Anatomical and histological study on thymus development in the embryo and adult mice

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Abstract

Background: The thymus is the location of T cell development, where lymphoid precursors mature and are exported to the periphery through a sequence of interactions with the thymic epithelium.

Objectives: To assess the anatomical and histological study on the development of the thymus in the embryo and adult mice.

Materials and methods: This study was developmental; fifty mice were divided randomly into 5 groups; group A: Pregnant female mice were dissected at the end of the 1st week of gestation to get their embryos. Group B: Pregnant female mice were dissected at the end of the 2nd week of gestation to get their embryos. Group C: Pregnant female mice were left to deliver their fetuses. Group D: Fetuses was left to complete their 1st week. Group E was designed as the control group in which the adult male and female mice were dissected and prepared for histological examination to compare their thymus with the embryonic and fetal thymus.

Results: All animals of the group A had no thymus gland while all the other mice had thymus gland. Thymus appeared lobulated with no differentiation between the cortex and the medulla in the newborn age. Hassle's corpuscles could be detected at the 6th postnatal age.

Conclusion: Age-related differences in the morphology and histology of TH reflects the development of T cell and immunity and its crucial role in immune disorders. Mice TH gain full maturity by the end of 1st postnatal week and involution appears in the 6th postnatal week of age. The distinction between the medulla and cortex gradually disappears as people age.

Keywords: Anatomical; Histological; Thymus; Embryo; Adult mice

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Introduction

The primary lymphoid organ of the immune system is the thymus (TH). T cells or lymphocytes develop within the TH. The acquired immune system, in which the body adjusts particularly to outside invaders, depends on T cells. TH is bilobed shape, divided into lobules. Every lobule is composed of cortex and medulla**(Barros et al., 2022).**The TH primordium in the mouse first appears around the 10^{th}embryonic day (E10) as an epithelial bud from the 3^{rd} pharyngeal arch. TH most probably arise from the pouch endoderm and potentially the third branchial cleft ectoderm **(Romano et al., 2013; Sakr, 2022).**

Following E11, the neural crest-derived mesenchyme and bilaterally produced TH primordia move away from the throat to form a bilobed organ that is situated above the heart**(Anderson et al., 1996).**After lymphoid stem cells migration into the epithelial primordium, the process of thymic lymphopoiesis begins around E11–E12 and is accompanied by TH migration and accelerated epithelial cell proliferation **(Ma et al., 2013).**Following their multiplication, these stem cells rearrange and alter the genes that code for their Ag (antigen) receptors and eventually transform into functionally mature T cells. The development of T cells and the differentiation of the thymic epithelium are also related processes **(Petrie & Zúñiga-Pflücker, 2007;Kadouri et al., 2020).**

The E12 mesenchyme surrounding the thymic epithelial rudiment has been shown to be essential for thymic growth in organ explant cultures. A high rate of proliferation from E12 till delivery causes the thymic mass to significantly expand in all directions and give rise to many lobules. The emergence of the first Hassall's bodies indicates the late maturation of TH**(Stefaniak et al., 2019).**

Optimal T cell formation and, consequently, proper thymus function depend on the patterning and arrangement of the TH stromal cells (Thymic Epithelial Cells, Vasculature, and Mesenchymal Cells). Because errors in thymus form and function can have major health repercussions, such as immunodeficiency, it is crucial to comprehend the developmental mechanisms that create proper thymus structure**(Ribatti, 2015)**

Despite the focus on T cell differentiation within the TH, research into thymus organogenesis has advanced more slowly. The goal of this revived interest in TH organogenesis is to comprehend how the thymus develops to support T cell maturation and regeneration. So, we aimed to assess the development of the TH in the embryo and adult mice.

Materials and methods

This study was developmental**;** fifty mice were divided randomly into 5 groups A, B, C, D and E. Group A: Pregnant female mice dissected at the end of the 1^{st} week of gestation to get their embryos to be dissected and prepared for histological examination.Group B: Pregnant female mice were dissected at the end of the 2^{nd} week of gestation to get their embryos to be dissected and prepared for histological examination. Group C**:** Pregnant female mice were left to deliver their fetuses which was dissected immediately and prepared for histological examination.Group D: Fetuses were left to complete their postnatal 1^{st} week and then dissected and prepared for histological examination.Group E was designed as the control group: Adult male and female were dissected and prepared for histological examination to compare their thymus with the embryonic and foetal thymus. Each group formed of 10 mice; 9 females and 1 male to fertilize them. Daily vaginal smear was taken for detection of copulation.

