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Relation Between Cadmium and Mercury and the Deficiency of Human Sperm Nucleus

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Abstract

Objectives: Mercury (Hg) and cadmium (Cd) are metals found in the environment from natural and anthropogenic sources. They are highly toxic to humans and other living beings. Most human exposures come from the consumption of contaminated seafood or occupational exposure. It has been accepted that exposure to heavy metals leads to damage the male reproduction. Therefore, the aim of this study was to evaluate the role of Hg and Cd in infertile men.

Materials and Methods: In general, 62 men were included in this study among whom, 31 cases were infertile and within the age range of 23-38. The blood samples were collected to measure the concentrations of Cd and Hg in the serum using atomic adsorption spectrophotometry. In addition, semen analyses were performed according to the World Health Organization criteria, followed by sperm characteristics such as motility, head morphology, validity, and total count for at least 200 spermatozoa of each sample. Statistical analysis was done with SPSS software, version 17. Finally, the Kolmogorov-Smirnov test was used for parametric distribution.

Results: A considerable level of Hg and Cd was detected in the serum of infertile men compared with the control and there was a statistical difference between them and the control group ($P \leq 0.05$) Based on the results, there was a correlation between the high level of heavy metals and impairments in seminal quality. Further, the DNA damage was evaluated using chromatin condensation staining assay and the results showed a high percentage of DNA damage for infertile men in accordance with the levels of Hg and Cd ($P \leq 0.05$).

Conclusions: According to our results, Cd and Hg cooperate in affecting the sperm and leading to the DNA damage of the sperm.

Keywords: Mercury, Cadmium, Male infertility, Chromatin condensation, Protamine, Seminal quality

Introduction

Heavy metals are considered as the elements that are naturally found in the Earth's crust but could be introduced to the environment by human activities. Besides other sources of heavy metals such as occupation exposure, the largest source of heavy metals is dietary habits (1). Generally, it has been accepted that heavy metals affect oxidative stress (2,3).

Heavy metals are one of the main causes of infertility in men (4-6). There are several publications that suggest a low dosage of these metals have adverse effects on the male reproductive system. For example, cadmium (Cd) can lead to a decrease in the semen quality and damage to the DNA of the sperm (7), or mercury (Hg) is connected with sperm abnormality in humans (8).

Hg and Cd are two specific heavy metal elements that besides to other heavy metals are toxic for human sperms and cause infertility and have negative effects on the chromatin of sperm DNA (9,10). DNA is susceptible to oxidative stress and heavy metals can induce a strong oxidative stress in the body cells by analysis of the lipid bilayer of the cells (7). Any damage to the DNA of the sperm results in the impairment of fertility and could induce men infertility, cancer, or other disadvantages in the long term (11,12).

Unless the poisoning effects of Hg and Cd on human reproductive health, there is limited or rare information about the mechanisms of their functions. The alteration in DNA through binding to proteins and changing their percentage might be one of the possible mechanisms in this respect. The present study aimed to determine Hg and Cd in the serum of infertile men and to investigate the possible interaction of these elements in histone to protamine replacement and chromatin condensation.

Materials and Methods

Study Population

The study was performed from 2017 to 2018 at the Laboratory of Omid Clinic Center in Iran and consisted of 62 men aged 23-38 years old. All participants were divided into two groups of 31 proven fertile and 31 infertile men who had been in problem for having a child during 3 to 6 years of marriage and their female couples were proven as fertile. All men were from the same district, Bushehr

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province, Iran. On the other hand, men with any symptoms of a systemic disease, alcohol addiction, prostatitis, or any reproductive disorder were excluded from the study. Any men with a body mass index of more than 30 or below 17 were excluded as well. Written consent was signed by two groups before any further examination.

