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Determination of Lead and Arsenic Concentrations in the Serum and its Effect on Sperm Parameters and Sperm Quality in Patients Referred to Omid Persian Gulf Infertility Center of Bushehr

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Abstract

Objectives: Arsenic (As) and lead (Pb) are widespread in the environment and human exposure to these elements has a broad range of toxic effects. However, the knowledge of possible mechanisms of As and Pb in male reproductive toxicity is generally negligible.

Materials and Methods: The aim of this study was to investigate the possibility of the presence of Pb or As in the serum of infertile men and the relation between them and DNA damage in the sperm by using the enzyme-linked immunosorbent assay, atomic adsorption, and chromatin attaining.

Results: These results showed that there is a significant correlation between high levels of Pb and As in the serum and semen quality in infertile men. Finally, the results of chromatin condensation (CMA3) staining demonstrated a high level of damage in the sperms of infertile men.

Conclusions: It was hypothesized that Pb and As have interaction effects on histone to protamine replacement and prevent the chromatin condensation, resulting in a reduction in male fertility.

Keywords: Lead, Arsenic, Male infertility, Chromatin condensation, Protamine, Seminal quality

Introduction

Almost 30 million males worldwide are infertile. Male infertility is the incapability to cause pregnancy in a normal female partner (1). This could lead to unexpected outcomes in the health and social life of patients and their families (2). Many factors affecting male infertility, including physical health, abnormality in sex hormones, heavy metals, and the like.

There has been a significant decline in semen quality during the 50 past years (3,4), and some studies suggested the possible role of environmental pollutions and industrial poisoning materials. Heavy metal pollutions produced by industrial activities and technological improvement are poisoning and difficult to biodegrade, therefore, they seriously threaten the environment and public hygienic (5). They can be introduced to cells through normal transition through calcium channels or competition to binding the sites of protein (6).

Heavy metals such as lead (Pb) and arsenic (As) are toxic to human and animal health. Both of them are naturally occurring elements that are found on the earth. A low dosage of As and Pb is desired for a human diet. However, exposure to higher doses of these elements leads to adverse effects on different organs including skin, cardiovascular, lung, and the reproductive system (7-9). They can pollute the air, soil, and water, and then enter the food chain that can remain for decades (10,11). Some studies suggested that Pb effects on spermatogenesis, chromosomal damage, and infertility in men (12-14). Further, several studies have confirmed a decrease in the quality of the semen and sperm numbers in As-exposed males (7,15,16).

Heavy metals can reach the nucleus and DNA of the sperm and thus damage the proteins since they are able to produce reactive oxygen species (ROS) (17,18). Generally, this valuable genetic material is packed in spermatozoa to avoid any damage to DNA. Histone proteins around DNA will gradually be replaced by protamine for effective condensation of the sperm DNA (19). Protamine binding to DNA is stronger than histone binding (20), which results in the protection of chromatin from oxidative damage (21). In the human mature sperm, the replacement of histone by protamine is nearly 80-85%. Some factors including smoking and aging, and some disorders such as varicocele and heavy metals can affect this replacement and lead to abnormal sperm morphology

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Key Messages

- Many people, specially men who work in industrial cities, are in danger of chemical contamination that is harmful for fertility, sperm quality and density.

and motility (17,22-24). ROS produced by heavy metals attacks the spermatozoa causing damage to sperm DNA (25). Spermatozoa are more susceptible to ROS attack and DNA damage when they have high protamine or poor disulfide cross-links (26,27). Many people, specially men who work in industrial cities, are in danger of chemical contamination that is harmful for fertility, sperm quality and density.

The aim of the present study was to evaluate the effect of the serum concentration of Pb and As, as prevalent heavy metals in men who are working in industries of the south of Iran, on semen quality such as sperm motility and morphology, and the DNA damage of sperm. Considering that the correlation among Pb and As concentrations, semen quality, and protamine production is unknown, the present study focused on this subject.

