ORIGINAL ARTICLE

Effects of Lead Toxicity on Serum Testosterone and LH levels in Adult Male Rats

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ABSTRACT

Objective: To find out lead toxicity affects all levels of reproductive axis including both testosterone and Luteinizing Hormone production in adult male rats.

Study Design: An experimental study on animals

Place of Study: The study was carried out at Islamic International Medical College, Rawalpindi from January 2010 to November 2010.

Materials and Methods: Thirty adult male Sprawgue Dawley rats, purchased from NIH, Islamabad were divided into two groups A and B each containing 15 rats. Group A served as normal control receiving plain tap water. Group B received 0.3% lead acetate in drinking water. Five rats from each group were sacrificed at the end of 2nd, 4th and 6th weeks. Serum testosterone and LH levels were analyzed using ELISA technique. Results were analyzed using SPSS version 13.

Results: Serum testosterone level was significantly decreased in lead treated group as compared to control group whereas LH levels showed no significant change.

Conclusion: Lead toxicity in male rats suppressed serum testosterone levels at all duration whereas LH levels at all durations manifested no significant change. This might be a result of direct testicular toxicity acting at testicular enzyme level alone or involving the hypothalamic-pituitary axis as well.

Key words: *Lead toxicity, testosterone, luteinizing hormone, male rats.*

Introduction

Lead (Pb) is one of the oldest and commonest environmental pollutant, which is reported to cause damage in multiple body systems.[1] In a developing country like Pakistan, people are specially exposed to lead pollution through air, water and soil.[2] Studies which have been conducted in Pakistan, have revealed that major population have blood lead levels above the internationally acceptable limits.[3] It can cause serious health problems including high blood pressure, damage to the brain, nervous system, kidneys and reproductive system.[1]

Like other toxic metals, lead causes oxidative damage and disrupts the prooxidant/ antioxidant balance which has been demonstrated in multiple studies.[4]

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Lead toxicity imposes adverse effects on male reproduction and fertility both in clinical and animal studies.[4]Testosterone production by testicular Leydig cells is essential for reproductive function and vitality of male. Lead causes reduction in semen volume and density, sperm count and increased morphological abnormalities of spermatozoa both in humans[5] and experimental animals[6]. It has also resulted in reduction of serum LH and FSH along with serum testosterone levels, thus showing that it also targets hypothalamic-pituitary axis.[7] It may have direct testicular toxicity thus decreasing testosterone levels or indirect effects through targeting the endocrine control of reproduction or both.[8] Studies in male rats have shown that lead intoxication disrupts testicular steroidogenesis by inhibiting the activities of testicular steroidogenic enzymes.[8]

The present work was conducted to study the impact of lead intoxication on plasma

testosterone and LH levels of male rats to evaluate its mechanism of action.

Materials and Methods

Thirty adult male Sprawgue Dawley rats between the ages of 60-90 days, weighing 130-200 gms were randomly selected. They were divided into two groups with fifteen rats in each group. Group A served as normal control whereas group B was given 0.3% lead acetate dissolved and added in drinking water, which was given in clean, inverted bottles specific for the rat cages. Both groups were fed on standard pellet diet and water ad libitum in the Animal House of NIH and kept in separate standard cages designed accordingly. Drinking water consumption in both the groups during the previous 24 hours was recorded daily and rats were weighed on weekly basis. Treatment in both the groups continued for 42 days with 5 rats being sacrificed in each group at the end of 2nd, 4th and 6th weeks.[9] They were sacrificed 24 hours after the last experimental day by an overdose of ether. Three to five ml blood was drawn by intracardiac catheterization. Samples were immediately transferred into labeled gold top vacutainers without anticoagulant kept in an ice packed rack. Samples were then shifted in an hour from NIH to Riphah Diagnostic and Research Lab at Riphah College Islamabad. Serum was separated by centrifugation, transferred into labeled 1.5 ml eppendorf tubes, frozen and stored at -80o C till assayed. Testosterone and LH levels in each group were quantitatively determined using solid phase Enzyme-Linked Immunosorbent Assay (ELISA) as described in the kit instructions leaflets provided by the manufacturer[10]. Testosterone and LH ELISA kit were purchased from DRG International, Inc. (Lot # 16k096). Testosterone ELISA kit is based on the principle of competitive binding. All reagents and samples were brought to room temperature. Desired number of micro titer wells were placed in the holder. 25 µL of each standard control and samples were pipetted into appropriate wells of the strips. 200 µL of HRP testosterone conjugate was added to each well. After though mixing, it was then incubated for 60 minutes at room temperature. The contents were then briskly shaken out and rinsed with wash solution and then 200 µL of substrate solution was pipetted to each well. After incubation the enzymatic reaction was stopped by pipetting stop solution. It was then read at 450 nm with the help of an ELISA reader (strip reader das, model C serial #961, 2005). Using semi-algorithmic graph paper a standard curve was constructed by plotting the mean absorbents obtained from each standard against its concentration with absorbance values at Y-axis and concentration on the horizontal X-axis. Thus the corresponding concentration for each sample was determined from the standard curve. The DRG LH ELISA kit is based on the sandwich principle, all instruction provided in the leaflets was followed and the corresponding concentration of LH levels were determined from the standard curve constructed.