Study Setting: The study was conducted at Department of Human Anatomy, Faculty of Medicine, South Valley University.

Inclusion criteria: Male and female mice, aged: one and two weeks of prenatal, newborn, one week postnatally and adult (6 weeks).

Exclusion criteria: Mice older than 10 weeks age.

Study tools: Animals preparation, experiments, fixation of the samples and microscopical examination

Animals: The animals were maintained in the Animal House of Faculty of science, South Valley University under normal day and night cycles. They were housed at appropriate temperature, fed mice chow ad libitum. They were allowed free access of water.

Fixation of the samples: Specimens were taken from both the neck and thoracic regions together as one block from each animal. Specimens were immediately fixed in 10% formalin and Bouin's solution, dehydrated in graded alcohols, cleared in methyl benzoate and embedded in paraplast. Serial transverse sections 5 µm thick were made from tissue blocks using a Leica microtome and stained with the following staining methods: Haematoxylin and eosin for routine histological examination and immunohistological methods for T lymphocytes. For immunohistological methods, Cytokeratins (CK 18) are markers for thymic epithelial cells**(Lee et al., 2011).**

All steps of tissue preparation and staining methods were done according to Bancroft and Gamble **(Bancroft and Gamble, 2008).**

Ethical Approval: This experimental study was fully approved by the Local Ethical Committee and by the Institutional Review Board of Faculty of Medicine, South Valley University.

Microscopical examination: Examination of the stained tissue slides will be done by Leica image analyzer.

Research outcome measures: Primary (main): Study of thymus and thymocytes and **secondary (subsidiary):** Study of other cells in the thymus.

Statistical analysis

With the aid of the IBM SPSS software package version 20.0 (IBM Corp., Armonk, New York). Data were fed into the computer and evaluated. Number and percentage were used to describe qualitative data. The normality of the distribution was examined using the Kolmogorov-Smirnov test. The range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) were used to characterize quantitative data. At the 5% level, significance of the results was determined. The applied tests were:

-Studentt-test: For normally distributed quantitative variables, to compare between two studied groups

-F-test(ANOVA): For normally distributed quantitative variables, to compare between more than two groups

Results

Prenatal 1st week age (E7)

Light microscopic examination of 1st prenatal week embryo (E7) showed that there was no TH gland. The mouse embryo at E7 showed an embryonic disc which was surrounded by two embryonic sacs (**Fig.1**).

