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Seminal Plasma Metals Concentration with Respect to Semen Quality

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Abstract The aim of the current study was to assess relationships between multiple metals burden in human seminal plasma and semen quality parameters. Levels of five metals (lead, manganese, copper, arsenic, and selenium) in human seminal plasma were determined by inductively coupled plasma mass spectrometry (ICP-MS), and the correlations between the metal concentrations and semen parameters (sperm concentration, sperm motility rate, and sperm morphology) were analyzed. The activities of acid phosphatase (ACP) and of α-glucosidase in human seminal plasma were also determined. Of the 100 subjects, 21 had fertility problems according to the World Health Organization criteria and were designated as "abnormal group." Significant inverse correlations were found between the concentrations of Cu, As, Pb, and the sperm concentrations (r_{Cu}=-0.312, P_{Cu}=0.029; r_{As}=-0.328, P_{As}=0.021; r_{Pb}=-0.377, P_{Pb}= 0.008). Moreover, the Cu, Mn, and Se concentrations were significantly higher in the abnormal group than that in the normal group (P_{Cu}=0.024, P_{Mn}=0.002, P_{Se}=0.002). The ACP activity was significantly higher in the normal group than that in the abnormal group (P=0.021). We also found a significantly negative correlation between α-glucosidase activity and the levels of As (r=-0.367, P=0.023). These findings provide evidence for relationships between human semen quality and metal exposures. These relationships are consistent with animal data, but additional human and mechanistic studies are needed.

Keywords Human semen quality · Metals · Acid phosphatase · α-Glucosidase

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Introduction

Semen parameters, which include sperm concentration, sperm motility rate, and sperm normal morphology rate, are important targets for analyzing men's ability of reproduction. There is growing concern about the considerable decrease in sperm concentration and semen volume over the last 50 years in general populations worldwide, especially in the USA and in Europe [1–4]. Although the sperm quality of fertile men in China is better as compared with reports from other countries for the same period, the speed of decline is higher [5]. Possible explanations for the decrease in semen quality include increased stress, lifestyle factors, and a variety of endocrine-altering chemicals in the environment. A study indicates that exposure to some metals in the environment can be linked to decreased male reproductive capacity [6].

Lead (Pb) and arsenic (As) are highly toxic metals for humans and other mammals. These metals are pervasive in the human environment and accumulate in the human body over a lifetime. Many experimental animal studies show that Pb and As can adversely affect the mammalian male reproductive system [7, 8]. Inverse correlations between metal burdens and male reproductive parameters have been found in epidemiological studies, and the reduction of semen parameters, such as sperm mobility and sperm count, are linked to elevated metal exposures [9–11]. Some other metals, such as copper (Cu), manganese (Mn), and selenium

(Se), are essential elements for animal and human health but may be harmful above certain levels [12–14]. For example, Cu and Mn, which act as cofactors for a variety of important enzymes, have been associated with reduced semen quality in rodents and in humans [15].

Acid phosphatase (ACP) in seminal plasma is mainly synthesized and secreted by the prostate [16]. The determination of ACP in seminal plasma has been used for the forensic identification. The level of ACP in seminal plasma will decrease if the prostate suffered from the inflammation [17–19]. Furthermore, the level of ACP could be used as a marker for the evaluation of the effects of various drugs or stimulators, such as Pb, male contraceptives, tobacco consumption, clomiphene citrate treatment, and testosterone intake, etc., on the male infertility [20]. The α-glucosidase in seminal plasma is secreted by the epididymis. According to World Health Organization (WHO), seminal α-glucosidase is a marker of human epididymal secretary function. It is significantly correlated to sperm concentration [21]. Moreover, the determination of α-glucosidase might be helpful to evaluate functions of the epididymis [22].

The potential exists for a number of metals to positively or negatively affect male reproduction have been extensively studied. However, most of these studies are based on high doses and not commonly encountered by the general population. Data regarding the male reproductive capacity of metals at environmental level are still limited. For this reason, in our study, the semen quality parameters, ACP and α-glucosidase activities, and the levels of different metals in seminal plasma were measured. In addition, correlation calculations between the metal concentrations and semen parameters (sperm concentration, sperm motility rate, and sperm morphology) were performed.

