LEAD, CADMIUM AND ARSENIC CONTENT IN SEMINAL PLASMA AND ITS EFFECTS ON SEMINOGRAM

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Statement:

All procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committees have approved them. Informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects have always been observed.

ABSTRACT

This study is to evaluate the levels of heavy metals, such as Pb (Lead), Cd (Cadmium) and As (Arsenic) in seminal plasma and its correlation with semen parameters such as volume, concentration and motility. This study is specifically focused on in vivo effects of heavy metals on total seminal and sperm cell quality. Semen samples were collected from male partners of infertile couples. Heavy metal constituents of separated seminal plasma was been analyzed by atomic absorption spectrophotometry and corresponding seminogram was done according to WHO guidelines. It has been found that In normozoospermic subjects Pb level in seminal plasma was below detectable limit compared to the subnormal samples. Similarly subjects having less seminal volume, showed considerably higher amount of Cd in their seminal plasma, compared to the subjects having normal seminal volume. Among the three heavy metals Cd concentration was also found to be much higher in all subjects. Samples having considerable motility problems had significantly high concentration of As. Recent studies have suggested that exposure to certain chemicals is associated with reproductive toxicity including changes in semen parameters.

1. INTRODUCTION

Studies confirm that environmental factors, such as pesticides, exogenous estrogens, and heavy metals may negatively impact fertility potential. Heavy metals have been identified as factors affecting human fertility. Heavy metal exposure has increased manifold in the past few decades due to environmental pollution and lifestyle habits and is one of the main causative agents for impaired reproductive function. Different forms of heavy metals are also considered human carcinogens and are believed to act in different mechanisms. Certain heavy metals act as mutagenic agents and sperm or oocyte DNA/chromatin appears to be the principal target for its effects. In contrast, some other heavy metals like arsenic (III) is considered nongenotoxic, but is able to target specific cellular proteins, principally through sulfhydryl interactions. Hence, heavy metals can simultaneously play a dual role in causing impaired reproductive function by altering genetic constitution and at the same time upregulating premalignancy.

During the last few decades there has been quite an amount of debate regarding the issue of declining semen parameters. Declining sperm count was first reported in 1974 by Nelson and Bunge (1), and this was thought to be due to changes in the environment and lifestyle. Since then numerous works have been carried out to establish a relation between sperm function and environmental and occupational exposure (2,3). In urban population, the study has attained importance after the observations that the human semen showed significant concentrations of
environmental toxins such as heavy metals and chlorinated pesticide (4). In 1992 Carlsen et al and Swan et al reported a global decline in sperm density between 1938 and 1997 (5,6). In that analysis they found significant declines in sperm density in the United States, Europe and Australia. However, they found no decline in sperm density in non-Western countries, for which data was very limited.

Heavy metals have been identified as factors affecting human fertility. Humans are exposed occupationally and environmentally to metal aerosols including lead (Pb2+) and cadmium (Cd2+). These toxicants accumulate in male reproductive organs. Susan Benoff, one of the leading figures in reproductive toxicology, was instrumental in analyzing the effects of lead, cadmium and other heavy metals on male factor infertility. In a prospective, double-blind study of the metal ion levels and sperm function; she showed that long term exposure (365 days) may cause testicular and spermatoxic effect (8).

The increasing concentrations of heavy metals (chiefly Pb) due to rise of vehicular pollution and food sources (9), can also hamper overall semen parameters. Hernández-Ochoa et al. (10) and Eibensteiner et al. (11) showed that sperm parameters are negatively associated with increasing lead concentrations. Recent research findings indicate that DNA damage due to oxidative stress can be one of the major causes of the decrease in sperm motility (12).

The mechanism by which heavy-metals like cadmium can affect motility parameters has been studied by previous authors (13-15). Aberrant sperm motility was correlated with altered expression of L-type voltage-dependent calcium channel isoforms found on the sperm with calcium and cadmium influx (13).

Arsenic is also contaminated through occupational sources like welding, metallurgy, furnace and ground water contamination, (Central Pollution Control Board, New Delhi, India, February 2002). In the present study, investigation has been made to show if arsenic can cross the blood-testes barrier and can get accumulated in the seminal plasma.

In the present study we have examined the correlation between seminal quality (including semen volume, sperm concentration, and sperm motility parameters) with lead, cadmium and arsenic concentration in seminal plasma. This study is specifically focused on the in vivo toxic effect of heavy metals on total seminal and sperm cell quality. This study aims in finding out how far these potent toxic metals can bio-accumulate in the seminal plasma crossing the blood-testes barrier and how much adverse effect do they have on semen parameters thus influencing reproductive potential of the individual.

