ORIGINAL RESEARCH PAPER

Biochemistry

SEMEN QUALITY, REPRODUCTIVE HORMONES AND LEAD IN BLOOD AND SEMINAL FLUID OF INFERTILE MEN WITHOUT OCCUPATIONAL LEAD EXPOSURE

KEY WORDS: Semen analysis, Lead, FSH, LH, Testosterone

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ABSTRACT

Almost 40-50% of infertility cases in modern urban Indian society is due to male partners and an important reason for this is suggested to be heavy metal, Lead (Pb) poisoning. Pb negatively impacts sperm motility and quality through complex and multiple pathways.

The objective of this study is to evaluate the level of Pb in semen and plasma of infertile males, changes in seminal parameters and correlation with reproductive hormonal levels like FSH, LH and Testosterone.

Except LH level, which was co-relatable in people with normal and statistically higher levels of blood Pb, all other parameters tested showed significant differences.

Increased Pb in subjects lead to abnormal sperm counts, progressive motility, total motility and morphology, while having no effect on the seminal quantity. Pb is a toxic substance for testicular tissue and functions, including spermatogenesis and mature sperm function. Serum FSH concentrations in cases and controls showed significant difference. Pb can reduce the amount of sulphated steroids excreted in the urine and can cause a reduction in testosterone levels and sperm concentrations, which reflects in the study.

Introduction:

Infertility is an ever increasing phenomenon that plagues the modern urban society. Almost 40-50% of it is due to the male partners, of whom around 2% exhibit suboptimal seminal parameters[1] One of the important reasons of male infertility of unknown etiology may be attributed to various environmental and occupational exposures to toxic substances, such as Lead (Pb). Occupational exposure to PB has been suggested to negatively impact on sperm quality and infertility[2]. Pb exerts multi-systemic toxic effects by inhibiting enzymatic activity (sometimes as a consequence of binding to sulfhydryl groups) interfering with the action of essential cations (particularly calcium, iron and zinc) and by altering the structure of cell membranes and receptors[3]. The effect of Pb on reproductive system seems to be complex and may involve multiple pathways[4].

The objective of this study is to evaluate the level of Pb in semen and plasma of infertile males without occupational exposure and to correlate their levels, and correlation between blood Pb level and hormonal levels, like Luteinizing hormone (LH), Follicle-stimulating hormone (FSH) and Testosterone in the test group.

Materials and Methods:

Study sample: A total of 86 participants were enrolled in the study. Sample size was determined using sample size determination in health studies formula and a prevalence of 10%[5].

All subjects aged 28 to 40 years who had history of infertility for more than year, sperm count 20 x 10^6 cells/ml and few or no leukocyte count per field were recruited as study population. Individuals with no history of infertility and normal semen analysis with at least 50% motility and 30% normal sperm morphology and count of ≥ 20 x 10^6 cells/ml were recruited as controls.

The study was carried on in AMRI hospitals, Kolkata, India, over a period of 2015 January to 2017 November.

Exclusion criteria: Male patients who were known to be diabetic or have varicocele, patients with orchitis, known congenital syndromes, or patients who were exposed to chemotherapy or radiation were excluded. In addition, those who had occupational exposure of Pb or who had taken any hormonal therapy were not part of the study group.

All subjects were married and infertile couples without occupational exposure of Pb. The subjects were male partners of infertile couples where there was no abnormality in female partners that may affect fertility.

A questionnaire was arranged for demographic data such as age, duration of marriage and infertility, whether or not having occupational exposure, smoking and previous medical and surgical history.

After taking consent from the patients, seminal fluid was collected by masturbation after five days of abstinence for detailed analysis. Blood was collected for blood Pb analysis and hormonal estimation of LH, FSH and Testosterone.

Routine semen analysis was done to assess sperm quality parameters, including semen quality, sperm density, sperm motility and sperm morphology as recommended by the World Health Organisation[6] after liquefaction at 37°C for 30 minutes and within one hour of semen collection. Thereafter the sample was spun at 12000 g for 20 minutes to obtain seminal plasma. The seminal plasma was stored at -20°C for Pb analysis.

The concentration of Pb in seminal plasma was determined using a Flame Atomic Absorption Spectrophotometer (Perkin Elmar; 2130 AAS).100 µl of seminal plasma was digested with 500 µl of super-grade 0.8 (M) HNO_3 in a glass tube. The residue was dissolved in 1 ml of 1% HNO_3 which was applied for detection of Pb. The recovery of Pb in spilled semen samples was 97%. The instrument was calibrated using 0 µg/dl, 5 µg/dl, 10 µg/dl and 20 µg/dl standards for Pb respectively. A sample blank was prepared each time a set of samples to control is the assayed in order to avoid possible metal concentration for external sources.

Blood Pb measurement was done using Lead Care Analyser (ESA, USA; Version 3.3), which includes the Lead Care Kit. 50 µl of whole blood in the EDTA was added to the treatment reagent in special tubes supplied with the kit.

Serum LH, FSH and Testosterone were measured by Roche Cobas 6000 Modular System, based on ECLIA principle.

This study utilised both internal and external quality control

procedure and obtained consistently satisfactory results, using quality control sera of BioRad, USA.