Materials and Methods

Subject Recruitment

Semen samples were recruited from 100 participants at the Reproductive Medicine Center of the 174th Hospital of PLA, Xiamen, China. The study population was between 23 and 39 years of age. Each subject completed a questionnaire including age and smoking (Table 1). The protocols of the study were approved by the committees on research ethics at all participating institutions, and informed consent was obtained from all participants.

Semen Parameters

Semen samples were collected using standard protocols. Semen parameters including sperm concentration, normal sperm morphology rate, and motile sperm rates were analyzed following the WHO protocol [23]. The 100 subjects were dichotomized as either greater than or less than WHO reference levels for concentration (20 million sperm ml^{-1}), and motility (50% motile sperm). Of the 100 subjects, 21 had fertility problems according to the WHO criteria. Metals level, ACP, and α-glucosidase activities in seminal plasma of 49 subjects (including 21 abnormal and 28 normal chose randomly from the 79 which had no fertility problems) were analyzed.

Table 1 Demographic categories of the general population

Category	Abnormal (n=21)	Normal (n=79)
Age [years (mean±SD)]	34.5±2.6	33.8±3.1
Duration of smoking [no. (%)]		
Never smoker	11 (52.4)	37 (46.8)
Smoker	10 (47.6)	42 (53.2)
Ever smoker	3 (14.3)	10 (12.7)
Current smoker	7 (33.3)	32 (40.5)

Measurement of Metals

All reagents were of analytical reagent grade unless otherwise specified. Double deionized water with a final resistivity of 18.2 MΩ cm^{-1} was provided by a Milli-Q mixed bed ion-exchange system (Millipore, Bedford, MA). All apparatus and material used (plastic and glass) were washed with 10% nitric acid overnight before analysis. A programmable 1200 W microwave (MARS 5, CEM, Matthews, NC, USA) with a rotor for 14 Teflon-lined vessels rated at 210°C and 350 psig (HP-500 Plus, CEM) served as the closed vessel digestion system. Samples were homogenized into 4 ml nitric acid with Rh (2 ppb) as internal standard and added into microwave polytetrafluoroethylene tube. In the first stage, the temperature was ramped to 120°C within 5 min followed by a dwell time of 5 min (with a pressure setting of 200 psig). Then the temperature was ramped to 180°C within 10 min followed by a dwell time of 10 min. After cooling, the sample was transferred into a measuring flask and diluted with Milli-Q water. Metal concentrations were determinated by inductively coupled plasma mass spectrometry (ICP-MS) [24]. We used a PE Sciex ELAN DRC Plus ICP-MS instrument (Perkin-Elmer, USA) for the measurement of metals. The limits of detection (LOD) for the seminal plasma metal levels were as follows: As 10 ng l^{-1}, Pb 1 ng l^{-1}, Mn 10 ng l^{-1}, Se 0.1 μg l^{-1}, and Cu 10 ng l^{-1}.

Determination of ACP and α-Glucosidase Activity

Semen samples were centrifuged at 3,000×g for 15 min to obtain seminal plasma for the detection of ACP and α-glucosidase activity. The ACP activity was analyzed following

WHO protocol [25]. Seminal plasma was diluted for 10,000 times with citrate buffer (0.09 mol l^{-1}), then 5 µl diluted sample was added into test tube with 50 µl balanced solution (nitro phenol sodium). Diluted sample was replaced with p-nitro phenol (0.1 mg ml^{-1}) to serve as standard and replaced with distilled water as blank. All tubes were incubated at 37°C for 30 min. The reaction was terminated by adding 0.5 ml of 0.1 mol l^{-1} NaOH solution. The activity was measured by Synergy™ HT multidetection microplate reader (BioTek, USA). The absorbance of solution was read at 520 nm. The α-glucosidase activity was analyzed using a commercial kit from Nanjing Xindi Biological Pharmaceutical Engineering (Nanjing, China).