Sperm and Serum Collection

Semen samples were obtained by masturbation and stored in sterile containers. The semen was examined directly after liquidation according to the World Health Organization guidelines (2010). Semen samples were obtained from the men who were restrained from sex or masturbation for 3 days. The sperm motility, morphology, viability, and sperm count were evaluated for at least 200 spermatozoa from each sample. For each measurement, a 5-mL aliquot was loaded on a counting chamber. The samples were then put in the chamber and covered with a glass lid. The concentration, motility, and complex motion were analyzed at ×20 magnification.

Further, blood samples were collected on the same day to determine the concentration of Hg and Cd. Similarly, the electrothermal-atomic absorption spectrometry method was conducted to measure these elements (7). For measuring purposes, the blood samples were digested by the Mars-5 microwave accelerated reaction system. For operation, radio frequency was set at 1, 100 W, the auxiliary gas flow was 0.89 L/minute, plasma gas flow: 15 L/minute, and the resolution was 0.6~0.7 amu. The number of scanning was 155 and it was repeated 3 times. Eventually, the analysis lasted for 5 minutes and each sample was measured in duplicate.

Sperm Chromatin Condensation (CMA3) Assay

Sperm chromatin condensation was assessed by the CMA3 staining. CMA3 can compete with the protamine for binding to DNA (13). The samples were air-dried and then fixed in Carnoy's solution at 4 ˚C. Afterward, each slide was soaked with the CMA3 solution (50 µL stock stain solution + 450 µL McIlvaine buffer, Sigma, St Louis, MO, USA) for 20 minutes and in a dark room (14). Finally, white blood cells were used as negative controls for protamine staining.

Statistical Analysis

Statistical analysis was done with SPSS software, version 17. The Kolmogorov-Smirnov test was used for parametric distribution and the difference between the groups was evaluated by independent-samples *t* test and Man-Whitney U test. $P \leq 0.05$ was considered statistically significant.

Results

Demographic Characteristics and Comparison of Seminal Parameters

The mean age of the participants was 31±0.5 years. Moreover, the duration of marriage was 6±1.2 and 4±1 years for infertile men and fertile men, respectively. Additionally, the mean value for body mass index was not significantly different between the groups (Table 1).

The results of the seminal analysis are presented in Table 2. For infertile men, a sperm total motility demonstrated a significant decline (12±1 vs. 28±3, P=0.01) and the total number of sperm was meaningfully higher in fertile men (225±35 vs. 63±6 P=0.01). Additionally, the mean percentage of abnormal morphology significantly increased (65%) in men with reproductive problems in comparison with fertile men (P<0.05). Eventually, the volume and agglutination of the semen samples of infertile men did not show any difference with the control group.

Atomic Adsorption Spectrophotometry

The results of this study represented a high level of Cd and Hg in the serum of infertile samples (Table 2). The serum concentration of Hg was assessed 0.0003 ppm for infertile men that was significantly higher than that of fertile men (0.00002) in the *P* value of lower than 0.05. In addition, the level of Cd was considerably higher in unhealthy people compared with fertile men (0.0227 vs. 0.0049, P<0.05), the related data are shown in Figure 1.

Table 1. Demographic Data in the Study Population

Variable	Mean Value
Demographic data	
Age (year)	31± 0.5
BMI (kg/m²)	27±1.5
Marriage duration (y)	6± 1.2 for IF, 4 ± 1 for F

Note. IF: Infertile; F: Fertile; BMI: Body mass index. Men aged 21-38 were divided into fertile (F) and infertile (IF) groups (from the couples who had no child for 3-6 years).

Table 2. Seminal Parameters of the Study

Parameters	Infertile Men Group (Mean ± SD)	Fertile Men Group (Mean ± SD)
Seminal physical parameters		
Agglutination	0-0.43	0-0.35
Total count	63±6*	223±35
Volume (mL)	7± 0.43	6± 0.32
Abnormal morphology		
Head (%)	66*	58
Neck (%)	13	11
Sperm motility		
Slow	12±1*	28±3
Immotile	10±0.3*	32±0.1
Serum concentration of mercury	0.0003	0.00002
Serum concentration of cadmium	0.0227	0.0049

Note. SD: Standard deviation.
*A significant difference. The results showed significantly bad effects for cadmium and mercury on the seminal parameters of the studied men. In addition, the serum concentration of mercury was assessed 0.0003 ppm for infertile men and the level of cadmium was 0.0227 ppm.

