Sperm DNA Fragmentation Index Relationship with Some Heavy Metals in Seminal Fluid of Infertile Men in Suez Canal University Hospital: A Cross-Sectional Analytical Study

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Abstract

Introduction: **Infertility affect between 8 and 12% of couples worldwide. Males are found to be solely responsible for 20-30% of infertility cases but contribute to 50% of cases overall. There are several factors affect male infertility one of them is heavy metals which considered a gonadotoxin and have bad impact on male fertility through affecting spermatogenesis and hormonal balance required for optimum sperm production.** ***Aim of work:*** **To demonstrate the effect of environmental exposure of some heavy metals (lead, cadmium, mercury and arsenic) on fertility and proper protection from its hazardous effect.** ***Methods:*** **A Cross-sectional analytical study has been conducted, that included two groups of patients. Group I: Infertile males with oligozoospermia, asthenozoospermia and or teratozoospermia group II: fertile men with proven fertility whose partners had conceived spontaneously. Semen samples had been collected after 3-5 days of abstinence and semen analysis had been performed according to the WHO laboratory manual 2021. Sperms DNA fragmentation had been assessed using aniline blue staining method. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) had been used to detect cadmium, mercury, arsenic and lead amounts in seminal plasma of all participants.** ***Results:*** **There is statistically significant positive correlation between levels of lead, cadmium, arsenic and mercury in seminal plasma among infertile group and level of sperm DNA fragmentation.** ***Conclusion:*** **heavy metals like lead, cadmium, arsenic and mercury had significant bad effect on sperm DNA fragmentation which in turn contributes in male infertility.**

Keywords: infertility, heavy metals, sperm DNA fragmentation

Introduction

Infertility is a medical condition defined as the inability to achieve a clinical pregnancy after 12 months of regular, unprotected sexual intercourse [(1)]. Approximately 8-12% of couples of reproductive ages around the world experience infertility, with men solely responsible for 20-30% of cases and contributing to about 50% of infertility cases in total [(2)].

Male subfertility has a wide range of potential causes, which may be due to congenital, acquired, or unforeseen factors that disrupt spermatogenesis. Various health conditions can negatively impact male fertility, highlighting the importance of evaluating patients to

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identify treatable or reversible lifestyle factors or underlying medical issues [3].

While semen analysis remains a key diagnostic tool for male infertility, advanced tests to assess sperm quality and function have been developed to enhance diagnosis and treatment [3].

Exposure to hazardous heavy metals, such as lead, cadmium, mercury, and arsenic, can adversely affect human reproductive health. These metals enter the environment from natural sources like rock weathering and volcanic eruptions, as well as human activities such as industrial emissions, mining, vehicle exhaust, and smoking [4]

Heavy metals can negatively impact various male reproductive functions, including sperm count, motility, viability, spermatogenesis, and hormonal balance, making them a significant concern in reproductive toxicology [2]

Exposure to toxic metals can lead to deficiencies in essential minerals like zinc (Zn^{2+}) and copper (Cu^{2+}), a reduction in antioxidant enzyme and vitamin levels, and an increase in immature spermatids. Additionally, these metals may cause sperm head actin loss, impair the acrosome reaction, increase apoptosis in testicular tissue, and damage the basal membrane of the seminiferous tubule [5]

Sperm DNA is crucial for embryo development and can be damaged before and during ejaculation. Factors such as reactive oxygen species, varicocele, smoking, heavy metal exposure, testicular heat, obesity, advanced paternal age, and testicular infections can all compromise male fertility [6]

Sperm DNA fragmentation (SDF) has been linked to recurrent pregnancy loss and lower rates of live births. The sperm DNA fragmentation index (DFI) is used to assess the extent of DNA damage in sperm. This is typically measured with dyes or probes that can detect breaks in the DNA. Elevated levels of SDF are more frequently observed in men with infertility [7]

In this research we choose these heavy metals because they are considered some of the most common heavy metals in our environment and demonstrate the effect of heavy metals on human semen parameters especially by focusing on sperm DNA fragmentation index.

