1999 guidelines [20, 21].

Heavy Metal Concentration Analysis

Semen was collected by masturbation into sterile heavy metal-free plastic containers. After that, the samples were subjected to 1,000 g centrifugation for 25 minutes to break the seminal plasma. Seminal plasma were added to portions of optima grade concentrated nitric acid in a glass centrifuge tube at 80º for removing protein in fume cupboard. The resulting aqueous digests were diluted 5-fold with 2% nitric acid to provide sufficient volume of each sample to determine several heavy metals by Graphite Furnace Atomic Absorption Spectroscopy (SHIMADZU GFA-7000, AA-70000, Japan). The main parameters for lead determination were wavelength 283.3 nm, current 4 mA, slit width 0.8 nm, drying at 90ºC/105ºC/120ºC, ashing at 600ºC, and atomization at 1,500ºC. The accuracy of the method was controlled by recoveries between 95% and 107% from the spiked blood samples.

Statistical Analysis

All analyses were performed using SPSS v13.0 (SPSS Inc., Chicago, IL, USA). Student-Newman-Keuls analysis of One-way ANOVA was used to compare intergroup values of semen quality and lead concentration. Spearman rank correlation analysis was used to evaluate the correlation between lead concentration in seminal plasma and semen quality. A $P<0.05$ was considered statistically significant.

Results

Semen Quality

The semen characteristics of the men in the exposure and control groups are listed in Table 1. Participants based in the exposure group had the lowest mean sperm concentration (46.302×10^6/mL), in control group one had 57.466×10^6/mL mean sperm concentration, and those in control group two had the highest mean sperm concentration (94.997×10^6/mL), and the difference was statistically significant. The semen volume, sperm motility in participants (include a, b, c, and a+b+c) from the exposure group were lower than those of other districts ($P<0.05$). Mean norm morph sperm rate was lower in pollution area than control groups one and two (57.342%, 62.691%, and 74.482%, $P<0.05$). However, the abnormal sperm rate, mix abnormal of men in exposure group was higher than controls; the head, tail, and body abnormal sperm rate of men in exposure group and control group one were higher than control group two ($P<0.05$).

Table 1. Mean semen characteristics by sampling districts [Mean (SD)]

District	Semen volume (mL)	PH	Sperm concentration ($\times 10^6$/mL)	Sperm rapid progressive motility (a%)	Sperm slow progressive motility (b%)	Sperm no progressive motility (c%)	Total motility (a+b+c)%	Normomorph sperm rate (%)	Abnormal sperm (%)	Head abnormal sperm (%)	Tail abnormal sperm (%)	Body abnormal sperm (%)	Mix abnormal sperm (%)
Exposure	2.833 (0.469)	7.57 (0.274)	46.302 (36.682)	13.684 (13.106)	8.29 (4.420)	13.836 (6.448)	35.836 (20.006)	57.342 (15.602)	35.992 (9.808)	25.126 (6.947)	2.206 (0.640)	4.331 (1.323)	4.331 (1.323)
Control one	2.297 (0.659)	7.466 (0.312)	57.466 (25.293)	21.258 (11.867)	15.361 (4.312)	20.631 (6.769)	57.253 (13.950)	62.691 (0.798)	37.309 (0.798)	26.313 (1.861)	2.83 (1.109)	4.36 (1.284)	3.807 (1.383)
Control two	3.464 (1.605)	7.088 (0.217)	94.997 (79.934)	27.538 (22.454)	10.343 (5.991)	20.673 (13.168)	58.559 (28.236)	74.482 (32.202)	13.397 (16.536)	11.32 (14.017)	0 0	0.91 (3.720)	1.192 (2.432)
P value *	0	0	0.001	0.006	0	0.006	0	0.005	0	0	0	0	0

* P values were derived by Student-Newman-Keuls analysis of One-way ANOVA to compare intergroup values. P < 0.05 was considered statistically significant.

Lead Concentration in Seminal Plasma

Each seminal plasma specimen was assayed in quadruplicate for lead (Fig. 1). The mean of seminal plasma lead value in exposure group were significantly higher than reference values for controls and populations not occupationally exposed to lead (6.328 ug/L, 3.354 ug/L, 1.356 ug/L).

The Correlation between Semen Quality and Heavy Metal Concentration in Seminal Plasma

Table 2 shows the correlation between semen quality and lead concentration in seminal plasma. Seminal plasma lead values displayed a significantly negative correlation with norm morph sperm rate (correlation coefficient: -0.379, P<0.05).