All data were tabulated using Microsoft Excel. Statistical significance was assayed with student's t test and results were deemed significant when $p < 0.001$. The analysis was done using SAS software[7].

The study was in accordance with Declaration of Helsinki[8] and guidelines on good clinical practice locally available. It was also approved by institutional review board and ethics committee.

Results :
The mean concentration of Pb in semen and sperm parameters in infertile males and control groups are depicted in Table 1 along with the blood Pb concentration and hormonal parameters.

Table 1: The base line data of seminal lead, seminal parameters, FSH, LH and Testosterone in infertile males and control group.

Measured parameters	Group A (infertile) N = 64	Group B (fertile) N = 22	p-Value
Age (Year)	36.09 ± 3.99	35.98 ± 4.01	0.911 (not significant)
Blood Lead (µg/dl)	16.62 ± 1.02	4.32 ± 1.21	0.001
Semen Lead (µg/dl)	7.32 ± 2.96	1.96 ± 0.35	0.001
Sperm Count (x 10^6 cells/ml)	12.6 ± 1.6	78.1 ± 12.8	0.001
Progressive Motility (%)	8.11 ± 1.62	59.2 ± 4.51	0.001
Total Motility (%)	17.03 ± 2.12	69.12 ± 3.44	0.001
Morphological abnormality (%)	13.92 ± 3.37	56.31 ± 3.11	0.001
Semen Volume (ml)	2.99 ± 0.31	3.01 ± 0.25	0.785 not significant)
FSH (mIU/ml)	14.13 ± 1.46	6.29 ± 1.52	0.001
LH (mIU/ml)	3.72 ± 1.62	3.84 ± 1.96	0.777 not significant)
Testosterone (ng/ml)	2.19 ± 0.64	5.12 ± 1.74	0.001

Values represents mean ± SD; Student's t test applied for determining the statistical significance between two groups (p 0.001)

The Pb levels were significantly higher in infertile males (p 0.001) compared to control group. While sperm counts, progressive motility and total motility were lower (p 0.001) in infertile males than control group. However no significant difference was detected in semen volume between infertile male and control group. With the increase in blood Pb level there was an increase in the hormonal level of FSH and it was a significant positive correlation (p 0.001). But no significant correlation with the hormonal level of LH was found. A significant negative correlation between blood Pb level and hormonal level of Testosterone (p 0.001).

Discussion:
This case – control study evaluated the levels of Pb in seminal plasma of infertile males without known occupational exposure and correlated their levels with semen quality. Blood Pb concentration was used as a biologic index for recent exposure and semen plasma Pb level was used as an indicator for the direct exposure of reproductive tissue. There was significant increase in seminal plasma Pb concentration in fertile males compared with normospermic controls. The increased level of Pb may have contributed to low sperm counts, progressive motility, total motility and morphology in these subjects. We observed no significant change in the semen volume of the subjects which inferred that Pb may have no effect on the seminal quantity. Similar observation was recorded by Salch et al[9] in their study. Because human sperm count, normal morphology and functions appear to be declining (a situation that may potentially jeopardise male fertility), increasing attention has been paid to male reproductive problems in recent years[10]. Many studies on reproductive system have reported Pb as a toxic substance for testicular tissue and functions, including spermatogenesis and mature sperm function. It was suggested that spermatogenesis regression is caused by increased inhibition B synthesis in highly Pb exposed subjects[11]. Average sperm motility and vitality has been affected in a negative correlation with blood Pb level, which could be explained by the direct toxic effect of Pb on seminal plasma[12]. The morphology of sperms is also affected by the increase in Pb level as the normal sperms shapes changes. The result goes with other studies demonstrating abnormal sperms in Pb exposed patients, because of the replacement of Pb for calcium as a second messenger, while leads to changes in protein conformation[13].

The chemical biomarkers of Pb in blood and semen were correlated with biochemistry markers (LH, FSH and Testosterone) and semen parameters. It was observed that depending on duration, environmental Pb exposure levels may lead to disruption of both hypothalamus and pituitary gland function in humans which may result in hormonal imbalance and hence poor spermatogenesis and sperm development[14].

Serum FSH concentrations in cases and controls showed significant difference. The low levels of FSH concentrations in cases is very much implied because of this reduction in gonadotropin secretion among individuals exposed to Pb, while moderated exposure only led to higher FSH levels[15].

No significant correlation of blood and semen Pb concentration with serum LH was observed. One study demonstrated a non-progressive increase in LH in individuals exposed for less than one year, while those exposed for more than three years showed a reduction in both testosterone and the testosterone – steroid transport protein ratio suggesting a correlation between testicular dysfunction and duration of exposure[16].

Pb can reduce the amount of sulphated steroids excreted in the urine can cause a reduction in testosterone levels and sperm concentrations[17], which reflects in our study.

These results draw a more definite relationship between seminal plasma Pb concentration and sperm count in men without occupational exposure to Pb having altered reproductive hormonal regulations.

In conclusion, Pb may represent reproductive toxicant in occupationally unexposed infertile males and create hormonal disturbances in those patients.

Limitation of study:
The inability to obtain large sample size. This is due to the nature of the cases, which are difficult to find considering all the inclusion & exclusion criteria.

Conflict of Interest:
The authors declare no conflict of interest in the present study conducted.

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