Original Article

Correlation of Blood Lead Levels with Atresia of Ovarian Follicles of Albino Mice

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Objective: To correlate blood lead level with the atresia of ovarian follicles of albino mice following exposure to different doses of lead.

Material and Methods: This experimental Study was carried out at Postgraduate Medical Institute, Lahore from 1st February to 31st March, 2004. A total of 40 adult virgin female albino mice of 6–8 weeks age weighing between 30 ± 2 grams were divided randomly into four groups of 10 each. Group I was not given lead acetate, whereas group II, group III and group IV were given lead acetate in doses 2, 4, 8 mg mg /kg/ day, respectively for 60 days by oral lavage. Blood samples were collected in EDTA tubes for the estimation of lead level. Both ovaries were removed by dissection and processed with the standard histopathological technique for the quantitative assessment of follicle numbers in response to toxic effects of lead.

Results: Blood lead levels were the lowest in group Id (0.16µg/ml mean SD 0.18 ± 2) and highest in group IV (0.62µg/ml mean ± SD 0.60 ± 0.015) and revealed significant statistical difference ($P < 0.001$) between groups I vs II , I vs III and I vs IV. Lead levels were found to be elevated with the increment in the dose of lead acetate. The counting of various stages of atretic follicle in the control group I and treated group II, group III and group IV of albino mice showed that as the blood lead levels increased, the percentage number of atretic follicles also increased as shown in the medium follicles of the control group I: 19.4%, group II: 29.7%, group III: 44.4% and group IV: 69.6% , whereas, the large follicles control group I revealed 4.8%, group II 9.4%, group III 17.4% and group IV 29.1%. Our results indicated that artesia of medium sized follicles reflected the extent of damage caused by the lead. Both large and medium sized follicles demonstrated a highly significant correlation ($r = 0.91$ and 0.97, $p< 0.001$) with the blood lead level respectively.

Conclusion: Oral administration of lead in high doses leads to reduction in the number of ovarian follicles. Our data revealed a strong correlation between blood lead level and atresia of ovarian follicles of albino mice which is consistent with the literature.

Keywords: Blood lead levels, Mice, Correlation, Atresia, Ovarian follicles

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Introduction

Lead is a known reproductive toxin since ancient times. There is evidence in the literature that lead poisoning reduced Roman reproduction.[1] Cases of sterility, frequent abortions and abnormal menses have been found to occur in woman working in lead based industries[2]. Reproductive anomalies range from sterility, menstrual disturbance, premature rupture of the amniotic membrane, premature delivery, decreased birth weight, chromosomal aberrations, macrocephaly, miscarriage, still birth and early death of offspring.[3-6] Maternal disorder in ovulation may also occur at blood lead level of about 40 microgram/dl[7]. Angel and Lavary[8] correlated birth outcome with maternal blood lead and reported premature membrane rupture, premature delivery and preclampsia at blood lead concentration of approximately 25 ug/100ml. Menstrual disorders and their cytomorphological characteristics in woman during occupational contact with inorganic lead are reported.[6,9] Chronic exposure to lead of cynomolgous monkeys resulted in decreased level of leutinizing hormone (LH), follicle stimulating hormone (FSH) and prostaglandins.[10] Alternation in menstrual cycle in the form of less frequent cycles, long more variable intercycle interval and fewer days of vaginal bleeding was also reported in the rhesus monkeys due to lead exposure.[11] Frank et al[12] reported suppression of luteal function in the form of decreased level of progesterone in lead exposed rhesus

monkeys. Hilderbrand et al[1] reported irregularity in the estrous cycle and reduction in the number of corpora lutea and in the development of ovarian follicular cysts in female rats at blood lead level of 0.30 µg/ml and 0.53 µg/ml respectively. Jacquet et al[13] demonstrated that direct effect of lead on ovarian tissue decreases the secretion of progesterone in mice, which may alter endometrium at times of implantation. Wiebe et al[14] found that lead exposure may significantly alter steroid production and gonadotrophin binding in the ovaries of adult rats. The ovaries of fertile female contain thousands of follicles in different stages of development. There is balance between follicular growth and atresia, resulting in the maturation of specific number of follicles in rodents capable of ovulating in each reproductive cycle. Quantitative assessment of follicle number has an indication of normal function as well as toxic response in ovaries.[15]

The present study was designed to correlate blood lead level with the atresia of ovarian follicles of albino mice following exposure to different doses of lead.