Statistical Analysis

We performed data analysis using SPSS, version 13.0. The correlations between all semen quality and metal concentrations were investigated using bivariate analysis. Differences between groups were tested statistically using Mann–Whitney *U*-test.

Results

Significantly negative correlations were found between the concentrations of Cu, As, Pb, and sperm concentrations (r_{Cu}=-0.312, P_{Cu}=0.029; r_{As}=-0.328, P_{As}=0.021; r_{Pb}=-0.377, P_{Pb}=0.008; Fig. 1). The correlations between metal concentrations and sperm motility rates showed that there were negative correlations between the concentrations of Cu, As, Pb, and sperm motility rates (r_{Cu}=-0.261, P_{Cu}=0.074; r_{As}=-0.328, P_{As}=0.075; r_{Pb}=-0.377, P_{Pb}= 0.068; Fig. 2). The average levels of all the five metals in the seminal plasma of the normal group were significantly lower than those of the abnormal group (Table 2), although only Mn, Cu, and Se showed statistically significant difference (P_{Cu}=0.024, P_{Mn}=0.002, P_{Se}=0.002). The ACP activity was significantly higher in the normal group than that in the abnormal group (P=0.021; Table 3). In the present study, we also found a statistically significant negative correlation between α-glucosidase activity and the levels of As (r=-0.367, P=0.023). No statistically significant negative correlations were found between metal

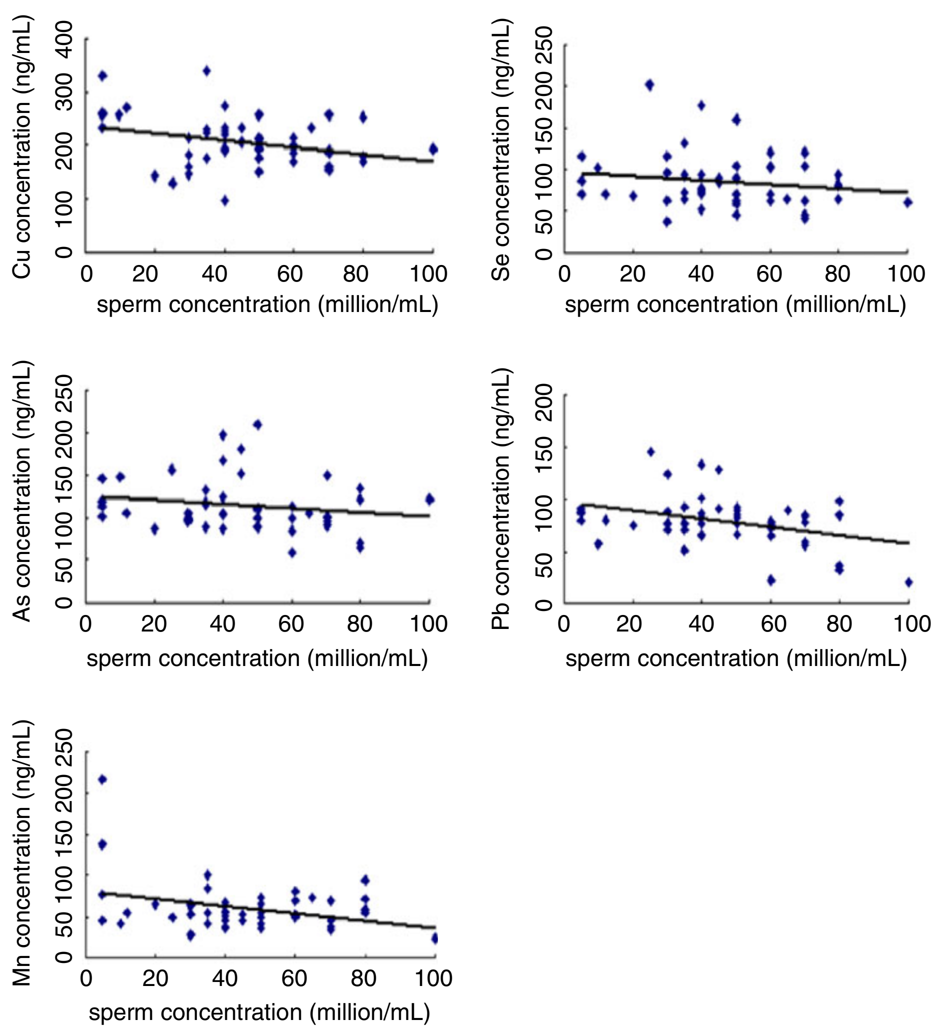

Fig. 1 Linear regression correlations between the observed metal levels and the sperm concentrations (n=49)

