The effects of high dose of iodine on the anatomical and histological features of the thyroid glands in rabbits during the prenatal and postnatal periods

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ABSTRACT

Background and objectives: Iodine is an essential nutrient for optimal thyroid function, the major health effects of concern with excess iodine ingestion are thyroid disorders. The purpose of this study was to investigate the effects of the toxic dose of iodine on the anatomical and histological features of the thyroid glands in rabbits during prenatal and postnatal periods.

Methods: Thirty two adult female rabbits were selected. Only sixteen female rabbits were given oral dose of iodine (11 mg/kg of body weight dissolved in 1ml of distilled water) twice daily for a period of one and a half month, then rabbit offspring's thyroid glands were studied anatomically and histologically.

Results: The study group showed significantly higher rate of thyroid glands developmental disturbances ($p<0.05$), significant increase in thyroid glands weight in the postnatal period only ($p<0.05$), and in the thyroid gland volume in the prenatal period at 21th day of gestation and postnatal periods ($p<0.05$). Histological analysis showed significant decrease in heights and increase in widths of follicular cells ($p<0.05$) in the study group at postnatal periods. The thyroid tissues at 21 th day of gestation in the control and study embryos were nearly similar in the diameter of the follicles, but the number of the follicles in the study group was significantly less than the control group ($p<0.05$). The study group in postnatal periods showed significantly lesser number of follicles but with larger diameter and the colloid showed cracking. A significant decrease in the blood vessels density was also seen ($p<0.05$).

Conclusion: High dose of iodine causes goitrogenic effect which may induces the blockade of hormone biosynthesis.

Key words: Iodine, thyroid gland, rabbit.

INTRODUCTION:

Iodine is an essential component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Thyroid hormones regulate many important biochemical reactions, including protein synthesis and enzymatic activity, and are critical determinants of metabolic activity [1]. Exposure to high amounts of iodine occurs via food [2], drinking water [3], medication [4] and iodized salt or iodinated oil [5]. Any increase in iodine intake will cause some increase in the incidence of hyperthyroidism in a previously iodine-deficient population. Doses of 30–250 ml of tincture of iodine (about 16–130 mg of total iodine per kg of body weight) have been reported to be fatal to human [6]. Hypothyroidism from excessive iodine intake is much more common than hyperthyroidism. Hypothyroidism is attributed to the prolonged suppression of thyroid hormone production as the result of excess iodine level [7].

Since the thyroid gland plays an important role, at least in later stages of development and growth, the onset and pattern of its biochemical (functional) differentiation in embryo and fetus have attracted much attention, as well as the interrelationship between the glands of mother and fetus in placental mammals. In pregnancy more iodine is required, to ensure maternal thyroid hormone (T4) production, and can be maintained at almost double that of the non-pregnant state. The fetus is entirely dependent on T4 transferred from the mother during the first and second trimesters and on iodine transfer for fetal thyroid hormone synthesis during the last trimester [8, 9].

This study is designed to investigate the effect of iodine toxicity on the gross anatomical features and histological picture of rabbit's thyroid gland during prenatal and postnatal periods in the study group compared with that of the control group.

MATERIALS AND METHODS:

The present study were done in the period between November/2010 and May/2011, and approved by the local scientific and ethical committee of the College of Dentistry in Hawler Medical University.

The total number of the animals used in this study was thirty two adult female rabbits (Oryctolagus cuniculus), weighing about (2.5-3kg), and not less than 10 months of age. The animals were acclimatized for one week to the laboratory conditions prior to experimental manipulation, with free access to standard laboratory diet and water and maintained in constant temperature controlled rooms (22±3 ◦C) with controlled lighting (12 h light/12 h dark).Pure iodine was extracted from povidone iodine solution [10]. After that a pilot study was conducted to find the standard highest tolerable, non lethal oral dose of iodine.

All females when paired with a male overnight are examined for a vaginal plug in the following morning. The day on which a vaginal plug was observed was designated day 0 of gestation. Sixteen female rabbits were given a constant oral dose of iodine (11 mg/kg of body weight dissolved in 1ml of distilled water)

twice daily, for a period of one and a half month (one week before mating , along the period of gestation and till the 7th day of the postnatal life).

