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RESEARCH ARTICLE

Analysis of semen quality, sperm DNA fragmentation and heavy metals in urine in 1202 males of premarital medical examination

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ABSTRACT

Background: Studies have shown that trace elements may adversely affect male reproduction, even at low levels. The toxic effects of heavy metals on the reproductive system have been mainly studied in animal experiments, and epidemiological evidence for populations exposed to the general environment is limited and inconsistent.

Objectives: Our aim in this study was to analyze the relationship between semen quality and multiple metals or metalloids (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, As and Se) in men undergoing premarital medical examinations (PME) and living in the same city.

Methods: Among 1202 PME men, semen quality and sperm DNA integrity were measured by using flow cytometry. Urine heavy metals were tested using a mass spectrometer at our infertility clinic. The urinary levels of various metals and the sperm DNA fragmentation rate of men with normal sperm parameters (NSP) and abnormal sperm parameters (ASP) were compared.

Results: Among1202 males, 42.0% (505/1202) were smokers, and 42.9% (516/1202) were alcohol users. A total of 594 men (594/1202, 49.4%) had NSP such as sperm concentration, total sperm, sperm motility and sperm morphology, whereas 608 men (608/1202, 50.6%) had some type of sperm-pathology (ASP); 600 had oligo- or astheno- or terato- or oligoasthenoteratozoospermia (OAT), 8 azoospermia 8/1202, 0.7%). The males of long-term outdoor work, smoking and drinking alcohol in ASP group were 252 (41.4%) , 308 (50.7%) and 283 (46.5%), respectively, and more than NSP group [(180, 30.3%), (33.1%) and (39.2%)] ($P<0.05$). There were no significant differences between ASP and NSP in semen volume, liquefaction time, pH, and round cells. The DNA fragmentation index shouwed a statistically significant difference between the two groups (13.3% ± 5.9%, 585 NSPs; vs 16.6% ± 14.1%, 585 ASPs, $P<0.001$). The mean concentrations of vanadium (V), chromium (Cr), cobalt (Co) and lead (Pb) and metalloid arsenic (As) were statistically significant between the two groups [(0.58 ± 0.42 μg, 483 NSPs; vs 0.65 ± 0.62 μg, 483 ASPs, P=0.043), (5.86 ± 15.14 μg, 483 NSPs; vs 11.94 ± 46.76 μg, 483 ASPs, P=0.007), (0.29 ± 0.26 μg, 483 NSPs; vs 038 ± 0.66 μg, 483 ASPs, P=0.008), (4.26 ± 1.90 μg, 483 NSPs; vs 6.47 ± 18.00 μg, 483 ASPs, P=0.008) and (39.69 ± 59.92 μg 592 NSPs; vs 51.85 ± 78.58 μg 598 ASPs)].

Conclusion: Our study suggests that high levels of vanadium, chromium, cobalt, lead and arsenic in urine may adversely affect sperm count, motility, morphology and sperm DNA integrity. Long time of outdoor work, smoking and alcohol consumption are more strongly associated with poorer sperm quality.
Keywords: Semen quality; Sperm DNA integrity; Urine heavy metals; Male fertility

1. Introduction

Male reproductive function is susceptible to various environmental and occupational factors. Evidence indicates that abnormal levels of metal elements in the body play a significant role in the decline of semen quality, and the reproductive system may be one of the organs targeted by heavy metals. However, studies on the toxic effects of heavy metals on the reproductive system have mainly focused on animal experiments involving exposure to high concentrations of heavy metals, while epidemiological evidence remains limited, and findings in populations exposed to general environmental levels are inconsistent. Various compounds have been identified as potential contributors to the decline in male fertility, such as pesticides, dioxins, solvents, and heavy metals [1,2]. Certain metals, such as cadmium, lead, arsenic, and mercury, are non-essential exogenous substances that can be detected in most general populations [3]. Consequently, the health and developmental risks associated with heavy metal exposure have become a topic of interest in the past century. In particular, the aforementioned chemicals can reduce semen quality, thereby affecting male fertility and leading to infertility [4]. Recent studies suggest a potential association between heavy metals and the decline in human semen quality [5,6], which may contribute to an increasing incidence of male infertility. Acute and chronic exposure to high levels of environmental pollutants may impair male fertility by affecting sperm quality, although the actual magnitude of this effect remains controversial [7].