Materials and Methods

Forty adult virgin female mice of 6-8 weeks age weighing between 30±2gm were procured from Veterinary Research Institute, Ghazi Road, Lahore. The animals were kept in animal house of Postgraduate Medical Institute, Lahore. The animals were randomly divided into different groups and kept in separate cages. They were numbered and weighed at one-week acclimatization and thereafter at 60 days. Mice were maintained in standard animal house conditions and were provided with animal feed (Punjab poultry feed no 3) and water at libitum. These animals were divided into four groups of ten animals each and were given lead (as lead acetate in deionized water) for 60 days. Group II, III & IV were given lead in dose of 2, 4 and 8 mg/kg/d respectively while group I was given deionized water only without lead at the same volume and frequency. The dose and duration schedule was based on as described by Junaid et al[2]. At the end of 60 days five sub-groups of two animals from each group were made by random selection (a, b, c, d, and e). For the estimation of blood lead level, blood was collected by puncture of the heart under ether anesthesia. Blood from two animals was pooled to make a sample so five sample were obtained from each group. Animals from each sub-group were sacrificed. Both ovaries were removed, saline washed and were placed in Bouin's fluid. After fixation, tissues were processed with the standard histopathological technique and serial of sections of both ovaries of each animal (5 µm thickness) were made and stained with haematoxylin and eosin stains. Sections of both ovaries were examined under light microscope for the counting, maturation and development of follicles. Every tenth section was counted for various stages of development of follicles as described by Mowad et al[16]. Follicles were classified into small, medium and large according to Chen et al [17]. Intra observer errors were also observed.

Lead estimation: Acid digestion method was used for the preparation of blood sample i.e. the blood sample was digested in nitric acid: hypochlorite (6:1) mixture and lead level was estimated using flame atomic absorption spectrophotometer[18] model Verian Spectra AA 250.

Statistical significance was evaluated by calculating standard deviation (SD) followed by Student's t-test. Correlation between two sets of data (r value) was done and the significance level was ascertained at $p<0.05$.

Results

During the course of study two animals in control group I and two in highest dose group IV died. The postmortem was performed but the cause remained unknown. The remainder animals were healthy with no significant change in body weight.

Atretic follicle: The counting of various stages atretic follicle in the control and treated groups of albino mice showed that as the blood lead levels increased, the percentage number of atretic follicle increased as shown as in medium follicles control group 19.4%, group II 29.7, group III 44.4% and group IV 69.6%, whereas, the large follicles control group showed 4.8% and group II 9.4%, group III 17.4% and group IV 29.1% as detailed in table 1.Our results indicates that artesia of medium sized follicles reflected the extent of damage caused by lead following 60 days of treatment with various doses of lead acetate i.e 2mg, 4mg and 8mg. Large follicles were affected at higher dose level (8mg, group IV 29.1%). However, the degree of atresia was maximum in group IV 69.6% of medium sized follicles having the highest lead level 0.62µg/ml at the dose of 8mg of lead acetate These findings showed a highly significant correlation ($r= 0.91$ and 0.97, $P<0.001$) of large and medium sized atretic follicles respectively with the blood lead level as shown in figures I and 2

Histopathological changes: Figures 3 described ovaries of control mice with normal histological structure .The ovaries of lowest dose group II (2mg lead acetate) showed undeveloped follicles places degenerated ova. In the highest dose group IV(8mg lead acetate) , ovaries revealed cystic spaces in medulla with undeveloped graafian follicles (Figure 4). However, the atretic follicle in the highest dose group (8mg lead acetate) also demonstrated the presence of homogenous fluid and blood in their cavities (Figures 5, 6).