All the samples were received an intramuscular injection of 0.5mg/kg of phenobarbital, after that a mixture of 50 mg/kg bodyweight of ketamine hydrochloride and 10 mg/kg body weight of xylazine 2% were used for subcutaneous injection in the neck. The fetal rabbits, in both control and study groups, were subdivided into four subgroups (four animals each) which represented those collected at the 14th and 21th day of gestation, at their birthday, and at 7th postnatal day.

The samples of the prenatal group were harvested by sacrificing the pregnant rabbits. After caesarean section, fetuses were collected; thyroid glands were exposed and examined for the presence of developmental disturbances, extracted from its position and directly weighed. Regarding the volume of the thyroid gland measurements, the mediolateral, anteroposterior and craniocaudal diameters of each lobe was recorded to represent the width, depth (thickness) and length respectively then the volume of each lobe was calculated using the formula of the volume of an ovoid (width*depth*length* π /6), then the summation of the volume of both lobes was determined to represent the total thyroid volume and the isthmus was not included in the volume calculation [11].

Thyroid samples were fixed in Bouin's solution, processed, sectioned, and stained by hematoxylin and eosin stain. Measurements of height and width of follicular epithelium were taken from at least 10 different randomly selected thyroid follicles for each rabbit (H&E, x 1000). The height of the cells was measured from the outside to inside edge and measured at four predetermined positions (12:00, 3:00, 6:00, and 9:00) [12]. The number of follicles and the vascular density were calculated in five randomly selected microscopical fields at 200x magnification, but the maximum diameter of the follicle was measured in ten follicles selected randomly (HE, x1000) using a stage and ocular micrometer. The means of all measurements were determined for each animal, and these measurements were also evaluated by two persons blind to the study.

Data were analyzed using Med-Calc statistical software. The data were non-normally distributed. Accordingly, non-parametric tests were used. Mann-Whitney U test was utilized to assess statistical significance in the median value between two groups and the P value of ≤ 0.05 was considered to be statistically significant.

RESULTS:

A.Anatomical features: In the prenatal period at 14th day of gestation, the thyroid gland anatomically was very hard to locate even with dissecting microscope and it was quiet impossible to study the presence of developmental disturbances, the weight and volume of the thyroid gland at this age. In other samples the thyroid was a bi-lobed organ located in the ventrolateral aspect of the trachea in the infrahyoid region just below the laryngeal prominence, near the cricoid cartilage with a narrow isthmus connecting the two lobes, and loose connective tissue was seen to enclose the gland which helps to fix the gland lobes to the tracheal tube. The absence of the isthmus was a developmental anomaly observed in 13 animals, (four in the control group and nine in the study group). Almost in all the samples, the lobes were extending from the 1st to the 3rd tracheal rings and only four cases in the study group seen extended to the 4th tracheal ring (Figure -1). No other developmental anomalies in the thyroid anatomy were noted. Statistical analysis showed significantly higher rate of developmental disturbances regarding the absence of isthmus or extension of the thyroid gland to the 4th tracheal ring associated with study group compared with that of the control group ($p<0.05$).

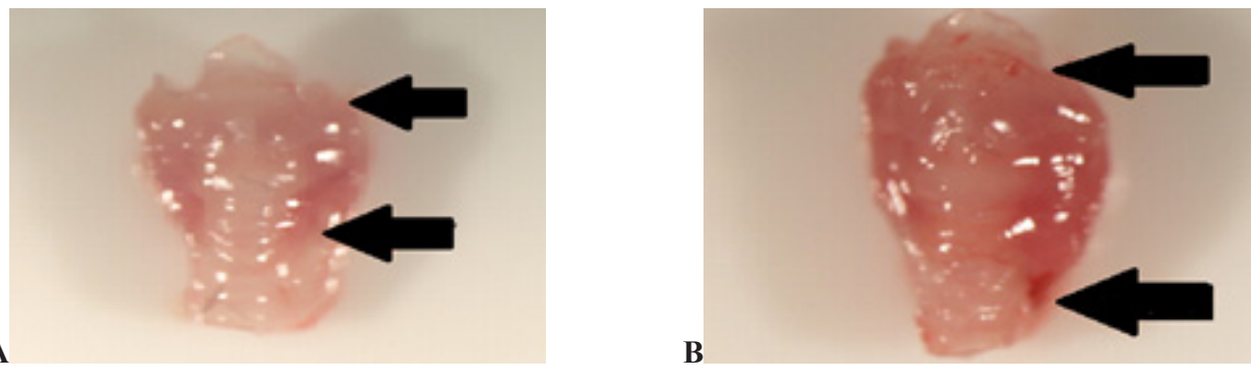


Figure- 1: The extension of the lobes of the thyroid gland. **A.** From the 1st to the 3rd tracheal ring (Arrows). **B.** To the 4th tracheal ring (Arrows).

