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Effect of lead exposure during perinatal period on kidney of adult offspring in rat: A stereological study

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Abstract

Lead is the most common toxic metal in nature, and its adverse effects on various organs, including the kidneys. As a complicated process, kidney development is influenced by various environmental variables. Although lead toxicity can occur at any age, it is important in pregnant mothers and infants. Therefore, the present study investigated how the low dose of lead administration could affect kidney offspring in rat model. To this aim, a stereology technique was used. A randomized sampling was used to assign animals to five groups. The first one (i.e., Group 1 as the control) was provided with ordinary drinking water plus glacial acetic acid (0.5 ml/liter) 0 as a lead acetate solvent. Animals in Group 2 were administrated 0.2% of lead acetate in drinking water for 30 days prior mating. Rats in Group 3 received drinking water with 0.2% of lead for 21 days during pregnancy. Animals in Group 4 consumed 0.2% of lead acetate in their water for 21 days within their lactation. Group 5 was provided with 0.2 % lead acetate in water in their pre-pregnancy (30 days), pregnancy (21 days) and lactation (21 days) periods. The left kidney was removed from male offspring 60 days after birth. The volume of the kidney, cortex, medulla, proximal convoluted tubules (PCT) as well as distal convoluted tubules (DCT), and also the length of PCT and DCT, were analyzed by means of stereology. The findings revealed a reduction in the volume and length of the DCT as well as some pathological effects in experimental groups, compared to the control group. Due to the ameliorating effect of lead in perinatal period even in low doses on offspring kidneys, cautiousness is needed in this period.

Keywords: kidney, lead, stereology, perinatal period

1. Introduction

Lead is known as the most prevalent poisonous metal existing in nature (1). As a result of human activities, the amount of lead that exists in nature during the past three centuries is estimated as 1000 times (2) Consuming contaminated food and drink is the basis of lead poisoning across communities. In addition, toxicity through inhaling contaminated dust and gases is prevalent (3). Toxicological studies have shown that lead affects central nervous system (4-6), peripheral nervous system (7, 8), cardiovascular system (9, 10), endocrine system (11, 12), immune system (13, 14), digestive system (15, 16), male and female reproduction system (17-20) and also urinary system (21, 22). The nephropathy induced by lead causes nephron malfunctioning, metabolic disorders and more protein excretion in urine (23, 24). Lead exposure is accompanied by pathological side effects such as the blockage of tubes, nucleus hetero-chromatization, increased diameter of renal tubules (25-29).

The kidney development is complex and progresses gradually followed differentiation into pronephrosis, mesonephrosis and metanephrosis (30). Many factors like environmental contamination can influence renal development result in malfunctions of the kidney. In this way, although prevalence of lead is considered as an ecological threat for all regardless of age (31) but pregnant women and young children are at the highest risk. A wide range of research showed that the level of lead in newborns exceeded 10 microgram / deciliter in blood and exceeded a standard limit (32).

The present research aimed to explore the toxic effect of a lower dose of lead during perinatal period on the morphometrical properties of the kidney of offsprings in rat model via an unbiased designed stereology. At present, stereology is a method of making unbiased and precise quantitative estimation of kidney structure (33). The structural features such as the number, size, length or nephron distribution are correlated with the renal function (34).

Assessing kidney volume, volume fraction of cortex and medulla, glomerular volume, length and the volume of proximal (PCT) as well as distal convoluted tubules (DCT) in kidney following lead toxicity during perinatal period (Pre-pregnancy, pregnancy and lactation) may contribute to knowledge for protections of mothers from lead toxicity and

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its adverse effects on neonates' kidney.

2. Materials and methods

2.1. Experimental animals and treatment

The male and female adult Wistar rats were purchased from the Pasteur Institute, Tehran, Iran. The rats were allowed to mate overnight. Those animals with vaginal plug were considered pregnant at gestation day 0. The rats were maintained in a temperature of 23–25°C with a 12-h light/12 h dark cycle provided with food and ad libitum. The experiment abided by the standard guide for the care and use of laboratory animals. The rats were divided randomly to five groups each with five animals:

Group 1 (control): This group was provided with ordinary drinking water plus glacial acetic acid (0.5 ml/liter) as a lead acetate solvent throughout the study.

Group 2 (pre-pregnancy): This group received lead acetate (0.2%) in drinking water for 30 days in advance to mating. After that, this group was provided with ordinary drinking water till the end of the treatment.

Group 3 (Pregnancy): This group received drinking water with lead (0.2%) for 21 days during pregnancy.