DNA is highly susceptible to chemical modifications by exogenous substances such as pesticides, hydrocarbons, and particularly heavy metals, which can induce oxidative stress. Specifically, DNA damage can compromise sperm quality, increase the risk of genetic and epigenetic abnormalities, and potentially contribute to certain diseases. As potent sources of oxidative stress, environmental toxins impair male reproductive function by disrupting the balance between reactive oxygen species (ROS) production and the antioxidant defense system. Many heavy metals may lead to DNA damage in sperm. For instance, vanadium (V) may be an essential trace element in humans, with a suggested maximum tolerable intake of 1.8 mg/day [8]. However, excessive exposure can adversely affect the physiology and histology of both animals and humans, including the male reproductive system [9]. Cadmium (Cd) has been linked to poor human semen quality and DNA damage. Lead (Pb) exposure not only negatively impacts semen volume, sperm concentration, total sperm count, sperm motility, and morphology but also compromises the structural and functional integrity of sperm DNA. These effects can lead to a significant decline in male fertility [5,10,11]. Chromium (Cr) is an essential nutrient for sugar and fat metabolism; excessive chromium levels can affect sperm motility, and chromium exposure has been shown to influence human sperm motility and morphology [11,12]. Cobalt (Co) can induce oxidative stress and alter markers of oxidative stress in semen, thereby reducing sperm motility and normal morphology[13]. Arsenic is a metalloid ubiquitous in the environment, primarily originating from drinking water and food. Exposure to arsenic can lead to health issues, particularly male reproductive dysfunction, as it reduces testicular weight, accessory organ weight, gonadotropin and testosterone levels, sperm count, motility, and viability. It also increases the production of reactive oxygen species (ROS), downregulates the expression of essential proteins, and disrupts several signaling cascades [14]. The oxidative stress induced by arsenic is associated with impairments in spermatogenesis. Arsenic is widely used in herbicides and insecticides due to its known toxicity to invertebrates, and arsenic exposure has also been linked to human infertility and miscarriage [15-17]. Currently, there are relatively few reports on the relationship between semen and heavy metals in premarital medical examinations (PME).

The objective of this study was to investigate semen quality and urinary trace element levels among men undergoing premarital medical examination (PME) in Jinan City. Semen quality parameters and sperm DNA fragmentation were assessed according to WHO standards [14]. The urinary concentrations of 11 potentially toxic and/or semen quality-affecting metallic elements—aluminum (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb)—as well as two metalloids, arsenic (As) and selenium (Se), were measured. The levels of various metals and metalloids in urine, along with sperm DNA fragmentation rates, were compared between men with normal sperm parameters (NSP) and those with abnormal sperm parameters (ASP).

2. Materials and Method

2.1. Study population

This study employed a cross-sectional design. From February 2020 to February 2023, a total of 1,202 men (aged 21–45) who requested premarital medical examinations (PME) were enrolled in our program. All participants had been living in Jinan City for over 10 years. Prior to the study, participants were informed about the investigation and provided their consent. The study protocol was reviewed and approved by the Ethics Committee/Institutional Review Board of the Maternal and Child Health Hospital of Shandong Province, China. All participants provided written informed consent. After signing the informed consent form, 1,202 subjects completed a psychosocial and behavioral factors questionnaire, which included basic demographic data, medical history, environmental factors, lifestyle, and diet. Their height, weight, and other indicators were measured. Participants with urinary system diseases, genetic disorders, or endocrine disorders were excluded.

2.2.Collection of semen samples

Semen samples were collected in sterile, non-toxic polypropylene containers and liquefied for 25 min. The computer assisted semen analysis (CASA) of Sperm Class Analyzer® (SCA, Spain) was used. The detection parameters mainly include normal semen parameters and sperm activity parameters.

2.3. Sperm morphology test

During the preparation of semen smears, 1 mL of the semen sample was transferred into a 10 mL plastic tube. The semen droplet was evenly spread along the surface of the first slide using a second slide held at a 45-degree angle, and the slide was labeled with the date and sample number. The smear was air-dried naturally and stained using the Diff-Quik method. Two hundred sperm cells were counted.

2.4. Sperm chromatin structure assay (SCSA) using flow cytometry

SCSA is a flow cytometry technique that identifies spermatozoa with abnormal chromatin packing, defined as being susceptible to acid-induced DNA denaturation in situ. The DNA fragmentation index (DFI) were detected. The flow cytometric method was performed based on the manufacturer's instruction and the SCSA procedure was described by Zhang et al. [15]. When the DFI exceeds 30 percent, sperm function is abnormal.