Materials and Methods

Study Population

The study consisted of 31 male partners of couples who referred to the Laboratory of Omid Clinic in Bushehr, Iran, for infertility problems during 2017-2018. In addition, 31 fertile men, they were healthy male partners of married couples without any problem of having a child, were considered as the control. An agreement was confirmed with all participants before sample collection. Demographic data were obtained from written consent forms. The inclusion criteria included having the same occupation, nationality, and good nutritional status. On the other hand, the exclusion criteria were any hormonal or anatomic disorders, varicosity, prostatitis, epididymitis, and urinary tract infections.

Sperm and Serum Collection

Semen samples were collected by masturbation, and the samples were carried in sterile containers. Fresh samples were analyzed according to the World Health Organization guideline (2010). Several parameters were measured, including pH, sperm morphology, sperm motility, sperm concentration, and vitality. Semen samples were liquidated at 37 ˚C and spermatozoa were separated from somatic cells. Then, sperms were centrifuged at 300 rpm for 30 minutes. Next, the sperm was re-suspended and used for chromatin condensation (CMA3) staining.

The peripheral blood of participants (5 mL) was taken on the day of semen collection after breakfast eating. After centrifuging the samples at 4˚C and 3000 rpm for 10 minutes, the serum concentration of Pb and As were determined by the atomic absorption spectrophotometer.

Sperm Chromatin Condensation Assay (CMA3)

Sperm chromatin condensation was evaluated by the CMA3 staining as described previously (28). In summary, the semen sample smear was prepared and air-dried at room temperature and then fixed in Carnoy's solution at refrigerator temperature for 10 minutes. Next, 100 µL of the CMA3 (Sigma, St, Louis, USA) staining solution (pH = 7) in the dark was added to each sample slide and incubated for 10 minutes. Then, the slides were washed in McIlvaine buffer and mounted with 1:1 phosphate buffered solution/glycerol and kept overnight at 4˚C. Using a fluorescent microscope, spermatozoa were analyzed with a yellow fluorescent filter. The CMA3 positive sperm (the bad sperm) was distinguished by a bright yellow stain. In this test, the good sperm is stained as dull yellow.

Statistical Analysis

The statistical analysis was done using SPSS software, version 17 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used for parametric distribution. The difference between the groups was evaluated by the independent-samples *t* test and the Mann-Whitney U test. $P \leq 0.05$ values were considered statistically significant.

Results

Demographic Information

The demographic information of all 62 men (31 fertile and 31 infertile cases) is presented in Table 1. There was no significant difference in the duration of marriage and body mass index (MBI) between the two groups. However, the body mass indices (BMIs) in infertile men were higher (29 ± 0.34 kg/m^2) compared to fertile men. The results showed a significant difference in the mean age between the two groups and the infertile men were older (35 ± 1.2 years) than fertile men (28 ± 2.3 years).

Semen Analysis

Infertile men had significantly lower sperm concentrations in comparison with fertile men (57 ± 8 vs. 223 ± 35 $P \leq 0.05$). However, semen volume, viscosity, and agglutination were the same between the groups. The percentage of sperms with abnormal head morphology in the infertile men group was 72 ± 6, which considerably differs from fertile men (58 ± 1). In addition, the motile and high-speed sperms in the fertile group were obviously higher compared with infertile men (10 ± 0.3 vs. 30 ± 0.1 and 28 ± 3 vs. 15 ± 1, $P \leq 0.05$), the details of which are provided in Table 1.

