SEMINAL PLASMA LEVELS OF LEAD AND MERCURY IN INFERTILE MALES IN BENIN CITY, NIGERIA

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ABSTRACT

Background/objectives: Studies on environmental exposure to toxic metals and their effects on male reproductive function are scare in our setting. This study evaluates the levels of lead and mercury in seminal plasma of infertile males who are non-occupationally exposed in Benin City, Nigeria and to determine the relationship between seminal quality and these toxic metals.

Materials and Methods: A total of 80 subjects participated in this study which includes 60 infertile males on routine visit to the infertility clinics in Benin City and 20 fertile males as controls. The concentration of lead in seminal plasma was assayed by atomic absorption spectrophotometer while the concentration of mercury was measured using inductively coupled plasma Mass spectrometry. Semen analyses were performed using standard techniques as recommended by World Health Organization.

Results: Mean seminal plasma lead and mercury levels were significantly higher (p<0.001) in infertile males compared with controls. Mercury and lead correlated negatively (p<0.001) with sperm count, progressive motility, total motility and morphology but not with semen volume. There was no significant correlation between toxic metals and sperm indices in fertile males (controls).

Conclusion: The levels of the studied toxic metals were higher in seminal plasma of infertile males and appear to have adverse effect on seminal indices in non-occupationally exposed males.

INTRODUCTION

There has been increasing concern regarding the decline in semen quality in the general population. Decline in sperm quality has been reported in both developed and developing countries including Nigeria[1-2]. Environmental factors such as exposure to oestrogen was linked to decrease in fertility in Europe [3] as well as increase prevalence of cryptorchidism and testicular cancer[4].

Occupational exposure to toxic metals such as lead and mercury has been suggested to negatively impact on sperm quality and infertility [5-7]. Toxic metals have also been reported to impair testicular function and sperm secretion in experimental animals[8]. Exposure to these toxic metals at low concentration could occur either voluntarily through supplementation or involuntarily through diet (eating of contaminated food, water) or contact with soil dust or air[9]. Even though not much study have been done regarding the effects of toxic metals on reproductive health in Nigeria, there appears to be growing concern for adverse reproductive health effects linked to low-level exposures experienced in the environment. Toxic metals could negatively impact the male reproductive system; either by disruption of hypothalamic-pituitary axis or by directly impacting adversely on spermatogenesis, resulting in poor semen quality which is associated to male infertility [10]. Studies have shown that toxic metal levels in blood may be inadequate to reveal their accumulation in the male reproductive tract [11-12]. Therefore levels of heavy metals in seminal plasma may provide a better index of exposure and effect in reproduction. This study was designed to evaluate the levels of lead and mercury in seminal plasma of infertile males in Benin City, Nigeria without occupational exposure and to correlate their levels with semen parameters.

MATERIALS AND METHODS

Type of study: Analytical study

Locus of study: The study was conducted at the Department of Medical Laboratory Science, School of Basic Medical Science, College of Medical Sciences, University of Benin, Nigeria.

Ethical approval: The study was approved by the Research Ethics committee of the University and informed consent was given by the individual subjects.
Sample size: A total of 80 participants were enrolled in the study. Sample size was determined using sample size determination in health studies formula and a prevalence of 10% (Lwange and Lemeshow [13].

Inclusion criteria: All consecutive subjects aged 18-50 years who had history of infertility for more than year, sperm count <20x106 cells/mL and few or no leukocyte count per field were recruited. 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 106 cells/mL were recruited as controls.

Exclusion criteria: Those with other specific genital and systemic disease such as genital infection, undecided tests, hepatic, renal, endocrine, autoimmune that may impair the reproductive capacity were excluded. Subjects with history of toxic metals exposure because they resided in areas known to have toxic metals contamination and cigarette smokers were also excluded from this study.