2.5. Metal content measurement in urine

The subjects were asked to collect 50 ml urine in a polyethylene plastic bottle with a lid， then transferred 2 ml urine into EP plastic tubes when back to laboratory，and preservation in -20℃ refrigerator. Urine content of 11 chemical elements potentially toxic and/or able to impact on semen quality - Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, As and Se were measured.

Each participant provided a 50 ml urine sample, which was collected in a sealed polyethylene plastic bottle. Upon returning to the laboratory, 2 ml of urine was transferred into an EP plastic tube and stored in a

freezer at -20°C. The urinary concentrations of 13 chemical elements—Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, As, and Se—with potential toxicity and/or possible effects on semen quality were measured.

Urine Sample Pretreatment: The frozen urine samples were removed from the freezer and placed in a constant-temperature water bath set at 45°C for 3 minutes. After water bath incubation, the samples were transferred to a high-speed centrifuge and centrifuged at 10,000 rpm for 3 minutes. Following centrifugation, 1.0 ml of urine supernatant was pipetted/transferred, and 0.8 ml of concentrated nitric acid and 2.2 ml of deionized water were added to a polytetrafluoroethylene (PTFE) digestion tube.

Analysis of Urinary Elements: The concentrations of Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, As, and Se in urine were analyzed using an iCAP Q inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Fisher, USA) equipped with an Octopole-based collision/reaction cell (Thermo Fisher Technologies, USA). Ultra-pure water was prepared using a Millipore Milli-Q water purification system (Millipore, USA).

Quality Control: For each batch of 50 urine samples, standard reference samples were included for quality control. The recovery rates for the target metals ranged from 82% to 117%, with a coefficient of variation below 10%.

2.6. Statistical analysis

The statistical analysis of the study was performed using an IBM SPSS (Version 22.0. Armonk, NY: IBM Corp.). Data are shown as the mean ± SD. The parametric data were statistically analyzed using Student's t-test. The paired test was used to compare data before and after sperm separation. Statistical significance was set at p-value < 0.05.

3. Results

Demographic Characteristics and Semen Parameters of Participants

A total of 1,202 men undergoing premarital medical examination (PME) were enrolled in this project. Their average age was 30.34 ± 4.16 years, and the mean body mass index (BMI) was 25.87 ± 3.94 kg/m². Additionally, 42.0% (505/1,202) of the participants were smokers, and 42.9% (516/1,202) were alcohol drinkers.

Computer-assisted semen analysis (CASA) revealed that 594 participants had normal sperm parameters (NSP), while 608 had abnormal sperm parameters (ASP). There were no significant differences between the NSP and ASP groups in terms of mean age, height, weight, or BMI (see Table 1). Among the 608 ASP cases, the following conditions were identified: Oligozoospermia (OS): 18 cases (3.0%, 18/608), Asthenozoospermia (AS): 131 cases (21.5%), Teratozoospermia (TS): 162 cases (26.6%), Oligoasthenoteratozoospermia (OAT): 289 cases (47.5%), azoospermi was identified in 8 cases (1.3% of the ASP group, corresponding to 0.7% of the total cohort). Among the 1,202 participants, 770 (64.1%, 770/1,202) were office workers, while 432 were manual laborers.

Table 1. Characteristics of the study population and sperm parameters

Variables	Total (n=1202)	NSP (n=594)	ASP (n=608)	p-Value
Age (years)	30.34 ± 4.16	30.28 ± 3.96	30.14 ± 4.11	0.555
Range (years)	21 - 45	21 - 45	23 - 45	
Height (cm)	177.35 ± 5.74	177.40 ± 5.60	177.3 0± 5.81	0.793
Weight (kg)	81.46 ± 13.61	81.41 ± 12.80	81.80 ± 14.31	0.630
BMI (kg/m^2)	81.46 ± 13.61	81.41 ± 12.80	81.80 ± 14.31	0.573
Occupation				