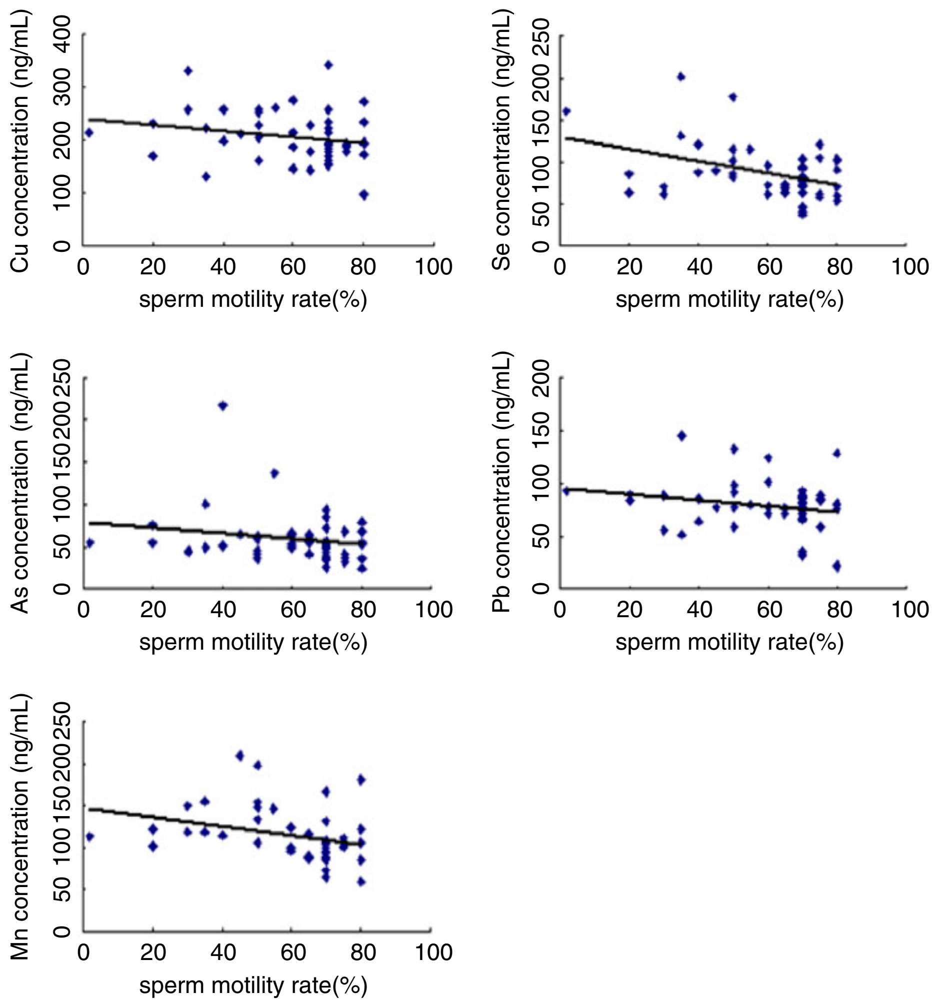

Fig. 2 Linear regression correlations between the observed metal concentrations and the sperm motility rates (n=49)

concentrations and sperm morphology rate (data not shown).