Patients and Methods

A. Research design:

Cross-sectional analytical study.

B. Description of study setting:

This study was carried out in the Andrology clinic, Faculty of Medicine – Suez Canal University.

C. Population and sample:

Target populations are males attending to the Andrology clinic. Patients' age ranges from 21 years old (legal age of consent in Egypt) to 60 years old as chromosomal aneuploidy and epigenetic phenomena occur more often in men older than 60 years. Patients were divided into two groups; group I: Infertile males with oligozoospermia , asthenozoospermia and or teratozoospermia group II: fertile men with proven fertility whose partners had conceived spontaneously without assisted reproductive techniques at least one time are considered control groups. Men recruited to the study are from Ismailia governorate and its surrounding areas.

D. Criteria of selection:

Inclusion criteria:

1. Married male patients.
2. Age between 21 and 60 years old.

3. Patients complain of primary or secondary infertility.

Exclusion criteria:

1. Patients with medical history of testicular dysfunction.
2. Patients with medical history of urogenital abnormality.
3. Patients with medical history of mumps or tuberculosis.
4. Patients with medical history of thyroid dysfunction. Subjects & methods
5. Patient with medical history of liver disease.
6. Patients with medical history of trauma to the testicles, radiotherapy, chemotherapy, orchitis and testicular carcinoma.
7. Patients with medical history of cryptorchidism, varicocele, hydrocele, undescended testicles.
8. Patients with medical history of using drugs known to affect gonadal function.
9. Patients with surgical history of genitourinary systems.
10. Patients with surgical history of thyroid gland, hypothalamus or pituitary gland.

E. Data collection tools:

We've collected data from eligible patients, adhering to pre-organized inclusion and exclusion criteria, using a structured data sheet which contained the following data. Two groups were included, and we collected the following data for each subject: (Full history taking, Clinical examination, Semen analysis, assessment of heavy metals levels in seminal plasma and Aniline blue DNA fragmentation test)

Assessment of Cadmium, Lead, mercury and arsenic levels in seminal plasma.

semen samples were centrifuged to obtain approximately 1 ml of seminal plasma, which was then stored at -20°C for the evaluation of heavy metals, including lead, cadmium, mercury, and arsenic. To prepare the samples for analysis, 1 ml of semen was digested using a mixture of perchloric acid, nitric acid, and sulfuric acid ($HClO_4$:HNO_3:H_2SO_4 in a ratio of 8:4:1) in an Aurora Transform 800 Microwave Digestion System (MW 800 closed vessels). The digested samples were then cooled, diluted to a final volume of 5 ml with distilled water, and analyzed for heavy metals using inductively coupled plasma-optical emission spectroscopy (ICP-OES) at the Micro Analytical Center at Al-Sadat City University, Cairo, Egypt [5]

Aniline blue DNA fragmentation test.

This test was conducted in the andrology clinic laboratory at Suez Canal University Hospital. To perform the staining, a fresh sperm smear was prepared for each case, air-dried, and then fixed in a 3% buffered glutaraldehyde solution in 0.2 M phosphate buffer (pH = 7.2) for 30 minutes at room temperature. Each smear was subsequently treated with a 5% aqueous solution of AB stain (5 g of powder in 100 ml of distilled water with 4% acetic acid, pH = 3.5) for 5 minutes. A minimum of 200 sperm cells were counted on each slide using light microscopy. Spermatozoa that were either unstained or stained pale blue were considered normal, while those with dark blue staining were classified as abnormal. The percentage of abnormal spermatozoa was recorded based on the evaluation of at least 200 sperm cells per slide [6]

Ethical committee approval:

The protocol received approval from the Institutional Review Board (IRB) of Suez Canal University, with the assigned approval ID 4760, prior to the commencement of the study.