Collection of semen sample: Semen was obtained by masturbation after five days[10] of abstinence into universal container ensuring that the sperm rich first part was not lost. The sample was left on the bench at 37 °C for 30 minutes for liquefaction after which semen analysis was done according to WHO standard13. Thereafter the sample was spun at 12000g for 20 minutes to obtain seminal plasma. The seminal plasma was stored at -20°C prior to toxic metal analysis between January and June 2014. Demographic and clinical examination findings were obtained using structured questionnaires.

Semen analysis: Routine semen analysis was done to assess sperm quality parameters, including semen quantity, sperm density, sperm motility, and sperm morphology as recommended by the World Health Organization [14], after liquefaction at 37°C for 30 min and within 1 h of semen collection.

Semen parameters evaluated included: sperm concentration, progressive motility, total motility, and percentage of normal forms using improved Neubauer counting chamber and microscope slide examination techniques respectively [14]. The criteria for normozoospermia were defined as a concentration of 20 x 106 /ml, with sperms of forward progressive motility more than 32% of spermatozoa, total motility more than 40% and normal morphology with oval-shaped head without abnormalities of tail in at least 30% of the spermatozoa.

Measurement of metals:

Determination of lead: The concentration of lead in seminal plasma was determined with electrothermal atomic absorption spectrophotometer and Inductively Coupled Plasma Mass Spectrometer. The instrument was calibrated using 0µg/L, 2µg/L, 6µg/L, 10µg/L and 30µg/L standards for mercury respectively. A sample blank was prepared each set of samples to control was to be determined to avoid a possible metal contamination from external sources. Approximately 20µL of the prepared sample was aspirated into the quartz spray chamber. The instrument was allowed to process the sample and display the concentration in µg/L.

Measurement of mercury: The concentration of mercury in seminal plasma was determined with Inductively Coupled Plasma Mass Spectrometer (Agilent 7500, Norwalk, U.S.A). The instrument was calibrated using 0ug/L, 2ug/L, 6ug/L, 10ug/L and 30ug/L standards for mercury respectively. A sample blank was prepared each set of samples to control was to be determined to avoid a possible metal contamination from external sources. Approximately 20µL of the prepared sample was aspirated into the quartz spray chamber. The instrument was allowed to process the sample and display the concentration in µg/L.

Quality control: Standard sample for each element was diluted to obtain serial dilutions of each sample and was used to calibrate and standardize the electrothermal atomic absorption spectrophotometer and Inductively Coupled Plasma Mass Spectrometer before running the analysis, and a graph was generated. Before being used all volumetric polyethylene (including the auto-sampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO3 for 24 hour. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container. Certified reference materials (CRMs) from (Le Centre de toxicologie du, Quebec) were analyzed. 86.5ng/mL and 7.42ng/mL was obtained as lead and mercury measured level from whole blood respectively while 93.2ng/mL and 8.02 were the certified value for lead and mercury respectively.

Statistical analysis: The data obtained were statistically evaluated using statistical package for Social Science Program (SPSS) version 16.0. Values obtained in this study were represented as mean±SEM for both test and controls. Student’s t test was used to compare the means while correlation was done with Pearson correlation coefficient. A P value less than 0.05 was considered statistically significant.

RESULT

Sixty (60) infertile males on routine visit to infertility clinics in Benin City, aged 38.9±0.98 formed the subject group while 20 fertile males aged 38.2±0.68 with evidence of parity were enrolled as controls.

Table 1 shows the mean concentration of lead and mercury in seminal plasma and sperm parameters in infertile males and control subjects. Lead and mercury levels were significantly higher in infertile males (p < 0.001) compared to control while Sperm counts, Progressive motility, and Total motility were lower (p<0.001) in infertile males than controls. There was however no significant different in the semen volume between infertile and fertile males.
Table 1: Seminal plasma lead, mercury levels and seminal parameters in infertile Males and Control