Variables	Total (n=1202)	NSP (n=594)	ASP (n=608)	p-Value
Office workers (%)	770 (64.1)	414 (69.7)	356 (58.6)	0.000[a]
Manual workers (%)	432	180 (30.3)	252 (41.4)	0.000[a]
Current smokers (%)	505 (42.0)	197 (33.1)	308 (50.7)	0.000[b]
Number of cigarettes		6.6 ± 1.4	10.6 ± 1.6	0.018
Drinking (%)	516 (42.9)	233 (39.2)	283 (46.5)	0.028[c]
Semen samples				
Liquefaction (min)	18.4 ± 3.1	17.7 ± 3.8	18.7 ± 4.8	0.076
pH	7.21 ± 0.23	7.21 ± 0.09	7.20 ± 0.32	0.526
Round cells	1.34 ± 0.67	1.34 ± 0.30	1.32 ± 0.95	0.847
Semen volume (ml)	3.83 ± 1.93	3.80 ± 1.50	3.91 ± 2.37	0.326
Sperm parameters				
Sperm concentration ($\times 10^6$)	50.62 ± 4.67	72.49 ± 3.80	40.83 ± 3.00	0.000
Total sperm ($\times 10^6$)	212.92 ± 17.9	269.21 ± 13.9	159.86 ± 15.6	0.000
PR (%)	36.82 ± 3.4	47.25 ± 9.1	27.23 ± 3.8	0.000
Sperm vitality (%)	76.34 ± 2.5 (n = 574)	80.82 ± 7.3 (n= 287)	72.55 ± 4.9 (n = 287)	0.002
Normal morphology (%)	5.15 ± 0.89	6.95 ± 0.43	3.38 ± 0.28	0.000
DFI (%)	15.07 ± 4.14	13.29 ± 2.93	16.56 ± 4.08	0.000
	(n=1193)	(n= 594)	(n = 599)	

Notes: *PR, progressive rate. DFI, DNA fragmentation index. NSP, normal sperm parameter. ASP, abnormal sperm parameter. a, $x^2 = 16.208$, $p<0.001$; b, $x^2 = 37.739$, $p<0.001$; c, $x^2 = 3.879$, $p<0.05$.*

Distribution of metal concentrations in urine

Heavy metal levels of Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb and metalloids As and Se in urine were measured in 483 men with ASP and 483 men with NSP. The concentrations of V, Cr, Co and Pb and As were significantly differences between NSP and ASP groups (see Table 2).

Table 2. The concentration of 11 metals and 2 metalloids in urine

Variables	NSP (n=483)	ASP (n=483)	*t*	p-Value
Al (μg/ml)	282.45 ± 144.00	282.45 ± 144.01	0.884	0.377
V (μg/ml)	0.58 ± 0.42	0.65± 0.62	2.031	0.043
Cr (μg/ml)	5.86 ± 15.139	11.94 ± 46.76	2.720	0.007
Mn (μg/ml)	3.59 ± 3.08	6.43 ± 39.15	1.588	0.113
Fe (μg/ml)	143.44 ± 278.67	160.05 ± 274.48	0.929	0.353
Co (μg/ml)	0.29 ± 0.26	0.38 ± 0.67	2.672	0.008
Ni (μg/ml)	13.75 ± 30.52	19.29 ± 101.13	1.152	0.250
Cu (μg/ml)	26.54. ± 135.93	21.96 ± 18.85	0.737	0.463
Zn (μg/ml)	643.18 ± 596.00	706.76 ± 678.71	1.637	0.102
Cd (μg/ml)	0.58 ± 0.47	0.61 ± 0.62	0.621	0.498
Pb (μg/ml)	4.26 ± 1.90	6.47 ± 17.98	2.681	0.008
As (μg/ml)	39.69 ± 59.92	51.85 ± 78.58	3.109	0.002
Se (μg/ml)	13.02 ± 7.23	12.38 ± 7.09	1.535	0.125

Notes: *NSP, normal sperm parameter. ASP, abnormal sperm parameter. Due to reagent limitations, urinary heavy metals and metalloids were measured in only 483 cases from each of the ASP and NSP groups.*

4. Discussion

This study investigated semen quality and urinary heavy metal levels in 1,202 men undergoing premarital medical examinations among the long-term resident population (over 10 years) in Jinan City. Basic information about the men was collected through questionnaires and physical examinations. Computer-assisted semen analysis (CASA) revealed abnormal sperm parameters (ASP) in 608 cases (50.6%). Questionnaire data showed that among these ASP cases, manual laborers accounted for 41.4%, which was higher than the proportion in the normal sperm parameter (NSP) group (30.3%). No statistically significant differences were observed between the ASP and NSP groups in terms of age, height, weight, or BMI (Table 1). However, there were statistically significant differences in the number of smokers and alcohol consumers between the ASP and NSP groups, with the former having significantly more individuals in both categories ($P < 0.001$). This suggests that occupation, smoking, and alcohol consumption have a more pronounced impact on sperm parameters. In Jinan, manual laborers generally earn lower incomes compared to corporate employees and office workers, and the former are likely exposed to higher levels of pesticides, fertilizers, and environmental pollution than the latter.