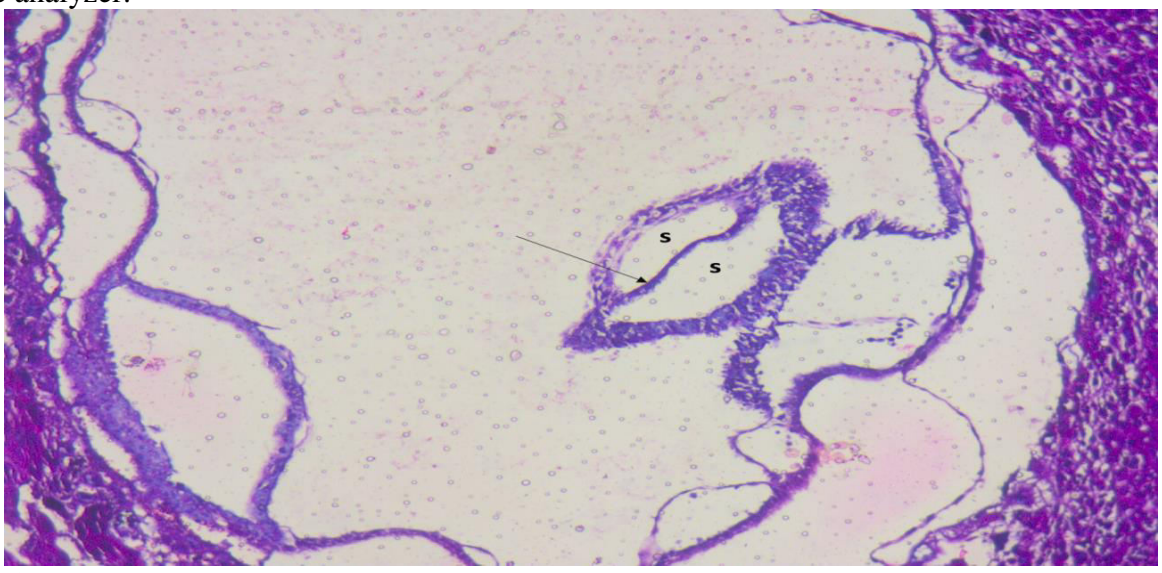

Fig. 1. A mouse embryo at E7 shows an embryonic disc (arrow), surrounded by 2 embryonic sacs (s).(H&E ×100)

Prenatal 2nd week age (E14)

In sections stained by H&E, the thymus gland of the mouse appeared as thymic primordium. This thymic primordium developed from the 3rd pharyngeal arch (**Figs.2&3**). In sections stained by immunohistochemical stain(IHC) CK18, most of the cell population were immune positive reticular thymic epithelial cells (rTECs) with golden brown stained, mostly stellate in shape and were interconnecting forming reticulum (**Figs.4&5**).

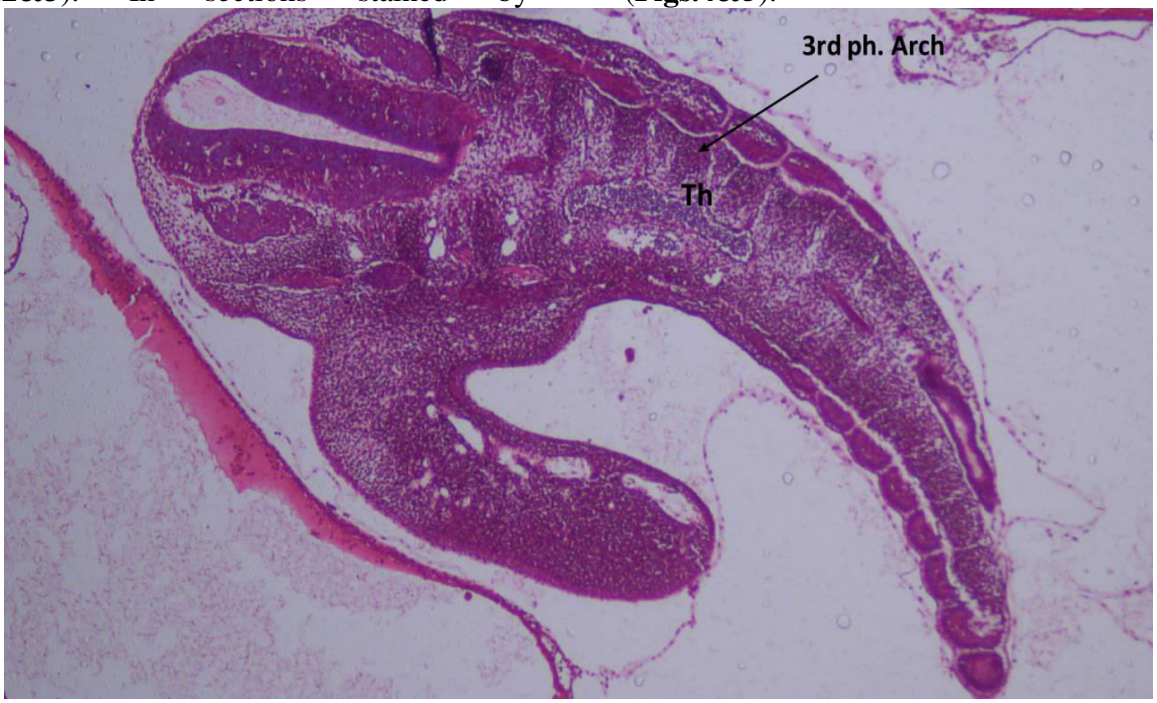

Fig.2: A mouse embryo at E14 shows pharyngeal arches. Thymic primordium (TH) arises from the 3rd pharyngeal arch. (H&E × 40)