Group 4 (lactation): This group received lead acetate (0.2%) in drinking water for 21 days during lactation.

Group 5 (pre-pregnancy, pregnancy and lactation): This group was provided with lead acetate (0.2%) in their drinking water during pre-pregnancy (30 days), pregnancy (21 days) and lactation (21 days) periods.

Lead acetate (0.2%) was freshly added to distilled water 0plus glacial acetic acid (0.5 ml/liter) to impede lead acetate sedimentation (35, 36).

After the postnatal day (PND) 21, neonates were separated from mothers in each group and maintained in separate cages and they received ordinary food and water.

2.2. Tissue sampling and stereological methods

Five male offspring in each group (one offspring from each mother) were selected randomly and were then anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) on PND 65. Animals were perfused with neutral formalin 10 % intracardially. The left kidneys were removed and weighted using a digital scale. Then, each kidney was immersed in the same fixative for more fixation.

After that each kidney was embedded in agar 6 % and isotropic, uniform samples were randomly taken via the orientator method (33). Each kidney was sectioned into parallel slabs in 2 mm thickness using a tissue slicer. A routine processing of slabs followed for a light microscopy. They were embedded in paraffin, cut into 5 µm-thick sections, and stained with hematoxylin-eosin and Periodic acid-Schiff. Geometrical probes were created using ImageJ package (https://imagej.nih.gov/ij/).

2.3. Estimation of the volume

The total volume of each kidney was calculated by changing its weight to a volume with the help of the specific kidney tissue density:

$$V(kid) = W(kid) / \rho,$$

in which ρ stands for the weight to volume ratio of kidney tissue (1.04 g/cm3).

The sections were scanned using a slide scanner (Optic lab H850, Plustek, China) and test point system was covered on each image (Fig. 1). Then, the fractional volume (Vv) of renal cortex and medulla was calculated using the following formula (37):

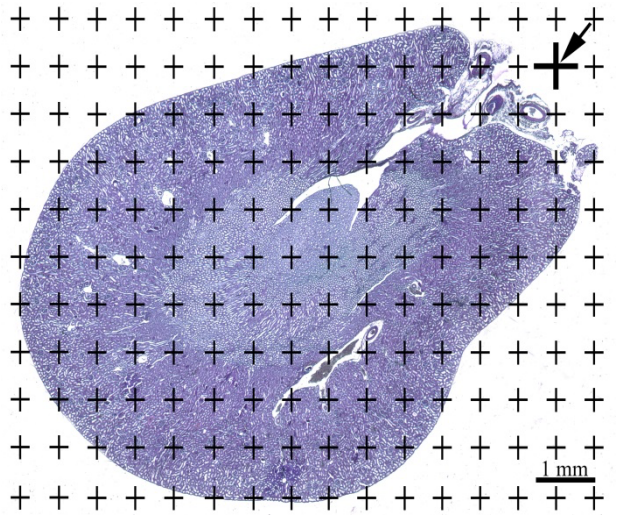

Fig. 1. Volume estimation. A point grid was superimposed on the sections. If tissue was found in the right upper side of a point of test (arrow), it was counted

$$Vv\ (\text{cortex or medulla}) =$$

in which ΣP cortex or medulla represents the total number of points that hit the cortex or medulla in kidney; ΣP (kidney) represents the total number of points that hit the entire kidney.

The total volume of renal cortex and medulla were obtained as:

$$V\ (\text{cortex or medulla}) = Vv\ (\text{cortex or medulla}) \cdot V\ (\text{kidney})$$

In order to estimate the volume of the glomeruli, PCT and DCT, we captured the systematic uniform random fields of view and moved the microscope stage in the same step lengths along the x and y directions for each section by a microscope (CX40, Olympus) linked with a digital camera (MB-2250, Germany). Then, the volume fraction was calculated by counting points hitting the glomeruli, PCT or DCT and reference section (Fig. 2a). Next, the total volume of glomeruli, PCT and DCT was estimated as the volume density multiplied by the total volume of kidney.

2.4. Estimation of the renal tubules length

For Estimation of length of PCT and DCT, unbiased counting frame were superimposed on chosen microscopic fields of

views. The number of profiles which is in the counting frame and does not reach the exclusion borders of the frame were counted (Fig. 2b) and the length density was estimated as:

Lv (tubules)=

in which ΣQ(tubules) stands for the total number of the tubule profiles sampled for the counting frame; a frame is the area related to a counting frame; P (kidney) represents the total number of points that hit the kidney. PCT and DCT total lengths were estimated as the length density multiplied by the kidney total volume (33).