The result also showed a significant increase in the thyroid gland weight in the postnatal period of the study group compared with that of the control group ($p<0.05$), but a non significant increase was found regarding the prenatal period at 21th day of gestation ($p>0.05$). No volume difference was observed between right and left lobes in all samples, but a significant increase in the thyroid gland volume in the prenatal period at 21th day of gestation and postnatal periods of the study group were seen compared with that of the control group ($p<0.05$) as seen in Table-1.

B. Histological features:

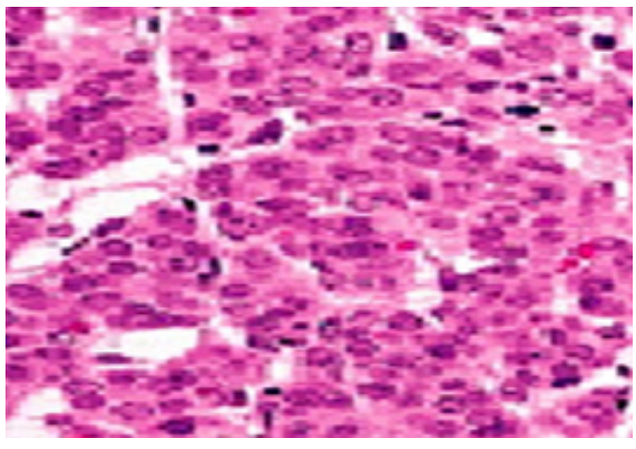
Follicular cell height and width: In the prenatal period at 14th day of gestation in both the control and study animals, the microscopical pictures showed that there were no significant microscopical signs of thyroid tissue development except for several clusters of columnar or cuboidal follicular cells that are not yet arranged to form follicles, yet although very little amount of first primordial follicle could be observed which indicates the starting point of thyroid tissue formation, development and differentiation (Figure-2).

In both the control and study groups, there were relatively no significant differences in the cells height and width in the prenatal groups, but there were significant decrease in the height and significant increase in the width of follicular cells ($p<0.05$) in the study group and the follicular cells appear flat compared with that of the control group which appear columnar or cuboidal shapes (Table-2). The toxic dose of iodine affected the thyroid gland of the study animals by inducing dysfunction and decreasing the cell height and increasing its width. The parafollicular cells population almost remained the same in both the control and study animal's thyroid samples.

Follicular number and diameter: Histological examination of the thyroid glands at the 21th day of gestation (Figure -3) showed that the glands in the control and study embryos were nearly similar in the diameter of the follicles, but the number of the follicles in the study group was significantly less than the control group ($p<0.05$). At birth, the thyroid tissues in the study group (Figure-4) significantly developed a lesser number of follicles but with a larger diameter ($p<0.05$). Seven days after birth, the thyroid tissue in the control group was normal in appearance and completely mature and the colloid was homogeneous and eosinophilic. In the study group, there were obvious differences in the affinity toward staining of the colloid with H&E stains in different follicles and even in the same follicle. Sometimes, different areas of the colloid in the same follicle showed obvious cracking. The number of follicles was significantly ($p<0.05$) less than that of the control group and the follicular diameter was significantly ($p<0.05$) larger with increase in the volume of colloid (Figure-5).

The analysis of H&E stained sections showed that the increased gland volume and weight in the postnatal samples of the study animals was associated with a widespread follicular enlargement and that the follicles were filled with colloid. This is accompanied by a decrease in the height of the follicular epithelial cells which appeared to be low cuboidal or even squamous in shape especially in the study samples that were aged seven days postnatal (Table-3).

The number of blood vessels: Statistical analysis showed a non significant increase in the thyroid tissue blood vessels number in the study group regarding the prenatal periods ($p>0.05$), but significant decrease was found in the postnatal periods compared with that of the control group ($p<0.05$), as seen in Table- 4.