Statistical analysis was performed by using SPSS version 13. The arithmetic mean and standard deviation of all observations were calculated. Difference in mean among control and treated groups was calculated by 'independent t-test'. The difference was considered significant if p-value was found

less than 0.05.

Results

At the end of day 14, serum testosterone level in lead treated (group B) was 1.5200±.49699 ng/ml as compared to control (group A) (3.0200±.19235 ng/ml) with a statistically significant decrease in p value < 0.001. Similarly at the end of day 28, testosterone level in group B (1.3200 ±.42071 ng/ml) manifested a significant decrease in p-value (<0.001) as compared to group A (2.9200±.55857 ng/ml). This statistically significant decrease in serum testosterone was persistent even at the end of day 42 (p-value <0.022) when lead treated group (1.9400±.23022 ng/ml) was compared to control group (3.0800 ±.72938 ng/ml) as shown in figure 1.

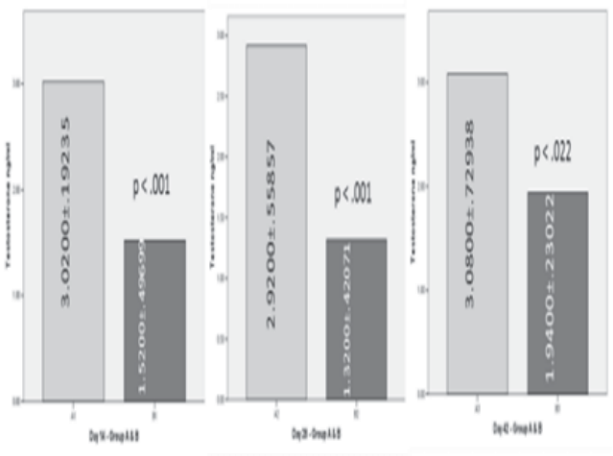

Fig 1: Comparison of mean serum testosterone level in control (group A) and lead treated group (group B) at 14, 28 & 42 days respectively. Values are expressed as mean ± SD.

Comparison of mean serum LH levels between group B (lead treated group) and group A (control group) revealed statistically insignificant difference (p>0.05) at all durations as shown in figure 2.

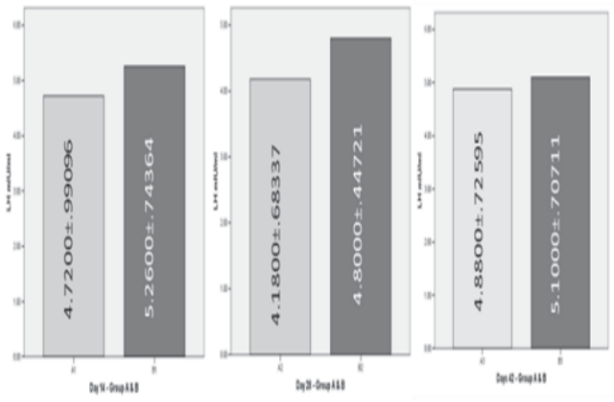

Fig 2: Comparison of mean serum LH level in control (group A) and lead treated group (group B) at 14, 28 & 42 days respectively. Values are expressed as mean ± SD.