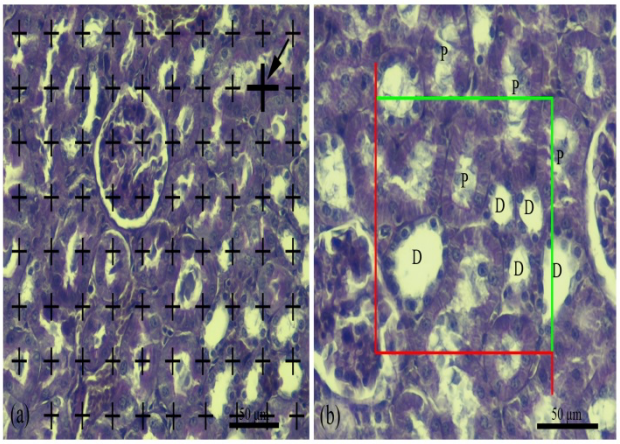

Fig. 2. Stereological estimation of volume fraction of glomeruli, proximal and distal convoluted tubules via the point counting method. The arrow shows the point on the right upper corner of the cross (a). Estimation of length of proximal and distal convoluted tubules using an unbiased counting frame with inclusion (green) and exclusion (red) lines (b). The tubular profiles inside the counting frame or touching the inclusion lines were counted versus those outside the counting frame or touching the exclusion lines which were ignored. Here four proximal (P) and five distal (D) convoluted tubule profiles were counted

2.5. Statistical Analysis

Kolmogorov-Smirnov test was used to check the data normality. Kruskal-Wallis and Mann-Whitney test and ANOVA followed by Tukey's post-hoc test were run to compare data between different groups in with abnormal and normal distribution, respectively. A $p<0.05$ was considered significant.

2.6. Ethical consideration

The present research project was performed based on the guidelines of the Ethics Committee at Tehran University of Medical Sciences (# 91-01-159- 18022). All the experiments were done in adherence to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

3. Results

3.1. Total kidney volume

The total kidney volume was estimated at 0.895± .108 cm3 in group 1, and 0.919 ± 0.560 cm3 in the group 2, 0.990± 0.148 cm3 in group 3, 0.829 ± 0.051 cm3 in group 4 and 0.930 ± 0.063 cm3 in group 5. Statistical analysis revealed no statistically significant difference in total kidney volume between different groups (Fig. 3).

3.2. Cortex and medulla volume

The cortex volume of the cortex is 0.510± .045 cm3 in group 1, 0.560 ± .031 cm3 in group 2, 0.606± .137 cm3 in group 3, 0.467± .061 cm3 in group 4, and 0.551 ± 0.064 cm3 in group 5. No significant difference was observed between the groups (Figure 1). The fractional volume of the cortex was calculated as is 57.28 ± 4.89 % in group 61.1 ± 4.38 % in group 2, 60.74 ± 7.1 % in group 3, 56.34 ± 5.06 % in group 4 and 59.27 ± 4.83% in group 5.

The total volume of the medulla was 0.384± .080 cm3 in group 1, 0.358 ± .059 cm3 in group 2, 0.386± .078 cm3 in group 3, 0.361± .041 cm3 in group 4 and 0.378 ± .044 cm3 in group 5. The fractional volume of the medulla was 42.69 ± 4.93 % in group 1, 38.86 ± 4.39 % in group 2, 39.260 ± 7.10% in group 3, 43.66 ± 5.06 % in group 4 and 40.72 ± 4.83% in group 5. The groups did not differ significantly in either the cortex or the medulla (Fig. 3, 4).

3.3. PCT and DCT volume

The total volume of the PCT was found to be 0.529± .091 cm3 in group 1, 0.511 ± .044 cm3 in group 2, 0.610± .089 cm3 in group 3, 0.511± .054 cm3 in group 4 and 0.515 ± .043 cm3 in group 5. The fractional volume of the PCT was 60.19 ± 14.27 % in group 1, 55.68 ± 3.38 % in group 2, 61.60 ± 2.02% in group 3, 61.56 ± 2.84 % in group 4 and 55.25 ± 1.13% in group 5. No significant divergence was found between the different groups in terms of the PCT volume.