2. MATERIALS AND METHODS

2.1 SUBJECT ENROLLMENT

A total of 38 men constituted the study. These 38 subjects were chosen very meticulously so that there is no selection bias. A detailed questionnaire was used to assess their lifestyle habits, occupational exposure and travel history. Since the morphology assessment procedure was not homogeneous, this parameter was omitted from the study. Samples having major liquefaction problem were also omitted. The semen analysis was carried out according to WHO guideline (16). To avoid regional variation, the study population belonged to the city of Kolkata.

2.2 SEMEN ANALYSIS

Each subject produced semen sample by masturbation into a sterile wide-mouthed plastic specimen container. The men were instructed to abstain from ejaculation for 2-5 days before producing the semen. The sample was allowed to liquefy at 37°C for 20 minutes before analysis. Measurement of both sperm concentration and motility were done in a pre-warmed (37°C) Makler counting chamber (Sefi Medical Instruments, Haifa, Israel). A standard WHO procedure (16) for semen analysis was performed which included assessment of: volume, sperm concentration, and percentage motility. The motility parameters were further classified into forward progressive motility (FPM) and non-progressive motility (NPM). Metals concentration was measured by Inductively Coupled Plasma Spectroscopy (Thermo Fischer, USA).

2.3 STATISTICAL ANALYSIS

Statistical analysis of the data was performed using Graph Pad Prism 5, and Microsoft Excel 2007. For every test of p value <0.05 was considered as statistically significant.

3. RESULTS

In normozoospermic subjects, Pb level in seminal plasma was below detectable limit compared to the subnormal samples (Mean ± SD: 2.29 ppm ± 2.06). Similarly subjects having less seminal volume, showed considerably higher amount of Cd (Mean ± SD: 0.73 ppm ± 1.24) in their seminal plasma compared to the subjects having normal seminal volume (Mean ± SD : 0.17 ppm ± 0.14) (Fig A). Samples having considerable motility problems (Mean ± SD: 5 M/ml ± 4.24) had significantly high concentration of As (Mean ± SD: 16.75 M/ml ± 14.14, P Value: 0.067) (Fig B). Among the three heavy metals, Cd concentration was found to be much higher in all subjects (Fig C). On an average Pb, Cd and As concentration was remarkably higher (Pb 172 times, Cd 99 times and As 3.5 times) than the WHO recommended permissible limit (Pb:0.01 milligram/liter, Cd:0.005 milligram/liter, As:0.01 milligram/liter) in seminal plasma as used in this study.

4. DISCUSSION

We have found that in normozoospermic subjects Pb level in seminal plasma was below detectable limit compared to the subnormal samples. Our finding in this regard confirms previous findings which indicated adverse effect of lead on spermatozoal characteristics. There has been a suggestion of the direct toxic effect of lead absorption on sperm production (17). Studies by Swan et al and Benoff et al, showed that multiple sperm parameters are affected as...
lead levels rise and that increased lead levels may contribute to the production of unexplained male infertility (6,7).

Sperm acrosome exocytosis is an absolute important mechanism by which it is determined that whether the final step of sperm membrane fusion cascade would be triggered by calcium channel and physiological fertilization will happen or not. As lead is a potent N-methyl-D-aspartate receptor (NMDAR) antagonist, can disrupt NMDAR-dependent brain-derived neurotrophic factor (BDNF) signaling. Hence, a soluble NSF attachment protein receptor (SNARE) complex protein, which is associated in vesicular exocytosis, may be impaired by lead exposure. Previous studies (18) confirmed that lead exposure produces the loss of synaptophysin / synaptobrevin complex. As this complex facilitates synaptobrevin to form functional (Soluble NSF Attachment Protein Receptor) SNARE complexes, due to lead exposure, may stop their activity and disrupts sperm acrosomal exocytosis (19,20).

Among the three heavy metals Cd concentration was found to be much higher in all subjects. In comparison to Lead and Arsenic, Cadmium can accumulate more readily crossing the blood test is barrier and thus shows in a higher overall percentage in the seminal fluid. Previous study also corroborates this fact (21).