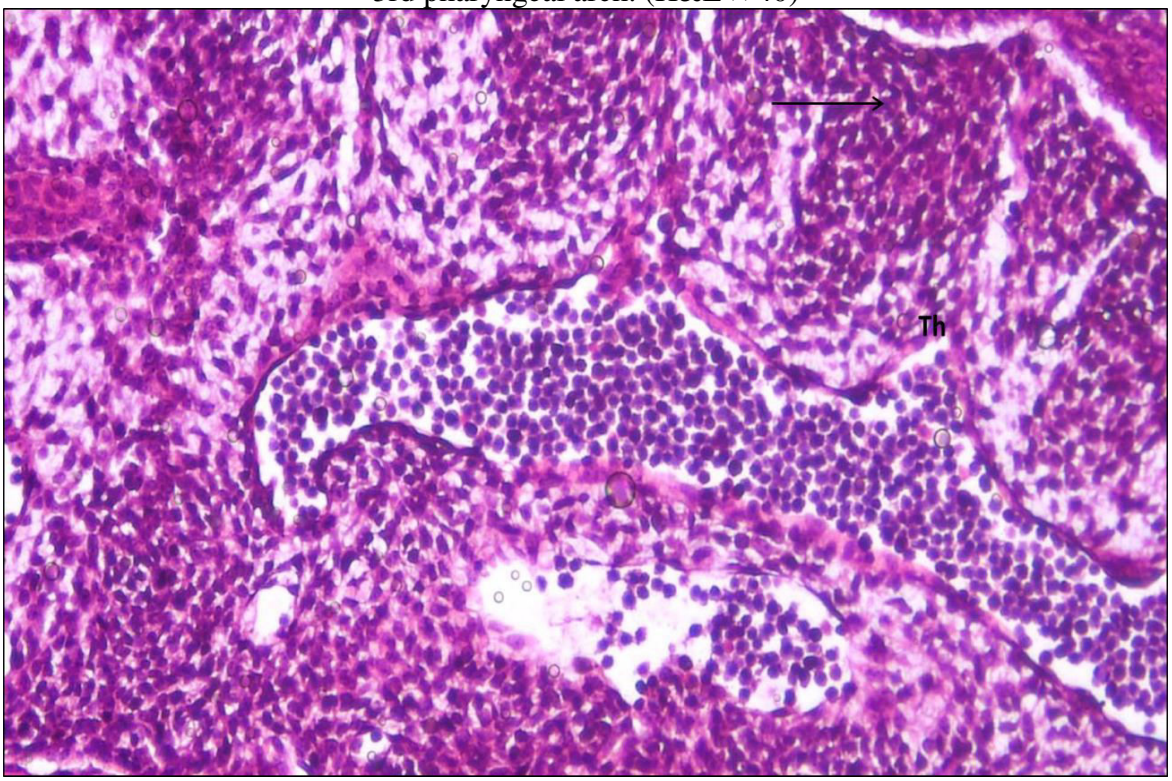

Fig. 3: Magnification of Fig. (2) showing the thymic primordium(TH). An arrow refers to the 3d pharyngeal arch. (H&E ×200)