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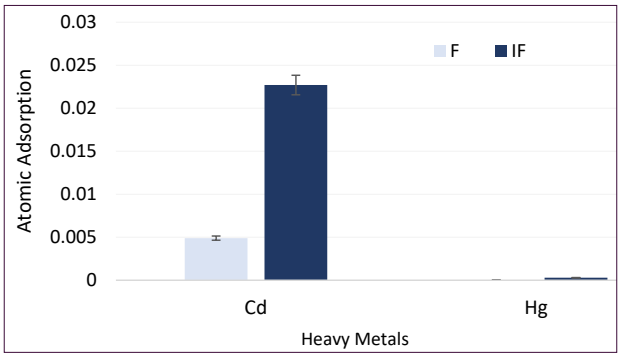

Figure 1. Serum Level of Cadmium and Mercury in Fertile and Infertile Participants

Note. Values are presented as the mean ± standard deviation. Cd: Cadmium; Hg: Mercury. The level of Cd and Hg for infertile men was considered significantly higher compared to fertile men.

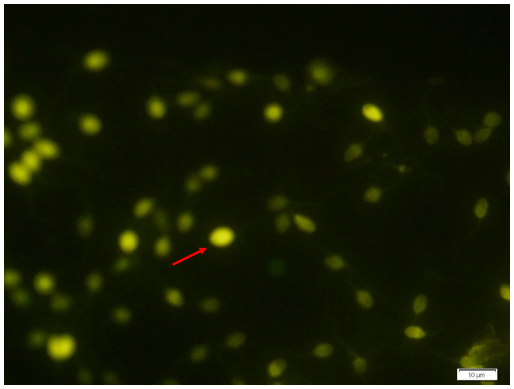

Figure 2. Chromomycin A3 (CMA3) Staining for the Detection of the DNA Damage of the Sperm

Note. The CMA3 positive sperm, abnormal sperm have yellowish color.

Comparison of Sperm Chromatin Condensation in Fertile and Infertile Group

The results of measuring CMA3 for infertile men showed that the mean percentage of this parameter significantly increased (38±2.3) in comparison with fertile men (12±1.12). The CMA3 positive sperm (bad sperm) was distinguished by the bright yellow stain in the semen samples of infertile men (Figure 2) and the dull yellow stained sperm was realized for fertile men. Further, a significant correlation was found between the concentration of Hg and Cd in the serum and the percent of non-condensed chromatin (r=0.32 and r=0.18).

Discussion

The present study distinguished the effect of heavy metals on the semen parameters and chromatin condensation of the sperm in infertile men and fertile men. The atomic adsorption results represented a high degree of Hg and Cd in the serum of infertile men. However, the percentage of these two elements was not considerably high in the serum of fertile men. On the other hand, a high percentage of non-condensed chromatin was detected in the sperm of infertile men that was in correlation with high levels of Hg and Cd. Based on the finding, the demographic data showed no specific differences between the two groups. Although the results of the volume and agglutination of the semen samples were the same in both groups, a significant reduction was found in the total count of the sperm. In addition, the other parameters of the sperm (e.g., the motility and head morphology of the sperm) demonstrated a significant difference in infertile men compared to the fertile men as the control group.

Heavy metals are broadly used in industries and can be introduced to the environment as toxic materials making them as the common pollutions (15). Cd and Hg are gaining more attention because of their different utilization in industrial activities. They might endanger the reproductive system directly or indirectly by affecting the gonads or hormones, respectively.