Statistical analysis

Data analysis was performed using statistical package for social sciences (SPSS) for windows version 25.0 (SPSS, Chicago, IL, USA). Numerical data were presented as mean ± SD and median (range), whereas categorical data were expressed by frequency and percentages. Fisher's exact test and chi-square test were used for statistical analysis of categorical variables as appropriate. Means differences between the investigated groups were assessed by Kruskal Wallis or Man Whitney U test. For all tests a probability value of less than 0.05 was considered statistically significant. Descriptive data were presented as mean ± SD or percentages. Fisher's exact test and chi-square test were used for statistical analysis of categorical variables as appropriate. Means differences between the investigated groups were assessed by independent t-test or Man Whitney U-test according to normality of the data.

Results

The present study was designed as a Cross-sectional analytical study that included healthy human males attending the Andrology clinic and other relative private sectors for treatment of infertility.

The study participants were divided into two groups

Group A: Infertile males

Group B: fertile men with proven fertility considered control groups.

Regarding socio demographic data, there was no statically significant difference between two groups.

Hormonal assays demonstrate that there was no statistically significant difference between the two groups.

Regarding semen parameters the infertile group had statistically significant lower sperm concentration, progressive motility, non-progressive motility, viability and normal forms compared to fertile group ($P < 0.001$) meanwhile the infertile group had statistically significant higher immotile sperms compared to fertile group ($P < 0.001$). There was no statistically significant difference between the two groups regarding other semen parameters.

Regarding the levels of seminal plasma heavy metals, the infertile group had statistically significant higher heavy metals (lead, cadmium, arsenic and mercury) levels compared to fertile group ($P < 0.001$). as demonstrated in table (1).

Table (1) Seminal plasma heavy metals in studied group

Variables (Heavy metals)	Group A (n= 50)	Group B (n= 50)	*P*
Lead	0.96±0.06	0.44±0.03	<0.001[a]
Cadmium	0.238±0.186	0.091±0.011	<0.001[a]
Arsenic	9.72±6.18	6.51±4.63	<0.001[a]
Mercury	0.049±0.002	0.029±0.002	<0.001[a]

Regarding the level of sperm DNA fragmentation index the infertile group had statistically significant higher sperm DNA fragmentation index level compared to fertile group ($P < 0.001$) as seen in table (2).

Table (2) comparison between levels of sperm DNA fragmentation among two groups			
Variables	Group A (n= 50)	Group B (n= 50)	*P*
Sperm DNA fragmentation index	34.51±8.24	13.43±10.58	<0.001[a]

Regarding correlation between semen parameters and level of lead among infertile group there is statistically significant negative correlation between level of lead and sperm concentration (r_s = -0.470/ P < 0.001), motility (r_s = -0.671/ P < 0.001) and vitality (r_s = -0.276/ P < 0.026) meanwhile there is statistically significant positive correlation between level of lead and sperm abnormal forms (r_s = 0.362/ P < 0.004) as seen in table (3).

Table (3) correlation between semen parameters and heavy metals (lead, cadmium, arsenic and mercury) levels among infertile group								
Variables	Sperm concentration		Sperm motility		Sperm abnormal forms		Sperm vitality	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*P*
Lead	0.470*	<0.001	-0.671*	<0.001	0.362*	0.004	-0.276*	0.026
Cadmium	-0.201	0.080	-0.426*	0.001	0.706*	<0.001	-0.192	0.090
Arsenic	-0.012	0.467	-0.304*	0.015	0.112	0.219	-0.106	0.231
Mercury	0.526*	<0.001	-0.574*	<0.001	0.431*	<0.001	-0.142	0.162

Regarding correlation between semen parameters and level of cadmium among infertile group there is statistically significant negative correlation between level of cadmium and sperm motility (r_s = -0.426/ P < 0.001) meanwhile there is statistically significant positive correlation between level of cadmium and sperm abnormal forms (r_s = 0.706/ P < 0.001) with no statistically significant correlation to other parameters.