Lead level: Lead levels were found to increase with the

increment of dose of lead acetate as detailed in table 2.The highest lead level was found in group IV 0.62 µg /ml SD 0. 60 ± 0.015.

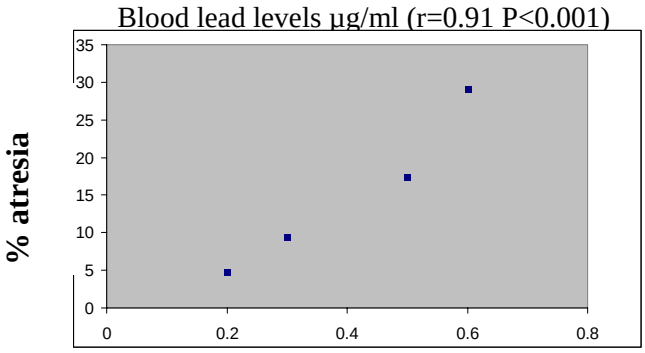

Figure 1: Correlation of atresia of large follicles with blood lead levels

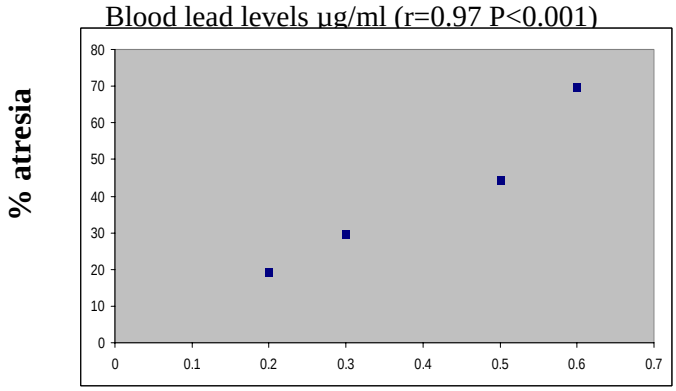

Figure 2: Correlation of atresia of medium follicles with blood lead levels

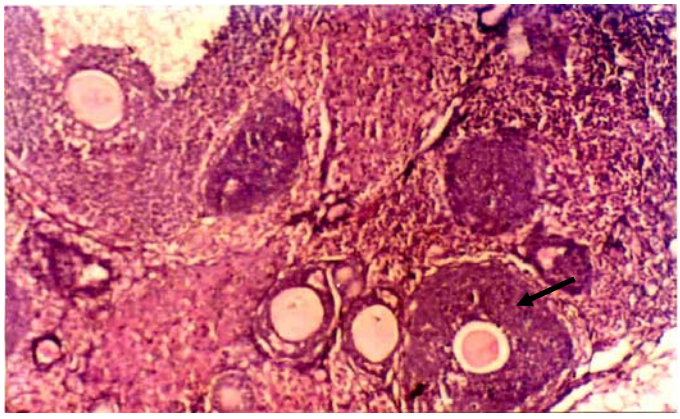

Figure 3: Photomicrograph showing normal histology of mouse ovary (H&E X 100).

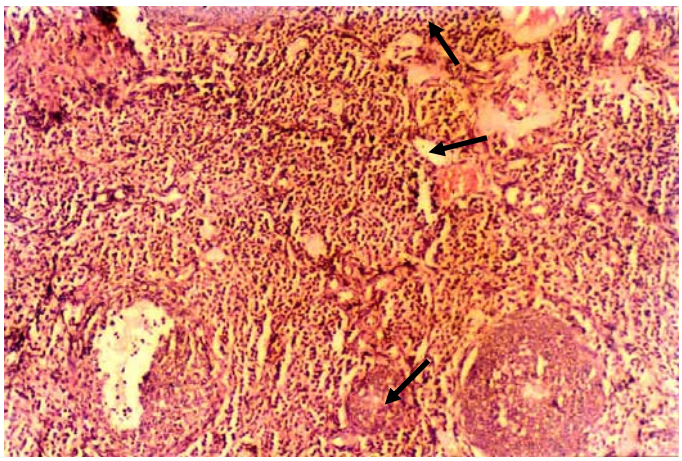

Figure 4: Photomicrograph showing cystic spaces,. Congestion and atretic follicles (H &E x 100).