DCT total volume was estimated to be 0.108± .010 cm3 in group 1, 0.106 ± .006 cm3 in group 2, 0.127± .005 cm3 in group 3, 0.115± .016 cm3 in group 4 and 0.144 ± .012 cm3 in group 5. The results indicated that the total volume of the DCT in the group 5 increased significantly in comparison to groups 1, 2 and 4 ($p<0.05$). The fractional volume of the DCT was 11.68 ± 0.950% in group 1, 11.82 ± 0.162 % in group 2, 11.54 ± .732% in group 3, 13.75 ± 1.320 % in group 4 and 15.53 ± 0.458% in group 5. The fractional volume of the DCT in group 5 indicated a significant increase in comparison with the other groups ($p<0.05$) (Fig. 3, 4).

3.4. Glomerulus volume

The total volume of the glomeruli was 0.019± .005 cm3 in group 1, 0.019 ± .001 cm3 in group 2, 0.022± .003 cm3 in group 3, 0.017± .002 cm3 in group 4 and 0.022 ± .005 cm3 in group 5. The fractional volume of the glomerular was estimated as 2.154 ± .778 % in group 1, 2.066 ± .159 % in group 2, 2.180 ± .240% in group 3, 2.034 ± .213 % in group 4 and 2.370 ± .494% in group 5. There was not significant difference between groups in glomeruli volumes (Fig. 3, 4).

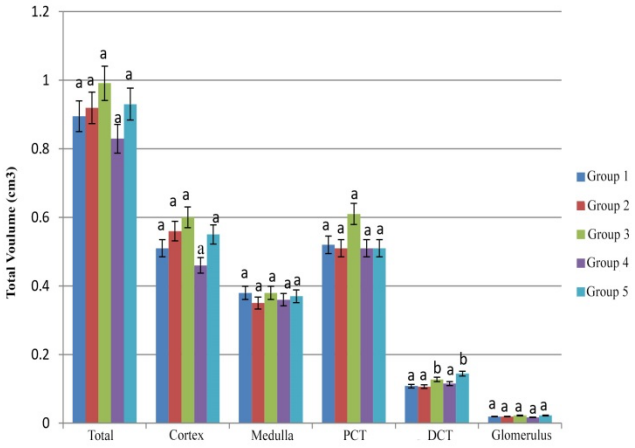

Fig. 3. Comparison of total kidney volume, total volume of the cortex, medulla, proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and the glomeruli between group 1 (control), group 2 (pre-pregnancy), group 3 (pregnancy), group 4 (lactation), group 5 (pre-pregnancy-pregnancy-lactation). Different letters represent statistically significant divergences ($p < 0.05$) between groups

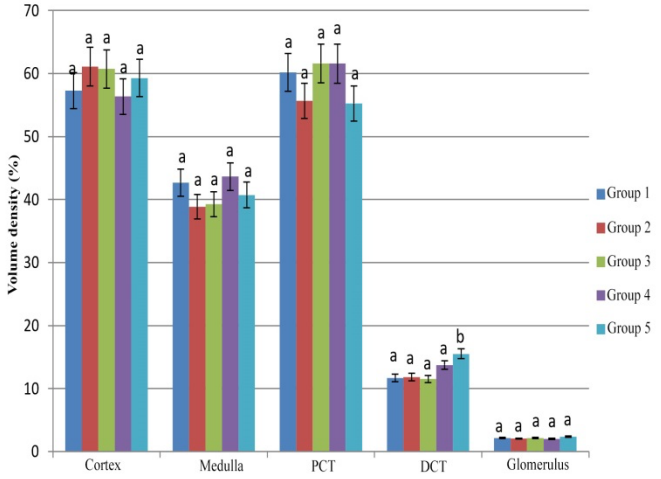

Fig. 4. Comparison of fractional volume of the cortex, medulla, proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and glomeruli between group 1 (control), group 2 (pre-pregnancy), group 3 (pregnancy), group 4 (lactation), group 5 (pre-pregnancy-pregnancy-lactation). Different letters point to a significant difference ($p < 0.05$) between groups

3.5. PCT and DCT length

The PCT length was estimated as 133.83± 30.78 m in group 1, 131.98 ± 21.66 m in group 2, 182.60± 32.91 m in group 3, 126.64± 23.18 m in group 4 and 154.01 ± 2.882 m in group 5. The between-group differences were not statistically significant.

The DCT length was 129.78 ± 14.75 m in group 1, 79.94 ± 14.57 m in group 2, 99.02± 20.87 m in group 3, 50.61± 5.25 m in group 4 and 98.220 ± 11.32 m in group 5. The DCT was shorter in all the groups receiving a treatment than the control (group 1), which was significant in groups 2 and 4 ($p < 0.05$) (Fig. 5).