Cadmium can directly be transferred to humans either from occupational environments or from inhalation like cigarette smoke. Cigarette smoke leads to the exposure of airborne environmental lead particles and cadmium. One single cigarette consists of 1.5 µg of cadmium (22,23). One tenth of the cigarette content is Cadmium and most importantly even though this sort of study wasn’t included in our case, it is already established in earlier study that even passive smoking is equally harmful (22). Though legislation against smoking in public area has been incorporated in 2003 in Indian law book, but due to under- manifestation of that law and non awareness, in India, there isn’t much constraint about smoking in public other than some highly restricted places like airports and petrol stations. Cadmium in ambient air also gets deposited onto earth surface, both in waters and soils. Some natural hyperaccumulator crops of this cadmium, like Lactuca sativa L (24), Rice plant (25), Bacopa monnieri L etc., use to mitigate this heavy metal from soil and water, then uptaking, accumulation, transportation through plant tissues and chelation through intracellular cadmium sequestration eventually makes the plant’s edible part toxic and finally after biomagnifications these heavy metals enter into the human body through the food chain.

This present study also found that, subjects having less seminal volume, showed considerably higher amount of Cd in their seminal plasma compared to the subjects having normal seminal volume. Cadmium in seminal plasma (CdSP) was also associated with low seminal volume (26). Cd was significantly correlated with cigarette-years and sperm volume (negative) (22).

In this study we have found that Cadmium significantly reduced the semen volume. The semen volume or the ejaculate is composed of an organic fluid, which contains secretions from the accessory sex glands namely, the seminal vesicle, the prostrate and the bulb-urethral glands. The seminal vesicle secretes mostly the fluid containing amino acids, citrate, enzymes, flavins, fructose, vitamin C etc. The prostrate secretes the fluid containing acid phosphatase, citric acid, proteolytic enzymes etc. Secretions from the bulbourethral glands consist of galactose, mucus, sialic acid and the pre-ejaculate. The functions of these structures are regulated by androgens (testosterone and DHT). It is an established fact that in mammal’s cadmium causes significant damage to accessory sex glands. (27). The effect of cadmium on the functioning of accessory sex gland is two-fold. Firstly it causes direct histological injury to these organs thereby reducing their functionality. The degenerative effect of cadmium is much pronounced because the biological half-life of cadmium is very long (more than 200 days). Secondly, cadmium interferes with the androgen feedback mechanism, which indirectly impairs the production of enzymes and other important constituents of these accessory sex glands. Studies have shown that Cadmium can mimic the effects of androgen and activate the androgen receptor thus interfering with the normal mechanism (28,29).

In this study, samples having considerable motility problems had significantly high concentration of As. Arsenic is a redox-inactive metal, which depletes the major antioxidants of cell, particularly thiol-containing antioxidants and enzymes. This causes an increase in production of reactive oxygen species (ROS) such as hydroxyl radical (HO.), superoxide radical (O²⁻) or hydrogen peroxide (H₂O₂). Enhanced generation of ROS can overwhelm cells’ intrinsic antioxidant defenses, and result in ”oxidative stress” (30), which can seriously affect sperm motility as observed here in our study. Evidence suggests that arsenic induces free radical formation and thus the generated reactive oxygen species (ROS) react with the polyunsaturated fatty acid (PUFA) rich spermatozoa, specially the mid spermatozoa and results in peroxidation which finally leads to destruction in spermatozoa causing reduced motility and viability (31).

5. CONCLUSIONS

Recent studies have suggested that, exposure to certain chemicals is associated with reproductive toxicity including changes in semen parameters. A previous study from our group has shown a decline in semen quality in the last two decades in the city of Calcutta, India. This present study clearly demonstrates the role of heavy metals in male semen quality. Among the three heavy metals studied, each one can have a singular or synergistic effect on seminal parameters. Moreover, chronic environmental or lifestyle exposure to these heavy metals can in course of time deteriorate the functional attributes of accessory sex glands as well as physiological well being of germ cells. Hence, general awareness among children and adult population is mandatory to avoid any untoward effect of such factors can have on reproductive functions and male fertility. More studies are needed to elucidate the molecular mechanisms underlying the deleterious effects caused by these heavy metals on reproductive function and spermatozoal integrity.
Fig A: Subjects having less seminal volume, showed considerably higher amount of Cd in their seminal plasma compared to the subjects having normal seminal volume.

Fig B: Samples having considerable motility problems had significantly high concentration of As.

Fig C: Among the three heavy metals, Cd concentration was found to be much higher in all subjects.

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