Discussion

The present study was conducted on adult male rats to study the effects of lead toxicity on serum testosterone and LH levels. Throughout the experiment lead treated group showed significant reduction in serum testosterone levels at all time periods as compared to control group. LH levels however, exhibited no statistically significant change. As it is known that serum testosterone levels are regulated via hypothalamic-pituitary-testicular axis, hence suppression in serum testosterone level indicate either a direct action of lead on testes, or an indirect action affecting the hormonal milieu at hypothalamic-pituitary level.

These observations are in agreement with other studies7,8 reporting similar effects on serum testosterone levels. The toxic manifestations of lead on testes resulted in degeneration of both spermatogenic and Leydig cells.6 The results presented herein are in agreement with another study reporting similar effects of lead on serum testosterone level in rats (Sokol 1990). She verified that increased duration of exposure

after 14 days did not further suppress serum testosterone levels or spermatogenesis.[11]

Another study reported lower expression of enzymes 3 and 17â HSD involved in testosterone biosynthesis as a result of lead intoxication.[8]This might be the reason of decreased serum testosterone level in our study as well. According to Roy Chowdhury lead adversely affects steroidogenesis either directly or through endocrinological system.[12]

However, in contrast to our study serum testosterone level remained unaffected in another study[13] while some others observed an increase in testosterone level after lead administration[14]. Their data could be viewed as a result of a mixture of specific lead toxicity (at the enzyme level) with other more general actions (at the level of hypothalamic-pituitary-testicular axis).

In present study serum LH levels in lead treated group, revealed no significant change when compared to the control group showing that the major target of lead intoxication are the Leydig cells with only a modest effect on pituitary axis. A rise in LH levels after lead intoxication showed that the negative feedback mechanism is trying to overshadow the toxic manifestation of lead. The non-significant increase in LH levels in the study after lead intoxication was similar to another study conducted by Allouche and her co-workers. In that study chronic exposure to lead acetate did not significantly affect LH levels along with impairment of testosterone levels again emphasizing that lead may target testicular function at Leydig cell level.[15]

No changes in serum LH was observed by Kempinas, as mentioned above.[14] Sokol reported that the signals between hypothalamus and pituitary gland are disrupted by lead exposure in an intact animal.[16] Hence, we cannot rule out the possibility of involvement of hypothalamic or supra hypothalamic area in our study. There could be a reduction in serum GnRH levels resulting in decreased serum testosterone synthesis by the Leydig cells and a secondary increase in pituitary LH secretion as observed in this study.

Contrary to our study of LH results, a decrease in LH was observed recently by some studies alongwith suppressed testosterones[6,17]. This difference in LH levels could be attributed to the longer duration of lead exposure, difference in technique of measuring hormones or the route of administration of lead. A study conducted on lead exposure in an occupationally exposed population suggested that lead toxicity initially produced a direct testicular toxicity followed by hypothalamic or pituitary disturbance with longer periods of exposure.[18]

The pathogenicity of lead toxicity on reproductive system can be explained by multiple mechanisms of action. Lead along with other commonly found persistent toxic metals like mercury, arsenic and cadmium damages cellular material and produce alteration in genetic material.12 The mechanism underlying in all these metals is common, involving oxidative damage. Studies have shown that lead poisoning disturb the normal balance of pro oxidants to antioxidants, thus affecting membranes, DNA and antioxidant defense systems of cells.[19]

Conclusion

Based on the findings of study presented

herein, it is concluded that lead intoxication resulted in testicular dysfunction as manifested by suppressed serum testosterone level with a modest effect on LH levels. Hence it seems that lead toxicity has a direct toxic action on Leydig cells along with some indirect effect by disturbing the hormonal milieu at hypothalamo-pituitary axis. The underlying mechanism of action of lead on reproductive system needs to be further evaluated to define new preventive measures against it.

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