<table>
<thead>
<tr>
<th>Measured Parameters</th>
<th>Infertile Males</th>
<th>Controls</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=60)</td>
<td>(N=20)</td>
<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td>38.9±0.98</td>
<td>38.2±0.68</td>
<td>=0.720</td>
</tr>
<tr>
<td>Lead (ug/L)</td>
<td>0.99 ± 0.04</td>
<td>0.47 ± 0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mercury (ug/L)</td>
<td>0.048 ± 0.002</td>
<td>0.032±0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sperm Count (x10⁶ cells/mL)</td>
<td>16.8 ± 2.51</td>
<td>74± 13.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>9.15 ± 1.78</td>
<td>57.1±3.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Motility (%)</td>
<td>21.50 ± 2.36</td>
<td>67.06±2.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>15.21±2.27</td>
<td>54.2±2.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Semen Volume (mL)</td>
<td>2.98 ± 0.18</td>
<td>3.04±0.31</td>
<td>=0.879</td>
</tr>
</tbody>
</table>

Table 2 shows correlation of lead with semen parameters. Semen plasma lead correlated negatively with sperm count, progressive motility, total motility and morphology (p< 0.001) while lead correlated positively with semen volume but the correlation was not however significant (p=0.434).

Table 2: Correlation of lead levels with Semen Parameters in infertile males

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Count (cells/mL)</td>
<td>-0.440</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>-0.541</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Motility (%)</td>
<td>-0.527</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>-0.504</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Semen Volume</td>
<td>0.089</td>
<td>=0.434</td>
</tr>
</tbody>
</table>

Seminal lead correlated negatively with sperm count, progressive motility, total motility and morphology while semen volume correlated positively with semen lead. The associations between lead and sperm indices were not statistically significant (table 3).

Table 3: Correlation of lead levels with semen parameters in fertile males

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Count (cells/mL)</td>
<td>-0.122</td>
<td>=0.597</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>-0.090</td>
<td>=0.698</td>
</tr>
<tr>
<td>Total Motility (%)</td>
<td>-0.123</td>
<td>=0.594</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>-0.265</td>
<td>=0.247</td>
</tr>
<tr>
<td>Semen Volume</td>
<td>0.068</td>
<td>=0.770</td>
</tr>
</tbody>
</table>

Table 4: Correlation of mercury levels with Semen Parameters in infertile males

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Count (cells/mL)</td>
<td>-0.341</td>
<td>=0.002</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>-0.382</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Motility (%)</td>
<td>-0.367</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>-0.375</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Semen Volume</td>
<td>0.113</td>
<td>=0.318</td>
</tr>
</tbody>
</table>

Table 4 shows that seminal mercury correlated negatively with sperm count (p<0.002), progressive motility, total motility and morphology (p<0.001) while the correlation(r=0.113;p=0.318) between mercury and semen volume was not significant (p=0.318).