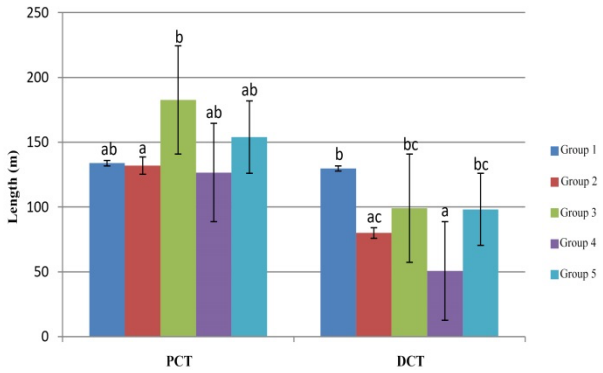

Fig. 5. Comparison of proximal (PCT) and distal (DCT) convoluted tubules length between group 1 (control), group 2 (pre-pregnancy), group 3 (pregnancy), group 4 (lactation), group 5 (pre-pregnancy-pregnancy-lactation). Different letters represent a significant difference ($p < 0.05$) between groups

3.6. Pathological findings

Histological examination revealed hemorrhage (Fig. 6a) and multifocal lymphoplasmacytic nephritis (Fig. 6.b) in the kidneys in lactation group. Microscopically, in some renal tubules of PPL group, acute cell swelling (hydropic degeneration) was observed in the tubular epithelial cells (Fig. 6c). Epithelial cell detachment and proteinuria were also seen in the sections in lactation group (Fig. 6d).

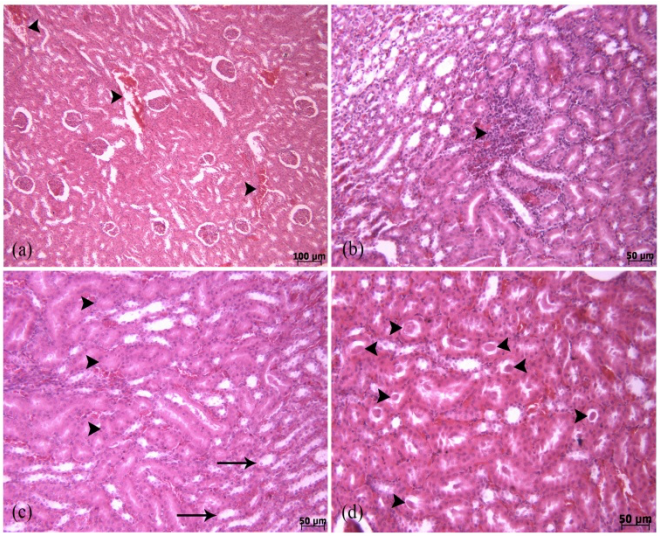

Fig. 6. Pathological examination revealed hemorrhage (a) and multifocal lymphoplasmacytic nephritis (b) in the kidneys of lactation group. Microscopically, in some renal tubules, acute cell swelling (Hydropic degeneration) was observed in the tubular epithelial cells of PPL group (c). Epithelial cell detachment and proteinuria were also seen in the sections of lactation group (d)

4. Discussion

Despite the negative effects of lead, it is almost impossible to avoid lead toxic exposure. Besides, the presence of asymptomatic lead in women who are pregnant leads to changes in the fetus that remain unknown. This study was conducted to assess the structure of kidney in offspring following lead poisoning in mothers during the perinatal period using unbiased designed based stereology.

In this study, no significant difference was found in the total volume or fractional volumes of the cortex or medulla between the experimental and control groups. However, Heidari et al. (38), showed that the mean total volume of kidney in 0.13% lead acetate groups, increased compared to the control group in adult male rat. It was significant in groups that received lead acetate in their drinking water for 8 and 12 weeks and not for 4 weeks. In addition, cortex and medulla volumes has been reported increased significantly in 12 weeks lead acetate administration compared with control group. It was assumed that renal hypertrophy is due to protein synthesis and proximal tubular epithelial cell hypertrophy (39). In contrary, Skröder et al. (40), showed that increased lead exposure at late gestation reduced kidney volume in children. It seems that the differences in the impact of lead on volume parameters in kidneys is related to the dose used and the duration of treatment. It was shown that kidney volume is related with glomerular filtration rate (41) and any change in cortex and medulla or renal volume can point to a renal pathology (42).