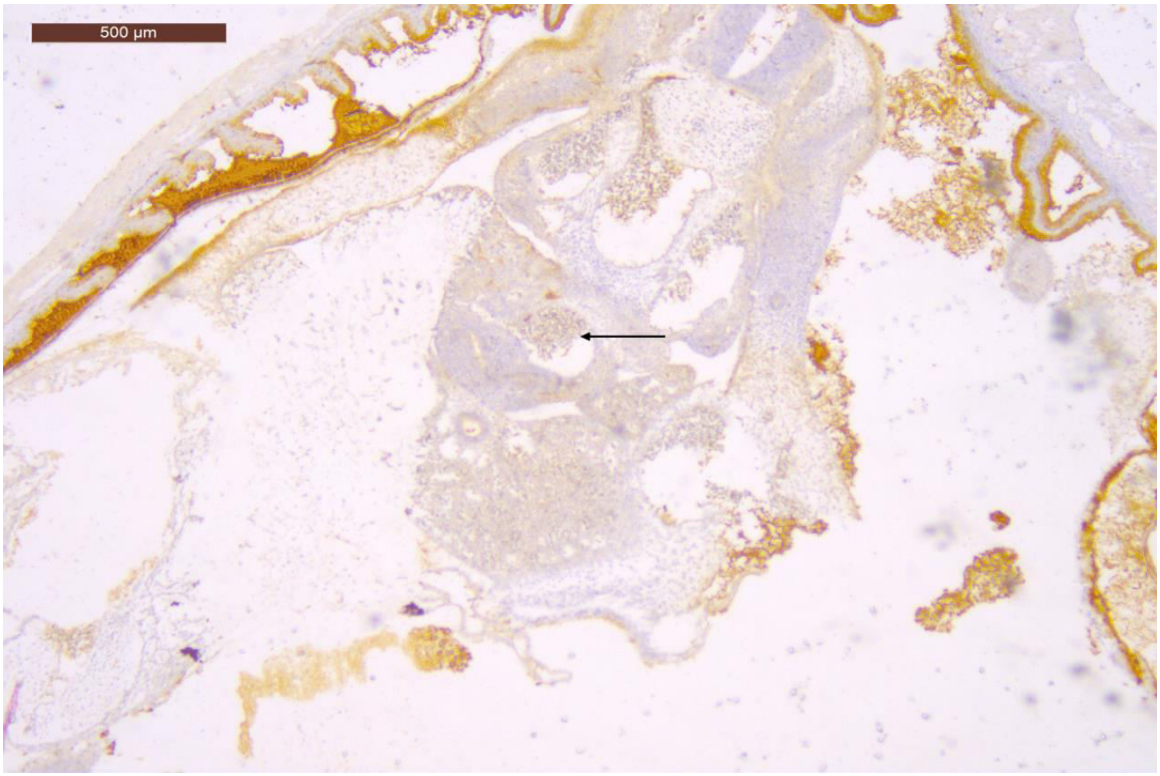

Fig.4. A mouse embryo at E14 shows thymic primordium (arrow) stained by immunohistochemical stain. (CK 18 ×40)