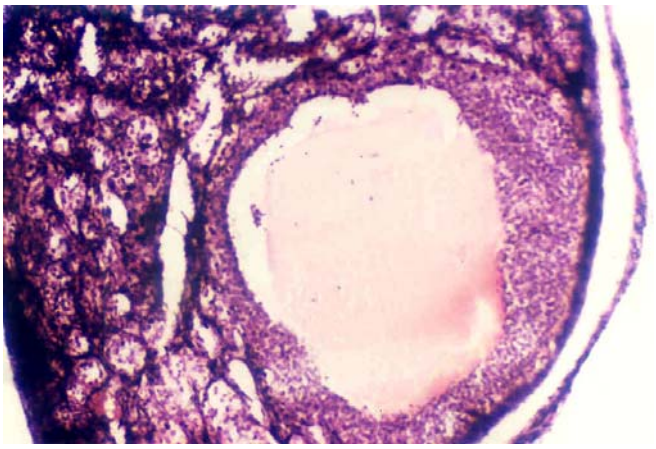

Figure 5: Photomicrograph showing atretic follicles filled with homogenous fluid. (H&E x 100)

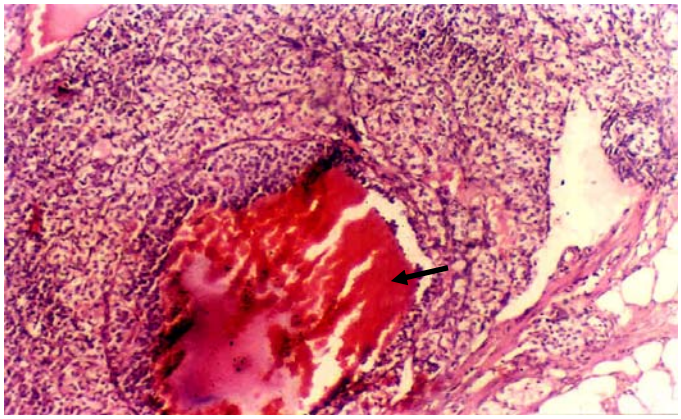

Figure 6: Photomicrograph showing atretic follicles filled with blood (H&E x 100).

Table 1: Blood lead levels (ug/ml) in mice exposed to different doses of lead for 60 days.

Group	Lead Dose	Blood levels in subgroups (ug/ml)					Mean ± SD
		a	d	c	d	e	
I	0 mg	0.19	0.21	0.18	0.16	-	0.18 ±.02*
II	2 mg	0.25	0.27	0.22	0.21	0.26	0.24 ±.02
III	6mg	0.46	0.46	0.44	0.47	0.47	0.46 ±.014
IV	8mg	0.62	0.59	0.60	0.60	-	0.60 ±.015
							p < 0.001

*p < 0.001 as compared with II, III, and IV

Table 2: Percentage of medium and large atretic follicles

Group	Atretic follicles	Sum of both follicle size	Atresia %
Medium follicles	(mean ± SD)		
Control	18.2 ± 3.7*	93.4 ± 8.15	19.4
2mg	20.2.± 3.2	67.8 ± 6.9	29.7
4mg	24.0 ± 3.4	54 ± 6.3	44.4
8mg	55.0 ± 12.9	79 ± 15.8	69.6
Large follicles			
Control	2.4 ± 1.3	49.2 ± 5.6	4.8
2mg	4.4 ± 1.71	46.6 ± 5.5	9.4
4mg	5.0 ± 1.7	28.6 ± 3.0	17.4
8mg	5.6 ± 1.76	19.2 ± 3.2	29.1

Discussion

Reproductive biologists are concerned with the lead because of its toxic effects on the reproduction system. Lead has been recognized as toxicant and injurious to health[23] and much effort has been devoted to reduce the use of lead in our daily lives. Lead also has damaging effects on fertility, pregnancy and fetal development in women living near or working in factories using lead.[24]

Reproduction anomalies range from sterility, menstrual disturbances, premature rupture of amniotic membrane, premature delivery and decreased birth weight to chromosomal aberration, macrocephaly, miscarriages, still birth and early death of offspring.3-6.