The infertile men showed a significant decline in sperm motility and sperm number, indicating that heavy metals could have an impact on the reproductive system of men in this study. Cd has disruptive effects on the endocrine system and produces reproductive toxicity in men, even in low dosage. In developed and developing countries, Cd is an important pollutant subject and previous research suggested a devastating effect of Cd on the reproductive system and considered it as one of the most important heavy metals for male infertility (16). In addition, a high level of Cd has been detected in azoospermia men (17). There is a relation between the serum concentration of Cd and the sperm count or motility of the sperm (18), and Cd can negatively affect sperm parameters such as adequate motility and the numbers of sperms.

High blood levels of Hg reduce 50% of sperm motility and sperm count, along with a high percentage of the abnormal morphology of the sperm (19). Further, Hg has inhibitory effects on sperm creatine kinase, which is a key enzyme for sperm normal metabolism, through binding to sulfhydryl sites (20). Furthermore, several publications have shown the impacts of Hg on the alteration of sperm morphology, as well as a decrease in sperm motility and seminal quality (21).

One of the main sources of Hg is fish and shellfish consumption (22). The population of the present study was from the same district, Bushehr, which is a coastal city, and fish is considered as the main dietary habitat of this population. The high serum level of Hg in this study might be related to this issue. Given that no information was available about the kind of food, the exact prediction of the role of the diet was impossible in this study.

The diagnosis of any damage to sperm DNA has been widely conducted as a supplement examination for semen analysis in many clinical laboratories and may predict the source of DNA damage that might be related to age, environmental contamination, or medical interference. By this method, it has been suggested that sperm damage results in men infertility (23). In the present study, the

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results of CMA3 staining revealed very high damage to the DNA sperm of the infertile group and there was a positive correlation between this damage and the high levels of Hg or Cd in the serum.

In this study, the chromatin condensation significantly decreased in the samples of infertile men and it could be related to the high level of Cd and Hg in their serum. Cd leads to the abnormal head morphology of the sperm (18). In the present study, the morphology of the sperm changed and CMA3 staining was positive for infertile males, indicating that Cd and Hg cut off the chromatin integrity of the sperm and increase DNA fragmentation. The inadequacy in DNA integrity could partly be related to the impairment of histone to protamine replacement. The higher amounts of protamine in the sperm means stability in the DNA structure and can reduce the damage of DNA by reactive oxygen species (ROS). Cd in the fish has a significant effect on DNA damage through oxidative damage (24) and has adverse effects on spermatogenesis via oxidative stress (25). However, knowledge about the effects of Cd on the DNA damage of human sperm is rare. To the best of our knowledge, this study was one of the first investigations on human sperm.

Hg impels the ROS and affects the antioxidant defense system of the cells by the inhabitation of sulphydryl groups. Some studies suggested that Hg causes DNA damage (26,27). However, these data are obtained from examinations on animal models, and there are a few studies on the human population and the effects of Hg on sperm DNA. Recently, mitochondrial DNA copy number and DNA damage have been shown in the white blood cells of the human exposed to high levels of Hg (28). On the other hand, there are several pathogenic mechanisms related to Hg including apoptosis, DNA damage, RNA damage, and epigenetic alteration (29). These findings suggest the potential role of Hg for inducing any changes in sperm morphology. This study did not merely focus on Hg, and thus it might be difficult to predict its role in altering the chromatin condensation. However, we can suppose the cooperation of Hg with Cd to influence the histone to protamine replacement and DNA damage.

Limitations of the Study

Some people did not like to attend the trial and the samples decreased due to the lack of information security. In this case, they were assured of the confidentiality of information.

Suggestions

In accordance with the results of the present study, it is suggested that people working and living in industrial zones perform the annual examination in order to avoid the side effects of their occupation.

Conclusions

Cd and Hg were found to play a crucial role in the generation of impairment in sperm parameters in infertile men in the present study. However, Cd and Hg probably had a negative effect on chromatin condensation and led to DNA damage in our study. Finally, a positive correlation was detected between these two heavy metals and deficiency to the human sperm nucleus.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

This study was approved by Kazerun Branch, Islamic Azad University Kazerun, Iran (Ethics No. 152305099710022).

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