Discussion

It has been reported that Cu deficiency in cows can delay estrus, and in rats and guinea pigs, it results in a greater incidence of fetal death and resorption [26]. A significant positive correlation between Cu concentrations in blood or semen and sperm motility is observed [27, 28]. However, Cu has also been identified as a highly toxic element for sperm, in its ionic form (Cu^{2+}), the trace element affects the fertilizing capacity of human gametes in vitro and interferes with the sperm–oocyte interaction leading to fertilization [29]. Many studies show that higher levels of Cu in blood or seminal plasma have significant adverse effects on sperm motility [30, 31]. In the present study, a statistically significant negative correlation was observed between the levels of Cu and the sperm concentrations. Furthermore, the levels

Table 2 Comparison of the observed levels of six metals in the seminal plasmas of normal men with those designated as abnormal (means±SD)

	Abnormal (n=21)	Normal (n=28)
Mn (ng/ml)	127.57±33.69	103.96±25.00[a]
Cu (ng/ml)	222.76±0.91	195.00±45.24[b]
As (ng/ml)	68.62±41.66	53.16±16.39
Se (ng/ml)	103.79±37.89	73.16±20.35[b]
Pb (ng/ml)	84.00±5.56	74.84±22.41

[a] Significantly different from abnormal populations, $P<0.05$

[b] Significantly different from abnormal populations, $P<0.01$

Table 3 Observed ACP and α-glucosidase activities in seminal plasmas of normal men and in those of men designated as abnormal (means±SD)

	Abnormal (n=21)	Normal (n=28)
ACP activity (U/ml)	19.24±9.26	25.43±8.51[a]
α-Glucosidase activity (U/ml)	40.61±13.91	44.92±12.17

[a] Significantly different from abnormal populations, $P<0.05$

of Cu in the seminal plasma of the normal group were significantly lower than those of the abnormal group.

Although Mn is an essential metal and necessary for the maintenance of health, high levels of this element exposure may cause a decrease in sperm motility and sperm concentration [15]. In addition, Wirth et al (2007) also report that ambient exposure to Mn is associated with reduction in sperm motility and concentration [32]. In the present study, Mn concentration was significantly higher in the abnormal group than that in the normal group, which suggested that exposure to Mn could be harmful to male reproductive health in human. Likewise, the essential trace element Se is indispensable for male fertility in mammals, it is toxic if taken in excess [33]. In the present study, the levels of Se in the seminal plasma of the normal group were significantly lower than those of the abnormal group.

Pb and As are highly toxic metals for the mammalian male reproductive system. Telisman et al. (2007) report nonoccupational Pb exposure, measured as blood Pb levels, to be associated with increased immature sperm and percentage of pathologic, wide, and round sperm in a study of Croatian men [34]. Another study in Mexico shows that Pb measured in spermatozoa or seminal fluid, but not in blood, is associated with decreased semen quality [11]. As is a cell toxicant, As^{3+} can block cell respiration by easily binding to sulfydryl to form a stable complex, block cellular metabolism, and finally inhibit some enzymes [35]. In a cross-sectional study of men attending infertility clinics in Michigan, USA, a significantly increased risk for low sperm motility is reported in men exposed to environmental level of As [36]. However, the role of these metals in male reproductive capacity is not clear. In the present study, a statistically significant negative correlation was observed between the levels of As, Pb, and the sperm concentrations.

The α-glucosidase in seminal plasma is produced mainly in epididymis. The α-glucosidase is significantly correlated to sperm concentration [37]. Elzanaty et al. [38, 39] show a significantly positive correlation between α-glucosidase activity and sperm motility. But no study has proved the relationship between α-glucosidase activity and metal concentrations. In the present study, a statistically significant negative correlation was observed between α-glucosidase activity and the levels of As (r=-0.367, P=0.023).

Taken together, statistically significant inverse correlations were found between the sperm concentrations and the levels of Cu, As, and Pb in the present study. In addition, the observed concentrations of Cu, Mn, and Se in the seminal plasma of the normal group were significantly lower than those of the abnormal group, suggesting that these trace elements also play roles in determining male reproductive capacity. Future work will involve examining these suggestive relationships with a larger sample size. Additional human epidemiologic studies, as well as mechanistic studies, are needed to confirm these findings.

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