Discussion

This study showed that the semen quality in men from the e-waste pollution area were lower than those of other control districts. The mean of seminal plasma lead value in the e-waste pollution area were higher than reference values for controls. Seminal plasma lead values displayed a significantly negative correlation with norm morph sperm rate.

Lead concentrations in the surface soils, surface water and sediments of e-waste recycling region were higher. Several studies showed residents living in the e-waste recycling site with soaring levels of lead. There is agreement that even moderate concentrations of lead reduce human semen quality. Fatima et al. observed that >40 μg/dL of lead in blood produced a decline of sperm count (<20×10^6 cells/mL), and lower motility (<50%) and morphology (<14%), with >35 μg/dL in whole blood [22]. Low lead concentrations in seminal plasma (0.2 μg/dL) were associated with impaired semen quality, 44% of motility, 32% of normal morphology, and 11×10^6 cell/mL of sperm concentration [1]. But in contrast, our study found levels of lead 10

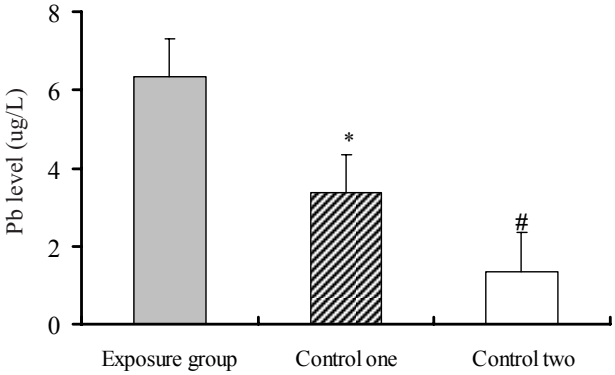

Fig. 1. Seminal plasma lead levels [ug/L Mean (SD)].
* the lead levels of men in exposure group was higher than control 1 (P < 0.05),
# the lead levels of men in exposure group was higher than control 2 (P < 0.01).

Table 2. Relationship between seminal plasma lead levels and semen quality*

	Semen volume (mL)	PH	Sperm concentration ($\times10^6$/mL)	Sperm rapid progressive motility (a%)	Sperm slow progressive motility (b%)	Sperm no progressive motility (c%)	Total motility (a+b+c)%	Normomorph sperm rate (%)	Abnormal sperm (%)	Head abnormal sperm (%)	Tail abnormal sperm (%)	Body abnormal sperm (%)	Mix abnormal sperm (%)
Lead	-0.030	-0.260	0.042	-0.279	-0.275	-0.038	-0.200	-0.379#	0.057	-0.115	-0.221	-0.097	-0.097
	0.875	0.166	0.824	0.135	0.142	0.840	0.290	0.039	0.765	0.545	0.240	0.611	0.611

*results of spearman correlation analyses: correlation coefficient and P values.
P values < 0.05

times higher in the seminal plasma (2.93 μg/dL) related with low motility, but not related with morphology (>14%) or sperm concentration (>20×10^6 cells/mL) [23]. Meeker et al. reported no effect on sperm concentration or motility with 1.5 μg/dL of lead concentration in whole blood [24]. Our study result showed that participants based in the pollution area had the lowest mean sperm concentration (46.302×10^6/mL) than two control groups (57.466×10^6/mL and 94.997×10^6/mL). Sperm motility in participants from the pollution area was lower than those of other districts (P<0.05). Mean norm morph sperm rate was lower in the pollution area than groups 1 and 2 (57.342%, 62.691%, and 74.482%, P<0.05). Seminal plasma lead values displayed a significant negative correlation with norm morph sperm rate.

In this study, we also found higher lead levels in control 1 compared to control group 2. We think the reason is the e-waste environmental polluted area close to the ocean, and the district that recruited control 1 group volunteers is located downstream from the polluted ocean. Although the control 1 area is 100 km away from the pollution area, pollution can spread to the control area. The district that recruited control group 2 individuals is 200 km away from the pollution area, has first-rate standard atmosphere quality in China, and is not a coastal region.

Our study showed that seminal plasma lead value in the e-waste pollution area were higher than reference values for controls, the semen quality of men from the e-waste pollution area were lower than those of other control districts, and there is correlation between the lead values of seminal plasma and norm morph sperm rate. So we deduce that male reproductive health may be threatened by environmental pollution, and it may influence local population diathesis.

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