Atomic Adsorption Spectrophotometry

The results of the serum concentration of As and Pb in the current study represented differences between infertile and fertile men. The serum concentration of Pb was higher in the infertile group compared with fertile men and a significant difference was detected in this regard ($P < 0.05$). On the other hand, higher serum As was

Table 1. Demographic and Seminal Data in the Study Population

Parameters		Infertile Group (Mean ± SD)	Fertile Group (Mean ± SD)
Demographic data	Age (y)	35 ± 1.2[a]	28 ± 2.3
	BMI (kg/m^2)	29 ± 0.34	26 ± 0.16
	Marriage duration (y)	8 ± 1	6 ± 1.5
Seminal physical parameters	Volume (mL)	7 ± 0.43	6 ± 0.32
	Agglutination	0-0.4	0-0.33
	Total count	57 ± 8[a]	223 ± 35
Abnormal morphology	Head (%)	65[a]	58
	Neck (%)	12	10
	Tail (%)	13	12
	Immature (%)	5	5
Sperm motility	Slow	15 ± 1[a]	28 ± 3
	Fast	40 ± 4.6	40 ± 7.1
	Progressive	15 ± 0.67	22 ± 1.8
	Immotile	10 ± 0.3[a]	30 ± 0.1
Serum concentration of heavy metals	Arsenic	0.9227	0.00093
	Lead	0.0002	0.0082

SD: standard deviation; BMI: body mass index.
[a] Significant.

found in infertile men compared with the control group ($P < 0.05$), the related data are shown in Figure 1.

Sperm Chromatin Condensation

The results of CMA3 staining are illustrated by the arrow in infertile men (Figure 2A) and fertile men (Figure 2B). There was a significantly higher bad sperm (histone replacement abnormality) in infertile men (38 ± 5.61) compared to fertile men (12 ± 0.78).

Correlation Between Heavy Metal Serum Concentrations and Sperm Chromatin

The mean As serum concentration was found to be significantly (r = 0.21) correlated with non-condensed chromatin (CMA3 positive, r = 0.36). Furthermore, the levels of non-condensed chromatin (CMA3 positive) are considerably higher in infertile men with a high As level (0.0213± 0.003) compared to fertile men.

In addition, a statistically higher significant correlation was observed between bad sperm (positive staining) and Pb serum concentrations in infertile men, and the level of positive CMA3 staining is significantly higher in the infertile men with high concentrations of Pb (0.0092±0.0007).

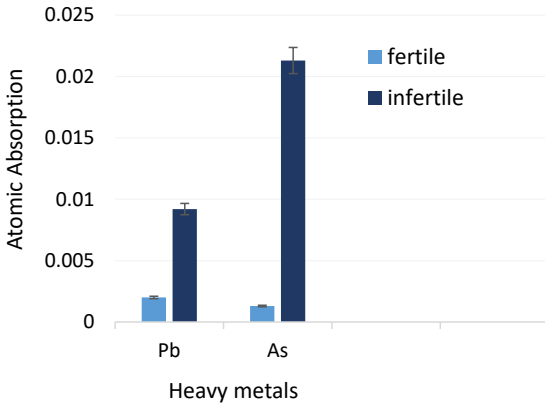

Figure 1. Serum Level of As and Pb in Fertile and Infertile Participants
Note. Pb: Lead; As: Arsenic. Values are presented as the mean ± standard deviation.

Discussion

Considering the role of heavy metals as one of the potential risk factors of male infertility, the current study investigated the effect of the serum concentration of Pb and As on sperm parameters, DNA damage, and sperm chromatin, as well as the possible correlation between these factors and semen quality in participants.

To the best of our knowledge, this was the first study that evaluated the histone replacement by protamine in infertile males exposed to high levels of Pb or As from the south of Iran. In this study, semen and blood samples were collected from 31 healthy men and 31 infertile men. Then, semen analysis, the atomic absorption of Pb and

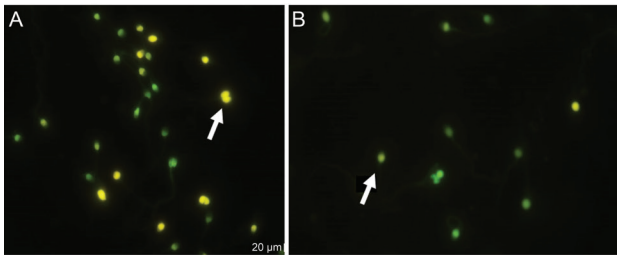

Figure 2. CMA3 Staining for the Detection of the DNA Damage of the Sperm Note. CMA3: Chromomycin A3; (A) The CMA3 positive sperm (abnormal sperm) in the infertile group and (B) The normal sperm stained as dull yellow in the fertile group.