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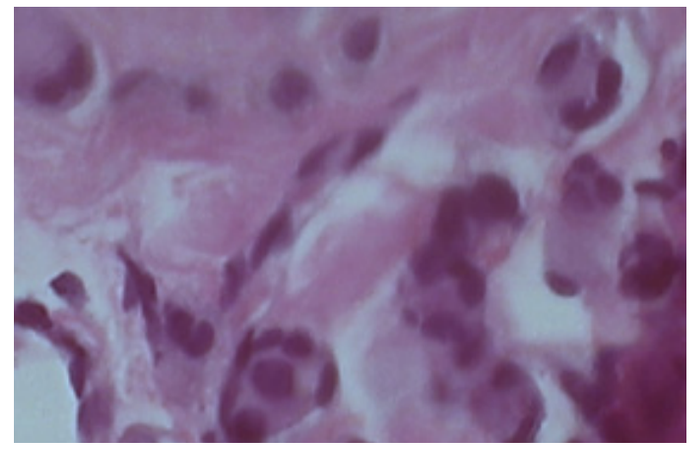

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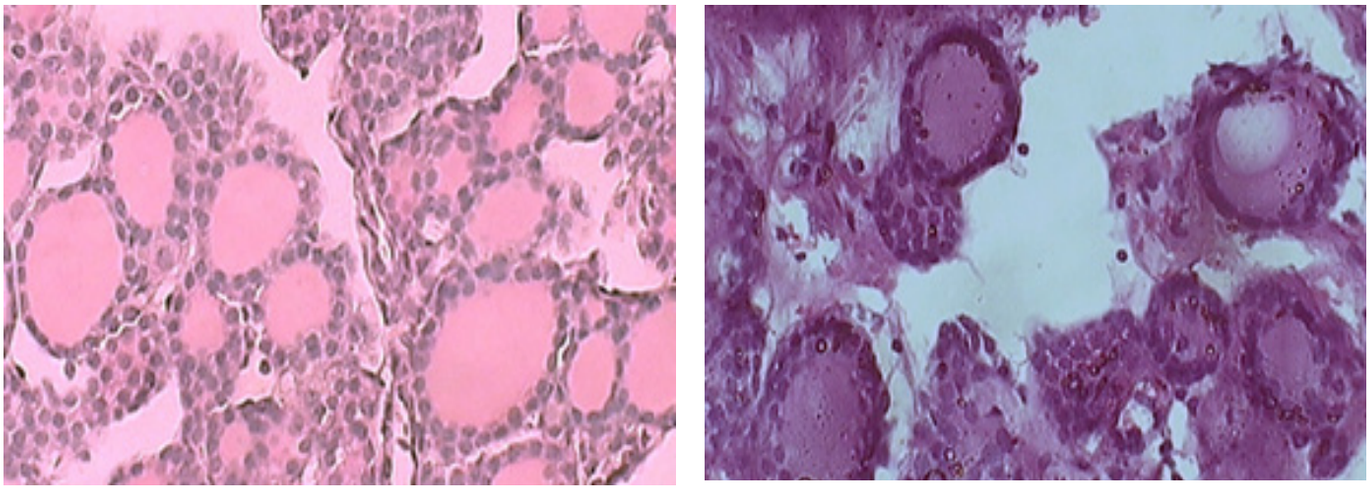
Figure -2: Thyroid tissue at 14th day of gestation **A.** Control group, the thyroid follicular cells are arranged in groups to form a primitive follicle (H&E, x 400). **B.** Study group, the follicular cells are less in number and irregularly distributed (H&E, x1000).



A B

Figure-3: Thyroid tissue at 21th day of gestation. **A.** Control group: The wall of the thyroid follicle build from cuboidal-shaped thyrocytes, the cavity of the thyroid follicle filled with colloid, and the parafollicular cells forming interfollicular clumps. **B.** Study group: Less number of thyroid follicles compared with that of the control group (H&E, x400).