The present findings revealed that the distal tube total volume in the PPL group increased significantly in comparison to the control group. Consistently, the related literature confirmed an increase in the volume of renal tubules due to heavy metal poisoning (27). In a study by Karimfar et al. (26), DCT, PCT, and collecting ducts were dilated in rabbits following prolonged exposure to lead acetate. Lead toxicity causes mitochondrial swelling in renal tubular cells and impaired the production of energy, which may be another reason for increased tubular volume (43). Higher doses of lead also impair the transport of salts and amino acids and atrophy of the renal tubules (27). It is important to note that tubular modifications happen sooner than glomerulus and interstitial tissue (44). Similarly, in this research, despite changes in the tubular volume, there was no change in the volume of the kidney, cortex or medulla.

In this study, no major change in glomerular volume was found between control and experimental groups. In a study, following the exposure to 0.5% lead acetate for periods longer than three months, no divergence was found in glomerular diameter between control and experimental groups with different periods of administration (39). This study also showed that the amount of glomerular filtration rate (GFR) increases from the third month onwards, along with kidney weight, which indicates renal hypertrophy (39).

However, renal hypertrophy is not associated with hypertrophy of the glomeruli, and the rise of kidney weight and volume is due to hypertrophy of the proximal tubules (39), which is due to protein synthesis in these areas (38). Goyer et al. (45) showed no changes in glomeruli, while signs of changes in glomerular cells became apparent 6 weeks after lead administration in rat.

Glomerular status following lead exposure differs according to factors such as differences in dose, duration of exposure, path of administration (oral or respiratory) and period of exposure or age (pre-pregnancy, pregnancy, postpartum, lactation, puberty) and gender (46-48).

Overall, glomerular volume changes usually occur during long periods of exposure. Unlike changes in other kidney tissues, the lack of changes in glomerular volume can be due to different physiological and histological conditions of glomeruli compared to other kidney tissues, which increases its resistance to toxins compared to other tissues. Glomeruli have a defense barrier due to their special structure (49).

In our results, the length of the distal tube in all experimental groups (pre-pregnancy, pregnancy, lactation and PPL) showed a significant decrease in comparison to the control in the pre-pregnancy and lactation groups. In general, the length of PCT and DCT in heavy metals decreased compared to control group. According to previous studies, lead acetate is a peroxidating agent and by damaging cell membrane lipids and membrane permeability leads to destruction and necrosis of renal tubular tissue (25, 50, 51).

Adverse effects of lead on the fetus varied according to gestational age. Lead crosses the placental blood barrier and is able to delay fetal development and has teratogenic effects on it. Maternal lead contamination through nutrition can also be transmitted to infants through milk (52, 53). Regarding shorter length of DCT of pre-pregnancy group in comparison with control group, it has been pinpointed that exposure to lead before pregnancy, even after the removal of lead during pregnancy, can still have effects on the fetus due to the very slow excretion of lead from the body (54).

Among the tissues, lead accumulates at the highest level in the kidney and causes marked pathobiological changes in the kidney structure and function (55). In the present study, multifocal lymphoplasmic hemorrhage and nephritis of the kidney were observed in the lactation group. In previous studies, exposure to leadwas observed hyperemia in interstitial tissue, renal arteries, and mild focal inflammation (56). Damage to kidney cells indicates impairment in mesangial tissue in the cell vascular pole (26). Lead has an inhibitory effect on several enzymes in the heme production pathway, reducing the life of erythrocytes by increasing membrane fragility and resulting in hemolysis. Finally, due to spasm and narrowing of cutaneous arteries, it causes bleeding and inflammation of renal arteries (57, 58). In pathological analysis some renal tubules of PPL group showed acute cell swelling (hydropic degeneration) in tubular epithelial cells. Epithelial cell detachment and proteinuria were also seen in the lactation group. These pathological findings are early signs of acute tubular necrosis, and damage to renal vascular endothelial cells causes ischemia and structural changes such as loss of the brush border, disruption of tight cell connections, and detachment of tubular epithelial cells in kidneys and the swelling of tubes (59, 60). In general, protein

kinase c activates a wide range of kinases and phosphatases that affect the process of cell division, proliferation and cell communication, and lead acts by disrupting the protein kinase c receptor system and causing tissue damage and changes in the kidney (55).

Knowing that lead passes through placental blood barrier and milk, this study confirms the effect of lead at low doses on offspring kidney following perinatal period. The results of this study can be useful in quantifying the changes in kidneys following lead poisoning and adopting appropriate methods to protect mothers from lead poisoning and its effects on all births.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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