Regarding correlation between semen parameters and level of arsenic among infertile group there is statistically significant negative correlation between level of arsenic and sperm motility (r_s = -0.304/ P < 0.015) with no statistically significant correlation to other parameters.

Regarding correlation between semen parameters and level of mercury among infertile group There is statistically significant negative correlation between level of mercury and sperm concentration (r_s = -0.526/ P < 0.001) and motility (r_s = -0.574/ P < 0.001) meanwhile there is statistically significant positive correlation between level of arsenic and sperm abnormal forms (r_s = 0.431/ P < 0.001) with no statistically significant correlation to other parameters.

Regarding correlation between level of sperm DNA fragmentation index and heavy metals (lead, cadmium, arsenic and mercury) levels among infertile group there is statistically significant positive correlation between level of lead (r_s = 0.403/ P < 0.002), cadmium (r_s = 0.291/ P < 0.020), arsenic (r_s = 0.241/ P < 0.045) and mercury (r_s = 0.511/ P < 0.001) in seminal plasma among infertile group and level of sperm DNA fragmentation as seen in table (4).

Table (4) correlation between level of sperm DNA fragmentation index and heavy metals (lead, cadmium, arsenic and mercury) levels among infertile group		
Variables	Sperm DNA fragmentation among infertile group (n= 50)	
	r_s	*P*
Lead	0.403*	0.002
Cadmium	0.291*	0.020
Arsenic	0.241*	0.045
mercury	0.511*	<0.001

Discussion

In this study we examined the effect of heavy metals not only on semen parameters but also on sperm DNA fragmentation in non-exposed population so the present study was designed as cross sectional analytical study included two groups group I: Infertile males with oligozoospermia, asthenozoospermia and or teratozoospermia group II: fertile men with proven fertility whose partners had conceived spontaneously without assisted reproductive techniques at least one time are considered control groups.

This study include included 100 males with age ranged from 21 to 60 years old. Patients had statistical insignificant difference in age, marriage duration, smoking index and hormonal profile.

Our key findings are as follows: First, there was a statistically significant difference ($P < 0.001$) in the levels of heavy metals between the infertile and fertile groups. This result aligns with the study by Bhardwaj et al., which demonstrated that heavy metals such as lead, cadmium, mercury, and arsenic negatively impact reproductive health by affecting several male reproductive functions, including sperm count, motility, viability, spermatogenesis, and hormonal balance. (5)

Additionally, the study by Sun et al. found that men with low fertility had higher levels of lead and cadmium in their seminal plasma. (8)

In the study by Ilieva et al., arsenic and mercury were shown to damage the reproductive system through mechanisms related to hormonal regulation and function, including their binding to sperm, interference with steroidogenesis, and direct effects on testicular cells. (9)

In the present study, the level of sperm DNA fragmentation was significantly higher in the infertile group compared to the fertile group ($P < 0.001$). Our findings are consistent with those of Ribas-Maynou and Benet, who described that various types of sperm DNA breaks, including single- and double-strand breaks, can lead to different clinical reproductive outcomes. (10)

The study by Kadioglu and Ortac also demonstrated that compromised sperm DNA integrity negatively impacts the biological structure of sperm, which can ultimately lead to poor pregnancy outcomes, miscarriages, and recurrent in vitro fertilization (IVF) failure. (11)

In the present study, a statistically significant negative correlation was observed between lead levels and sperm concentration, motility, and vitality. This finding is consistent with the study by Giulioni et al., which reported that lead (Pb) exposure significantly reduces total sperm count, concentration, and motility. (12)

The study by Tuncay et al. disagreed with our findings, as it reported no statistically significant association between seminal plasma lead (Pb) levels and sperm concentration, motility, morphology, or total progressively motile sperm count. This discrepancy may be attributed to differences in study design; Tuncay et al's study was prospective, while our study was cross-sectional and included a larger sample size. [13]