Sperm parameters are influenced by a multitude of factors beyond those observed here including age, genetic background, environment, occupation, and lifestyle. Numerous studies have indicated that over the past few decades, the quality of living conditions and environmental pollution (such as water, air, and soil) significantly affect organism adaptation, reproduction, survival, and the quality of sperm or oocytes, thereby impacting fertility [16-19]. Occupational and environmental exposure to toxic pollutants may lead to declines in sperm concentration, motility, and morphology [20-22].

Smoking is a known environmental exposure and an important environmental toxin. In vitro studies have demonstrated adverse effects of cigarette smoke compounds on sperm parameters. Smokers exhibit reduced sperm concentration and motility [23,24]. Several studies have shown a negative correlation between excessive alcohol consumption and semen quality. Ricci et al. [25], in a review of MEDLINE and Embase, concluded that alcohol intake adversely affects semen volume and normal morphology.

Environmental exposure to heavy metals, particularly lead and cadmium, has been linked to a decline in human semen quality (26). In this study, the urinary levels of V, Cr, Co, and Pb were significantly higher in the ASP group compared to the NSP group ($P < 0.05$), and the sperm DNA fragmentation rate was also significantly elevated in the ASP group ($P < 0.001$). This suggests that these heavy metals may exert adverse effects on sperm parameters such as concentration, total sperm count, motility, and morphology. Higher internal levels of these metals may result from exposure to pesticides, environmental pollution during manual labor, as well as smoking and alcohol consumption.

Animal studies have shown that vanadium (V) exposure induces male reproductive toxicity by generating excessive oxidative stress [8,27]. Human exposure to chromium (Cr) has been associated with certain effects on sperm morphology [12], and seminal plasma Cr levels have been negatively correlated with total sperm motility and normal morphology [12]. Research on environmental cobalt (Co) exposure and its relationship with oxidative stress parameters and antioxidant defense systems in fertile males indicates that Co can induce oxidative stress in sperm and alter markers of oxidative stress in semen (28). Studies also suggest that lead (Pb) may adversely affect sperm motility, morphology, and DNA integrity [29,30]. Even at low exposure levels, blood lead concentrations have shown a significant negative correlation with sperm motility and normal morphology rates [30].

Beyond direct toxicity, arsenic and lead may also disrupt the metabolism of certain essential elements, such as copper (Cu), zinc (Zn), and selenium (Se), by interfering with their absorption, distribution, and bioavailability. These metals can further promote oxidative stress and inhibit DNA repair mechanisms, ultimately contributing to male infertility.

The limitations of this study

This study only tested 1,202 men who underwent premarital examinations in the Jinan area, with regional restrictions. Therefore, it is necessary to continue extensive testing and investigation of men who underwent premarital examinations in other urban areas in the future. More detailed tests for outdoor workers and office workers are not comprehensive either. They only rely on the fact that outdoor workers' exposure to food, water and pollution sources is definitely higher than that of office workers, without considering the specific sources of heavy metal exposure (such as diet, drinking water, and occupational exposure intensity), etc. In view of this, it is necessary to expand the sample size for further in-depth research.

5. Conclusion

Results of routine semen parameters and urine heavy metal concentrations from 1202 males who were required to undergo a premarital medical examination showed that sperm motility, morphology, and DNA integrity were lower in those with higher V, Cr, Co, and Pb levels and metalloid As. Lifestyle habits, particularly smoking and heavy alcohol consumption, may pose a threat to male reproductive health and may affect the quality of local populations.

Author contributions

Zhi-Da Shi and Pu Wang conducted the study; Zhi-Da Shi and Ye Zhao wrote the original draft; Zhi-Da Shi, Pu Wang and Ye Zhao designed the study; Zhi-Da Shi, Pu Wang, Xiang-Yang Sun, Jian Li and Ye Zhao discussed and analyzed the data; Jian Li and Ye Zhao reviewed and edited the paper. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest

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