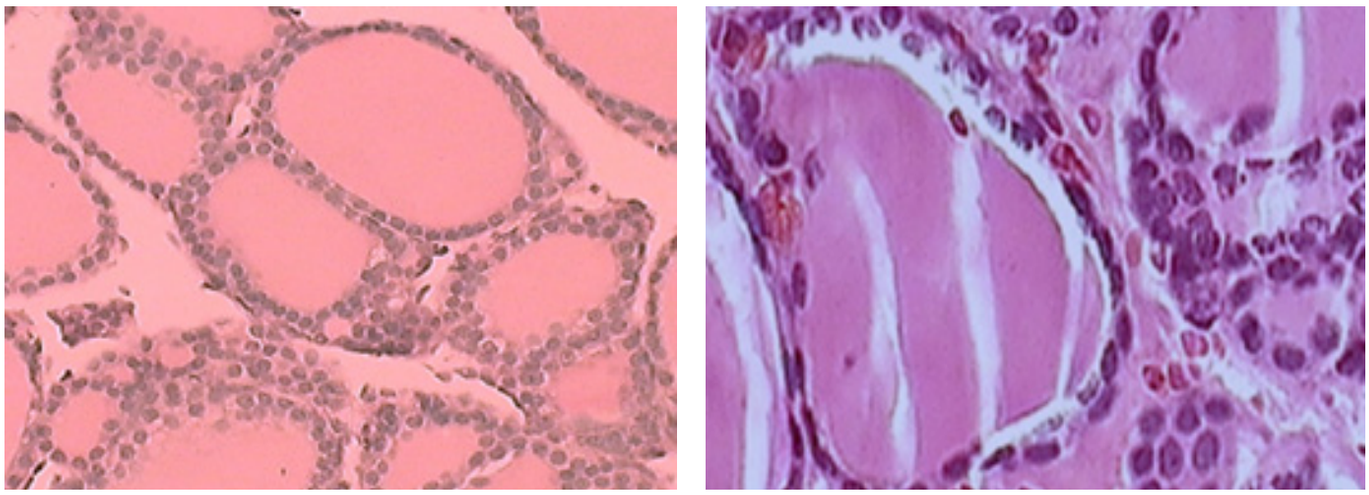


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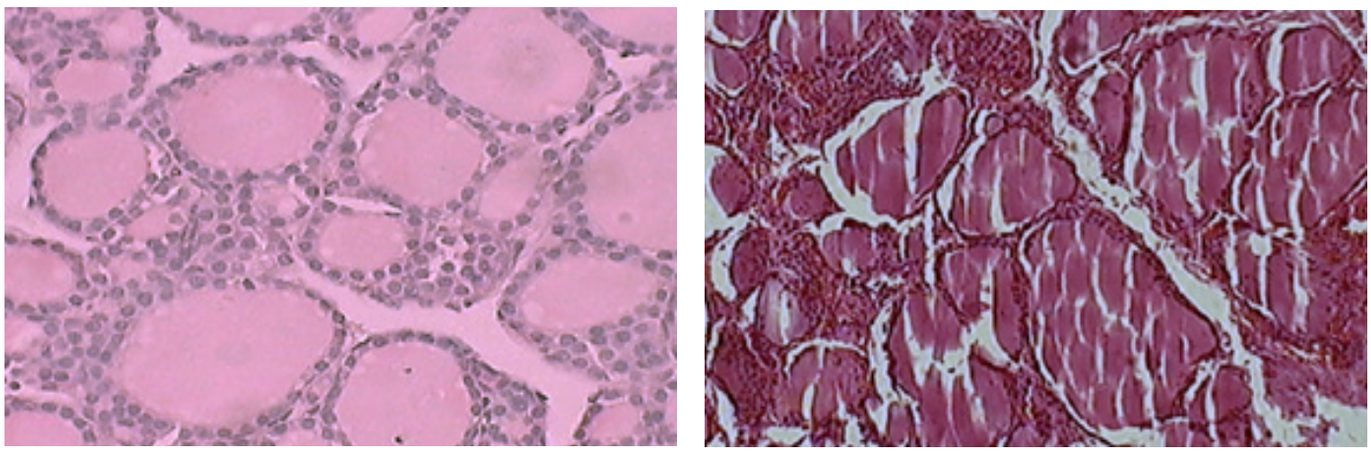
Figure-4: Thyroid tissue at birth. **A.** Control group: The follicular cells are cuboidal and the colloid materials are homogenous. **B.** Study group: The follicular cells are flat shape and the colloid materials are not homogenous (H&E, x400).



A B

Figure-5: Thyroid tissue of the control group seven days after birth. **A.** Control group: The follicular cells are cuboidal and the colloid materials are homogenous. **B.** Study group: The follicular cells are flat shape and the colloid materials are not homogenous with obvious cracking (H&E, x400)

Table -1: Thyroid glands weight (mg) and volume (mm3) in the control and study groups.