In our study, a statistically significant negative correlation was found between cadmium levels in seminal plasma and sperm motility, as well as a statistically significant positive correlation with the percentage of sperm with abnormal morphology. These results are consistent with the study by Li et al., which demonstrated that elevated cadmium (Cd) levels can reduce progressive motility and sperm morphology in men without occupational exposure to cadmium. [14]

Our findings were in contrast to those of Bazid et al., who reported a significant negative correlation between seminal cadmium levels and sperm concentration and viability. This discrepancy may be explained by differences in study design, as Bazid et al.'s study compared the effects of cadmium on semen parameters between smokers and non-smokers. Smoking introduces additional factors, such as other harmful elements in tobacco, which could also affect sperm concentration and viability. [15]

Our results indicated a significant negative correlation between arsenic levels and sperm motility, which is consistent with the findings of Zargari et al., who reported that arsenic exposure led to a reduction in sperm motility. [16]

Additionally, the study by Zubair et al. showed that arsenic binds to thiol groups in tissue proteins, impairing their function. This metal also affects mitochondrial enzymes, disrupting energy production, which in turn negatively impacts sperm motility. [17]

In the current study, a statistically significant negative correlation was observed between mercury levels in seminal plasma and sperm concentration and motility, while a statistically significant positive correlation was found with sperm abnormalities. These results are in line with the study by Ilieva et al., which reported that higher mercury concentrations in seminal fluid were associated with abnormal sperm morphology, reduced sperm motility, and decreased sperm viability. [9]

Our results revealed a statistically significant positive correlation between lead levels in seminal plasma and the sperm DNA fragmentation index. These findings are consistent with the study by Li et al., which demonstrated that lead exposure promotes the generation of reactive oxygen species (ROS) in semen. Elevated ROS levels in spermatozoa can damage sperm DNA, leading to increased DNA fragmentation in sperm cells. [18]

Our results showed a statistically significant positive correlation between level of cadmium in the seminal plasma and sperm DNA fragmentation index which in accordance with Oliveira et al. study that showed exposure to Cd increased DNA fragmentation in sperms. [19]

In the present study, we found a statistically significant positive correlation between arsenic levels in seminal plasma and the sperm DNA fragmentation index. These results are consistent with the findings of Bhardwaj et al., who demonstrated that spermatozoa

chromatin and flagellum proteins, which are rich in thiol groups, are significantly affected by arsenic-induced reproductive toxicity. This exposure leads to chromatin margination and the formation of giant spermatid cells. Bhardwaj et al. also reported that arsenic exposure induces the formation of free radicals, causing oxidative stress that ultimately results in DNA damage. [5]

Our results demonstrated a statistically significant positive correlation between mercury levels in seminal plasma and the sperm DNA fragmentation index. These findings are in accordance with the study by Hayati et al., which showed that as mercury concentration increased, sperm DNA fragmentation also increased.[20]

Financial issues make it difficult to include larger sample size. Despite the small sample size, there was positive correlation between levels of sperm DNA fragmentation index and level of heavy metals (lead, cadmium, arsenic and mercury) in seminal plasma. Another disadvantage of our study that we use aniline blue stain to assess level of sperm DNA fragmentation index which less accurate method than other tests like TUNEL test or Comet assay test.

Conclusion

According to our study results, it could be concluded that heavy metals like lead, cadmium, arsenic and mercury had significant bad effect on sperm DNA fragmentation which in turn contribute in male infertility.

Recommendations

The limitations of this study were relatively small sample size in addition to fund which were needed for more accurate DNA fragmentation measuring test and in order to confirm these results, large randomized prospective studies are required. We recommend further studies that include other types of heavy metals and demonstrate its effect on male fertility. We also recommend further studies that use other tools to assess the level of sperm DNA fragmentation like TUNEL assay and COMET assay to get more accurate results regarding sperm DNA fragmentation assessment.

Further studies are recommended to demonstrate how to protect human male from the harmful effect of these heavy metals and how to treat exposed men and enhance their fertility condition.

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