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Cadmium level in seminal plasma may affect the pregnancy rate for patients undergoing infertility evaluation and treatment

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ABSTRACT

This study evaluated the relationship between pregnancy rate and semen cadmium concentration. This prospective and nonrandomized clinical study analyzed 341 male partners of infertile couples undergoing infertility evaluation and management. Semen samples were collected to analyze semen quality and cadmium concentrations. The main outcome was pregnancy during 60-day infertility treatment. Simple linear regression analysis revealed an association between semen cadmium concentration NS sperm count ($r = -0.150$, $P = 0.0416$) in nonsmoking subjects ($n = 184$). In both smokers and nonsmokers, semen cadmium concentrations were significantly higher in non-pregnant patients than in pregnant patients. In nonsmokers, Cox multi-variable fertility ratio analysis demonstrated an association between semen cadmium concentration and fertility (fertility ratio of log semen cadmium = 0.24; 95% confidence intervals (CI) = 0.12–0.47, $P < 0.0001$) after adjusting for related variables. Each tenfold increase in semen cadmium concentration was associated with a 4.17-fold increase in infertility ratio in nonsmoking patients. In smokers, Cox multi-variable fertility ratio analysis demonstrated that sperm count and semen cadmium concentration are associated with fertility (fertility ratio of log semen cadmium = 0.17; 95% CI = 0.04–0.63, $P = 0.0085$) after adjusting for related variables. In smokers, each tenfold increase in semen cadmium concentration was associated with a 5.88-fold increase in infertility ratio. In conclusion, low levels of cadmium accumulation in semen may contribute to male infertility by reducing sperm quality.

1. Introduction

Infertility affects approximately 10–15% of reproductive-age couples. In 25% of cases, infertility is attributable to poor semen quality [1]. The etiology of poor semen quality is complicated. Increasing evidence suggests that chemical and physical agents in the environment may affect male fertility in humans [2–4].

Mean sperm concentrations and volumes in normal males have dropped substantially over the past 50 years [5]. This decline in semen quality is believed to be related to exposure to environmental toxins. Rapid industrialization, motorized vehicular traffic and population growth are believed to have increased the toxic metals release into the environment. Heavy metals may also affect semen quality [6]. Cadmium exposure can adversely affect both male and female reproductive systems. Cadmium in seminal plasma may be increased by cigarette smoking as well as local nutritional or industrial exposure. Due to its direct effect on testicular function as well as by hormonal alterations, heavy metals exposure has been associated with impaired semen quality [7]. Although blood tests are standard procedure for toxicological study of heavy metals exposure, recent data indicate they may not adequately reveal heavy metals accumulation in the male reproductive tract. Consequently, seminal heavy metals concentrations could provide a better measure of reproductive toxicity caused by heavy metals exposure [8,9]. Semen analysis is the basic laboratory study for assessing male infertility.

Reports differ as to the accuracy of cadmium testing for assessing semen quality. The purpose of this study was to evaluate cadmium levels in the seminal plasma of male partners of infertile couples undergoing infertility evaluation and to clarify the relationships between sperm quality, pregnancy rate and semen cadmium concentration.

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2. Materials and methods

2.1. Study populations

All male partners of infertile couples attending the reproductive center of Lin-Kou Medical center, Chang Gung Memorial Hospital from January 2005 to December 2005 and undergoing infertility evaluation were recruited for this study. A questionnaire survey collected data regarding patient occupation, smoking status, body height, body weight, marital status, infertility history and other patient data. Patients with a history of heavy metals exposure or who resided in areas known to have heavy metals contamination were excluded from this study. Semen samples were collected to analyze semen quality to measure metals concentrations. All samples were collected by masturbation after 3–5 days of sexual abstinence. All patients underwent infertility treatment and 60-day follow-up. The primary outcome was defined as pregnancy. Patient medical records were reviewed by doctors unaware of patient cadmium concentrations to avoid possible bias. All participants provided informed consent.

2.2. Semen analysis

After liquefaction at 37 °C for 30 min and within 1 h of production, routine semen analysis was performed according to the World Health Organization (WHO) method [10] to assess sperm quality parameters, including semen quantity, sperm motility, sperm count and sperm morphology. The criteria for normozoospermia were $\geq 20 \times 10^6$ ml^{-1} concentration with grade A motility in 25% or grade A + B motility in 50% of spermatozoa and normal morphology in at least 30% of the spermatozoa.

2.3. Cadmium analyses

Approximately 100-μl of seminal plasma was digested with 500-μl of super-grade 0.8 M HNO3 in a glass tube. The residue was dissolved in 1 ml of 1% HNO3 and applied to a graphite tube for cadmium detection by atomic absorption spectrophotometer (Varian spectraAA 200Z, CA, USA). Cadmium recovery rate in spiked semen samples was 96%. The instrument was calibrated using 1, 2 and 4 μg/l standards for cadmium. A sample blank was prepared with each set of samples to control for possible metals contamination from external sources. The cadmium detection level was 0.01 μg/l. This study utilized both internal and external quality-control procedures and obtained consistently satisfactory results. A certified commercially available product (Seronorm Trace Elements, Sero AS, Billingstads, Norway) was employed to check intra-batch accuracy and ensure inter-batch standardization. The coefficient of variation for cadmium measurement was ≤5.0%. External quality control was maintained via participation in two programs: National Quality-Control Program conducted by the Taiwan government and an international program run by the College of American Pathologists.