DISCUSSION

This study evaluated the levels of lead and mercury in seminal plasma of infertile males in Benin City, Nigeria without occupational exposure and correlated their levels with semen quality. There was significant increase (p<0.001) in seminal plasma lead and mercury concentrations in infertile males compared with controls. Statistically significant inverse correlation (p<0.001) was observed between lead and mercury levels and sperm count, progressive motility, total motility and morphology in infertile males. The increased levels of toxic metals may have contributed to low sperm counts, progressive motility, total motility and morphology in these subjects. However there was no significant different in the semen volume in fertile males compared with controls (p=0.879).There was no statistical significant association between lead and mercury levels with sperm parameters in fertile control subjects. Although not much study has been done on the effect of lead on human reproductive function in Nigeria but the few available studies elsewhere presented conflicting findings [14,17]. However we observed statistically significant increased (p<0.001) in levels of seminal plasma lead in infertile males compared with controls. This is consistent with that reported by other authors [17,18-20].The subjects evaluated in this study had no history of lead poisoning none where they occupationally exposure to lead. This is an indication that low-level exposure to environmental lead may be implicated in the decrease semen quality. It was observed that environmental lead exposure levels depending on duration may lead to disruption of both hypothalamus and pituitary glands functions in both experimental animals and humans which may result in hormonal imbalance and hence poor spermatogenesis and sperm development[21-23]. When gonadotropins interact with specific receptors on the reproductive cell surface they stimulate gonadal steroidogenesis and gametogenesis, but increased lead concentration in the tissue have been observed to interact with these receptors on the surface of the cell membrane thereby preventing the gonadotropins from binding to receptors [24] or cause increase in the production of reactive oxygen species that affect membrane integrity [25]. This interaction impairs gonadotropic binding, steroidogenesis,
and gamete growth. Lead and cadmium can induce oxidative stress by their capacity to interact with reactive oxygen species (ROS) which has both physiological and pathological role, thereby increasing their oxidant activity affecting sperm membrane integrity thus poor sperm quality [26]. Lead has also been shown to increase ROS production which disrupts the inner and outer mitochondrial membranes [27]. The increased oxidative stress may results in the release of cytochrome C protein from the mitochondria, which activates caspases, an enzyme that induces sperm cells apoptosis [27]. Xu et al [28] earlier observed a positive correlation between lead level and degradation of DNA bases in sperm cells resulting in sperm cell death, consequently abnormal semen parameters. Others also demonstrated that lead decreases sperm function such as premature acrosome loss and decreased chromatin condensation [19]. In humans, zinc may contributes to sperm chromatin stability and binds to protamine 2. Lead competes with zinc and binds human protamine 2 causing conformational changes in the protein [29]. This decreases the concentration of DNA protamine 2 binding which probably leads to alterations in sperm chromatin condensation [30]. We however observed no significant change in the semen volume of the subjects, which inferred that toxic metals may have no effect on the seminal vesicle. This observation is consistent with that reported by Saleh et al [20].

Mercury has been observed to affect male reproductive function of occupationally exposed individuals and spermatogenesis in animals [31-32]. In vitro studies have also indicated that mercury is capable of inducing sperm abnormality [33], but the role of increased seminal plasma mercury levels is not fully elucidated in occupationally unexposed individuals. In this study we observed increased seminal plasma mercury levels in infertile males compared with controls. These finding is consistent with previous studies elsewhere [34-35]. The major mechanism involved in toxic reproductive effect of mercury is oxidative stress [36]. Oxidative stress is involved in many aspect of male infertility. A shift to a more oxidative state may lead to lipid peroxidation, DNA damage, membrane alteration, making worse the metabolism and inactivation of enzymes in spermatozoa [34,37-39]. Sharma and Agarwal [39] observed that oxidative stress mediated damage to sperm membrane and may account for defective sperm function observed in high proportion of infertility cases. Also Arabi and Heydarneja [35] observed that lipid peroxidation may result in altered viability and movement which may have accounted for the abnormal morphology and motility observed in this study. In addition, membranes of acrosomal cap, the mid-piece and the tail of the human sperm have been shown to be the potential binding sites for mercury [33]. Subsequently, disruptions of sperm membrane permeability, mitochondrial function, and DNA synthesis by the microtubules are possible mechanisms whereby mercury toxicity occurs[31,331]. Apart from the sperm themselves, supporting cells in the testis and epididymis are also possible targets of mercury toxicity [33,40], which may result to semen abnormality and clinical infertility.

The major possible sources of non-occupational exposure may include environmental discharge of toxic metals containing products such as petroleum products, the use of combination of fossil fuels (petroleum and coal) as well as municipal wastes may be contributing factors to airborne lead and mercury pollution [41]. Treatment options of subjects with heavy metals toxicity is evolving even though no agreement exist for now. Clinical protocol involved the use of EDTA, DMPS and DMSA chelators which had proved to be therapeutically beneficial [42].

Limitation of study: The inability to obtain large sample size due to the reluctance of prospective patients and control subjects to give consent for their samples to be collected and used was a major limitation in this study.

CONCLUSION

In conclusion, based on the result of this study lead and mercury may represent reproductive toxicant in occupationally unexposed infertile males in Benin City, Nigeria. Toxic metals may be routinely assayed when evaluating male subjects with idiopathic infertility.

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Conflict of Interest : None

REFERENCES