As, along with protamine staining were performed to provide valuable information on the relationship between the serum level of Pb or As and DNA damage. The seminal parameters were considered as providing data for infertility. The results revealed a significant association between Pb and As concentrations and impaired semen quality and impairment in histone replacement with protamine.

In the present study, although the BMI in both groups was in a normal range, it was slightly higher in infertile men. Previous studies have shown that there might be a correlation between increased BMI and infertility in men (29,30). Moreover, a decrease in testosterone in a high range of BMI has been suggested in all ages (31), and it has been largely accepted that men with a higher BMI are more prone to oligospermia or azoospermia (32,33).

In this study, alterations were observed in the semen quality such as the total count, abnormal head morphology of sperm, and the motility of sperm in correlation with increased Pb and As. The results of this study demonstrated that men with high serum levels of As have poor semen quality. The quality of sperm is defined based on the viability and motility of the sperm, coupled with intact DNA (16).

As, as a heavy metal, has drastic effects on the sperm number, along with the motility and morphology of the sperm (34-36) through accumulations in the epididymis, seminal vesicle, and the testis tissue (35). Nowadays, it is confirmed that As is correlated with infertility in men (37,38). As interrupts the spermatogenesis by producing a high concentration of H_2O_2 and disturbance in carbon-binding spermatid-binding protein 1 operation (39).

Some studies have shown that the concentration of Pb in the serum of infertile men is high (40) and it has a negative effect on sperm motility (41,42), the presence of abnormal sperm morphology, and the total count of the sperm (43). The results of the present study revealed a high level of Pb in the serum of infertile men and a positive correlation between the serum level of Pb and the poor quality of the semen. Thus, it can be claimed that Pb cooperated with As in producing abnormality in the sperm and resulted in infertility in the men of this study.

Based on the finding of the present study, a great reduction was found in chromatin condensation and there was a positive correlation between protamine replacement and the serum concentration of Pb or As. The condensation of chromatin protects the genetic integrity of the sperm in male and female reproductive tracts. Some investigations suggested that when the chromatin is not completely condensed, there might be a high degree of reduction in fertilization, and they are unable to fertilize the oocyte even in the case of a direct injection (44,45). Pb interferes with the histone-to-protamine replacement (46). In addition, it can compete with zinc in binding human protamine 2, thereby resulting in protamine alterations (47), and subsequently, infertility in humans (48). Our results are in accordance with those of Awadalla et al (13), representing that the concentration of Pb can reduce reproductive ability in men by its effects on the chromatin condensation process.

Several studies have shown that As can damage DNA through the generation of ROS (49,50). In this study, it was supposed that As, similar to Pb, has a toxic effect on histone to protamine replacement and chromatin condensation, therefore, results in a reduction in sperm numbers and motility in addition to an increase in the production of abnormal sperms. As binds to sulfhydryl or thiol groups on sperm proteins and decrease the quality of the semen (34,51). Chromatin in human spermatozoa possesses a rich part of thiol on protamine and sulfhydryl groups, which are important for sperm motility and stability (34). If our idea was true, As helps Pb in yielding toxic effects on sperm DNA and chromatin condensation.

Limitations of the Study

Some people showed no willingness to attend the trial. In that case, they were assured of data confidentiality. However, the samples were decreased due to this issue.

Conclusions

Overall, a significant increase in Pb and As concentrations was found in the serum of infertile men, and there was a correlation between poor semen quality and the abnormality of sperm morphology. Finally, a significant reduction was observed in histone to protamine replacement presented in the sperm of infertile men, leading to a decrease in chromatin condensation and fertility.

Suggestions

It is recommended that other studies be performed including a larger sample size and measuring other heavy metals such as copper and aluminum measured as well.

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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