Toxic effects of lead are preventable by reducing its exposure to the environment and at working place where lead is used.[25] Keeping this in mind, this study was carried out in 40 adult female albino mice to explore the deleterious and toxic effects of the lead on the number and development of female albino mice ovarian follicles.

The animals in control group I were sacrificed after 60 days (only deionized water was given). The blood samples were taken for the base blood lead level and ovaries were dissected to see the baseline changes for the comparison with other groups of experimental animals. The gross and microscopic examination revealed normal morphology of the ovary.

In the present study the number of atretic follicles was found to increase with the elevated blood lead levels in the ovaries of mice treated with lead acetate orally for 60 days. Though the precise reason for such a change cannot be derived but it may be postulated that such effect may be due to direct lead exposure to the ovarian tissue or mediated by an alteration in the endogenous pituitary gonadotrophin and / or steriodogenic secretion. Jacquet etal[13] reported direct ovarian toxicity due to this metal, with decrease in secretion of progesterone. In contrast to our study, Wiebe and co-workers[14] treated rats with lead chloride (20&200ppm) in their drinking water during pregnancy and lactation and female offspring were examined. Tissue (blood, kidney and bone) lead levels, ovarian steroidogensis, gonadotrophin levels and gonadotrophin binding receptors were determined. The results demonstrated that lead may effect gonadotrophin receptors binding and alter steroid production in ovaries of female rats. This hormonal affect may lead to reduction in the number and development of follicles. The metallic effects on the multiple sites along the hypothalamic-pitutiary-ovarian-endometrial axis have been suggested to result in ovarian atrophy.[19-20] High dietary intake of galactose[17] and polycyclic aromatic hydrocarbons[21] were reported to induce deleterious effects on small oocytes.

The blood lead level in the control group was high. This may be due to the presence of lead in the diet. On the other hand doubling of blood lead level between 2 and 4 mg/kg/day dose group and failure to observe a similar increase between 4 and 8 mg/kg/day dose group may be explained by the fact that the relative blood lead levels are nor linearly correlated with the dose administered[26]. Barltrop and Khoo[27] suggested that the mechanisms responsible for lead absorption might be saturable if large single doses are administered. Our data of blood lead levels (0.25, 0.46, 0.62 ug/ml in group II, III& IV) are comparable with level measured in the general population (0.43µg/ml)[22] and (0.25-0.30µg/ml).[23]

The results of our study are also consistent with the finding of Junaid et al[2] that lead seems to have damaging affect on ovaries i.e. follicular development and maturation if mice were exposed to high dose of lead. Similarly in another study, Vermande-Von Eck and Meigs[19] found that exposure of lead to monkeys resulted in flat, white atretic ovaries with no dominant follicles. Whereas, Hilderbrand and colleagues studied 120 sexually mature male and female rats and divided them

into control group, lead was not administered while other groups received 5 and 100 µg of lead dose for 30 days. At the end of study period, blood lead levels in the control group was 0.14µg/ml and in the treated group blood lead concentration was 0.19 and 0.30µg/ml respectively. The group with the highest blood lead concentration 0.19 and 0.30µg/ml) revealed persistent vaginal estrous and the development of ovarian follicular cyst with the reduction in the number of corpora lutea. These findings indicate the possible hazardous effects of lead acetate on sexuality and reproductive function. Our study also proves if higher concentration of lead administered orally, it hampers the follicular development and maturation.

Conclusions

Oral administration of lead in high doses leads to reduction in the number of ovarian follicles. Lead also affects maturation and development of ovarian follicles with increase in number of atretic follicles. We suggest exploring the effects of lead in experimental animals on endocrine and central nervous systems. It is worth to explore hematological aspects and changes in the estrous cycle with the different doses of lead.

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