2.4. Statistical analysis

Group differences were analyzed by Chi-square test combined with Fisher correction and Student's *t*-test. The Mann–Whitney *U*-test was employed (for data that were not OR to analyze data not) normally distributed. All P values were two-tailed, and all results were presented as means ± S.D. Logarithmic transformation of variables, such as semen cadmium concentrations, with non-normal distribution was performed. Relationships between logarithmic transformation of semen cadmium concentration and other variables were determined by simple linear regression. The Cox proportional-hazards model was employed to determine the significance of variables, which included age, body-mass index, semen cadmium levels, semen amount, sperm account, sperm motility, and sperm morphology, in predicting pregnancy during the study period. A *P* value <0.05 was considered statistically significant.

3. Results

Three hundred and forty-one males meeting the inclusion criteria were enrolled in the study. Table 1 presents demographic characteristics for all subjects. Age range was 28–44 years, and mean age was 34.9 (S.D. = 3.7). Approximately 46% of the subjects had a history of tobacco use. The mean body-mass index was 22.6 (S.D. = 1.6). Mean semen volume was 3.0 ml, mean sperm concentration was 63.4×10^6 ml^{-1} and the average percentage of total progressively motile sperm was 60.1%. The average cadmium concentration was 1.52 μg/l (Table 1). All subjects were married, and none had a history of cadmium exposure. Semen samples were evaluated according to WHO standards. In nonsmokers, sperm count correlated with semen cadmium concentration but not with age or body-mass index (Table 2). Table 3 shows the differences in semen cadmium concentrations between pregnant (0.55 ± 0.58 μg/l) and non-pregnant (1.79 ± 2.35 μg/l, $P < 0.0001$ by Mann–Whitney *U* analysis) patients who were nonsmokers. Similarly, semen cadmium concentrations in non-pregnant smokers (1.73 ± 3.33 μg/l) were significantly higher than those in pregnant patients who were smokers (0.55 ± 0.58 μg/l, $P = 0.0014$ by Mann–Whitney *U* analysis) (Table 4). Cox multi-variable fertility ratio analysis revealed an association between semen cadmium concentration and fertility (fertility ratio of log semen cad-

Table 1
The base-line characteristics of study patients ($n = 341$)

Variables	Mean ± standard deviation (range) or number (%)
Age (Y/O)	34.9 ± 3.7 (28–44)
Body-mass index (kg/m^2)	22.6 ± 1.6 (19.2–26.1)
Smoking	157 (46.0%)
Semen cadmium concentration (μg/l)	1.52 ± 2.63 (0.07–25.37)
Semen volume (ml)	3.0 ± 1.2 (0.3–8.0)
Sperm count (×10^6)	63.4 ± 49.6 (0.0–368.0)
Sperm motility (%)	60.1 ± 20.2 (0.0–97.4)
Sperm morphology (%)	58.0 ± 17.8 (0.0–94.9)
Sperm count <20 × 10^6	69 (20.2%)
Sperm motility <50%	95 (27.8%)
Sperm morphology <30%	29 (8.5%)

Table 2
The relation between semen cadmium concentration (logarithmic transformation of cadmium) and age, BMI, semen volume, sperm count, motility and morphology assessed by simple linear regression in patients without smoking ($n = 184$)

Variables	*R* values	*P* value
Age (Y/O)	−0.026	0.7256
Body-mass index (kg/m^2)	−0.090	0.2228
Semen volume (ml)	−0.068	0.3570
Sperm count (×10^6)	−0.150	0.0416
Sperm motility (%)	−0.088	0.2344
Sperm morphology (%)	−0.035	0.6329

Table 3
Means differences of variables between pregnant and non-pregnant group patients without smoking ($n = 184$)

Variables	Pregnant ($N = 48$)	Non-pregnant ($N = 136$)	*P*
Age (Y/O)	34.9 ± 3.5	34.5 ± 3.7	0.5378
Body-mass index (kg/m^2)	22.4 ± 1.2	22.3 ± 1.2	0.6992
Semen cadmium concentration (μg/l)	0.55 ± 0.58	1.79 ± 2.35	<0.0001[a]
Semen amount (ml)	3.1 ± 1.1	3.1 ± 1.3	0.8097
Sperm count (×10^6)	69.0 ± 45.0	61.9 ± 51.6	0.3990
Sperm motility (%)	64.5 ± 15.9	62.2 ± 18.7	0.4436
Sperm morphology (%)	62.1 ± 14.7	59.9 ± 16.9	0.4115

[a] Data by Mann–Whitney U method, and other data by the Student's *t* test.

Table 4
Means differences of variables between pregnant and non-pregnant groups patients with smoking ($n = 157$)

Variables	Pregnant ($N = 21$)	Non-pregnant ($N = 136$)	*P*
Age (Y/O)	36.4 ± 2.9	35.1 ± 3.8	0.1462
Body-mass index (kg/m^2)	23.4 ± 1.5	22.9 ± 1.1	0.0630
Semen cadmium Conc. (μg/l)	0.55 ± 0.58	1.73 ± 3.33	0.0014[a]
Semen amount (ml)	2.8 ± 1.0	2.9 ± 1.2	0.8268
Sperm count (×10^6)	58.9 ± 64.1	63.5 ± 46.9	0.2255[a]
Sperm motility (%)	64.1 ± 20.6	55.9 ± 22.1	0.1126
Sperm morphology (%)	54.8 ± 19.8	55.2 ± 19.0	0.9251

[a] Data by Mann–Whitney *U* method, and other data by the Student's *t*-test.