Age (Day)	Gender	Control group	Study group	P-value
21 days of gestation	Thyroid weight	61.925	64.124	0.248
	Thyroid volume	4.6	5.4	0.020
At birth	Thyroid weight	69.5	81.25	0.020
	Thyroid volume	5.80	9.0	0.020
7 days after birth	Thyroid weight	77.75	91.95	0.020
	Thyroid volume	6.65	12.90	0.020

Table-2: Follicular cell height (μm)and width (μm)of the thyroid tissues in the control and study groups.

Age (Day)		Control group	Study group	P-value
14 days of gestation	Average follicular cell height	1.25	1.24	0.248
	Average follicular cell width	1.475	1.52	0.052
21 days of gestation	Average follicular cell height	3.35	3.3	0.885
	Average follicular cell width	1.8	2.1	0.146
At birth	Average follicular cell height	3.85	2.5	0.020
	Average follicular cell width	2.05	3.6	0.020
7 days after birth	Average follicular cell height	5.5	2.32	0.020
	Average follicular cell width(μm)	2.45	3.4	0.020

Table -3: Median number and diameter of follicles in the thyroid gland in the control and study groups.

Age (Day)		Control group	Study group	*P*-value
21 days of gestation	Number of follicles	29.4	19.55	0.020
	Diameter of follicles	11.6	11.11	0.24
At birth	Number of follicles	44.75	32.8	0.020
	Diameter of follicles	14.55	27.1	0.020
7 days after birth	Number of follicles	52.45	43.82	0.020
	Diameter of follicles	18.96	32.3	0.020

Table-4: Median number of blood vessels in the thyroid gland of the control and study groups.

Age (Day)		Control group	Study group	P-value
14 days of gestation	No. of blood vessels	3.7	4.2	0.21
21 days of gestation	No. of blood vessels	6.2	6.5	0.23
At birth	No. of blood vessels	9.5	7.7	0.025
7 days after birth	No. of blood vessels	13.5	10.2	0.025

DISCUSSION:

Iodine toxicity significantly causes higher rate of developmental disturbances ($p<0.05$), this result comes in agreement with that of Nishiyama et al (2004) [13], and Xue *et al* (2006) [14]. The excessive iodine transferred by placenta may cause direct effect on the development of fetal thyroid.

In the study group, a non significant increase in weight of thyroid glands and significant increase in the volume were found regarding the prenatal period at 21th day of gestation ($p>0.05$), but this increases were significant in the postnatal period compared with that of the control group ($p<0.05$). This comes in agreement with the study of Guo et al (1991) and Zimmermann et al (2005) [15, 16]. In both the control and study groups, there were relatively non significant differences in the cells height and width in the prenatal groups, but a significant decrease in the height and significant increase in the width of follicular cells ($p<0.05$) in the study group were found, and the follicular cells appear flat and the follicles became distended with colloid, compared with that of the control group. Xue *et al* (2006) [14] studied the effect of toxic dose of iodine on the thyroid gland of the mouse and found the same result. The impairment of thyroid function caused by iodine toxicity leads to the inhibition of phagocytosis / pinocytosis of the colloid that contains thyroglobulin, causes it to accumulate in the follicular lumen and thereby diminishing the height of the follicular epithelium and increasing its width, and cause increasing the follicular diameter, and consequently the thyroid gland weight and volume were increased. This comes in agreement with the study of Zimmermann et al (2005) [16].

In the control and study embryos, the thyroid tissue at 21th day of gestation were nearly similar in the diameter of the follicles, but the number of the follicles in the study group was significantly less than the control group ($p<0.05$). At birth and seven days after birth the thyroid tissues of the study group showed a lesser number of follicles with larger diameter and the colloid showed obvious cracking. The decrease in the number of follicles and the increase in the size of follicles are due to increase in the colloid contents caused by a decrease in thyroid hormone secretion. This comes in agreement with results of Castillo *et al* (2003) [17].

Non significant increase in the thyroid tissue blood vessels number in the study group regarding the prenatal periods ($p>0.05$) was found, this might occur as a reaction of the body to the toxic dose of iodine, but significant decrease was found in the postnatal periods compared with that of the control group ($p<0.05$), and this might result due to the increased colloid contents of the thyroid follicles that raises intra thyroidal pressure and consequently reduces the vascularity in the area.

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