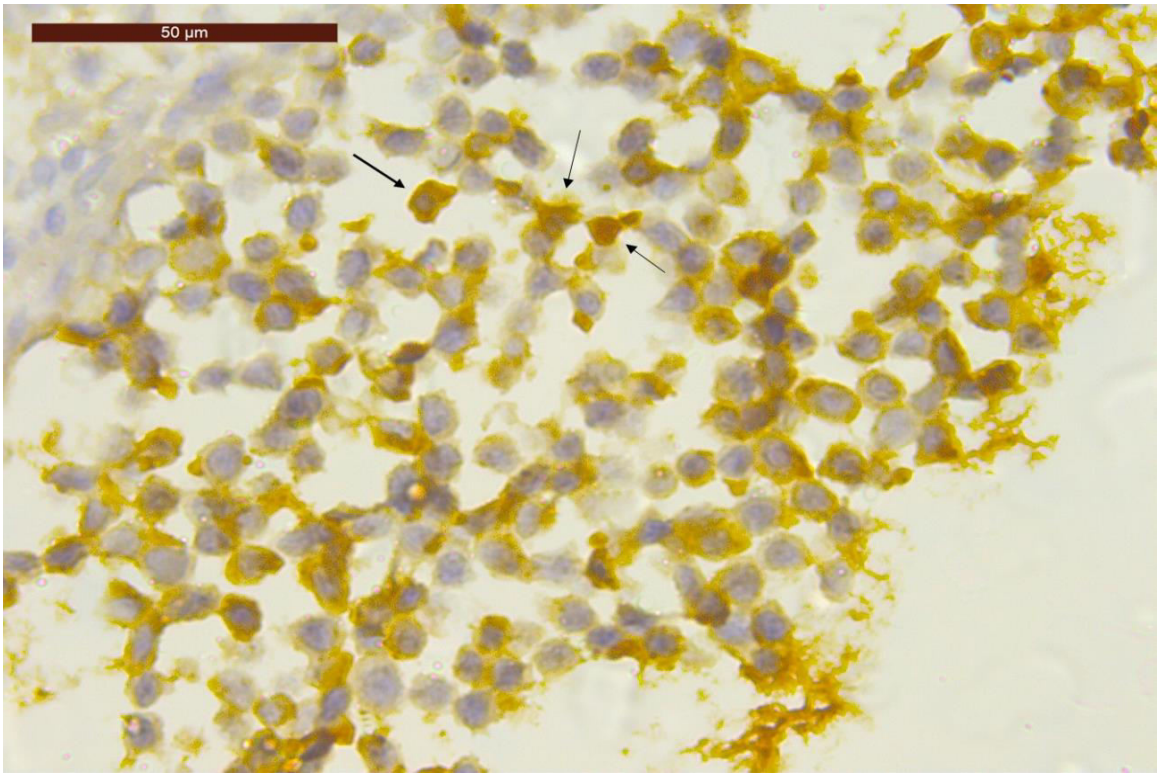

Fig.5. A magnified of Fig. (4) Showing thymic primordium stained by IHC stain. rTECs are stellate in shape (arrow) and are interconnecting forming reticulum. (CK 18×600)

Newborn age (P 1)

The newborn thymus gland of the mouse appeared lobulated, highly cellular and with many blood capillaries. Thymic lobules have no demarcation between the cortex and the medulla. The thymocytes appeared in small clusters separated by many rTECs . Thymocytes appeared as small cells having small dark nuclei with scanty cytoplasm and many of them were binucleated indicating active proliferation. rTECs appeared large cells with large vesicular nucleus, prominent nucleolus, and abundant cytoplasm. Also, many of them were binucleated (**Figs.6&7**). In sections stained by IHC stain CK18, Thymus appeared highly cellular and lobulated. There was no differentiation between the cortex and the medulla. TH showed immune positive rTECs , they were large with abundant cytoplasm, stained light brown and some of them were binucleated (**Figs.8&9**).

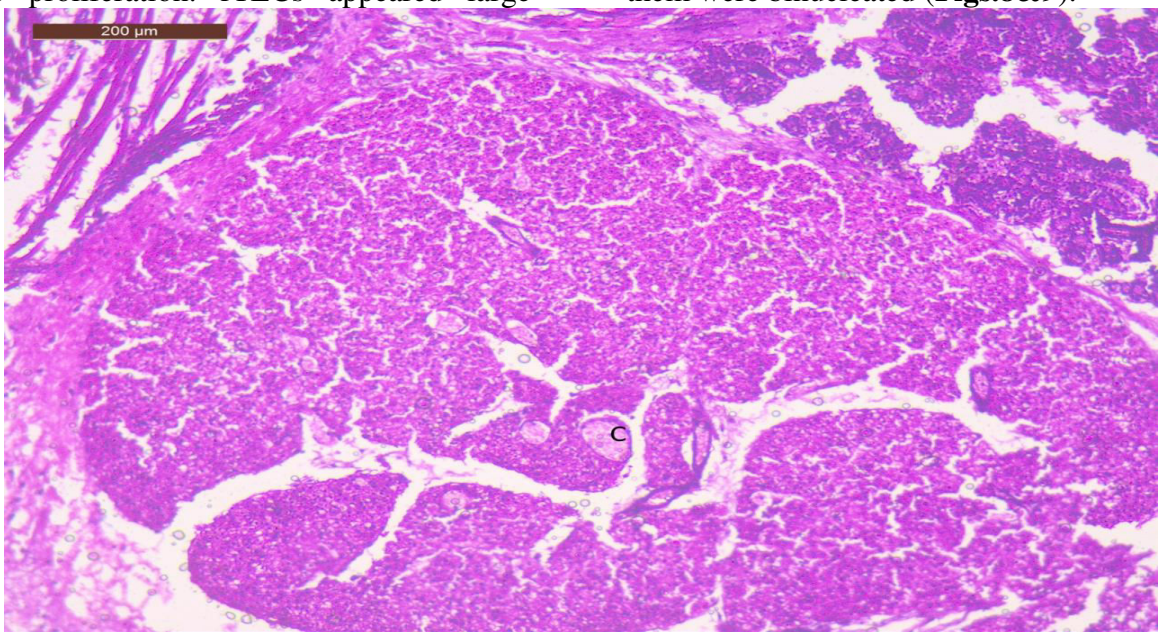

Fig.6. TH of newborn mouse shows many blood capillaries (C) inside lobules.(H&E X 40)

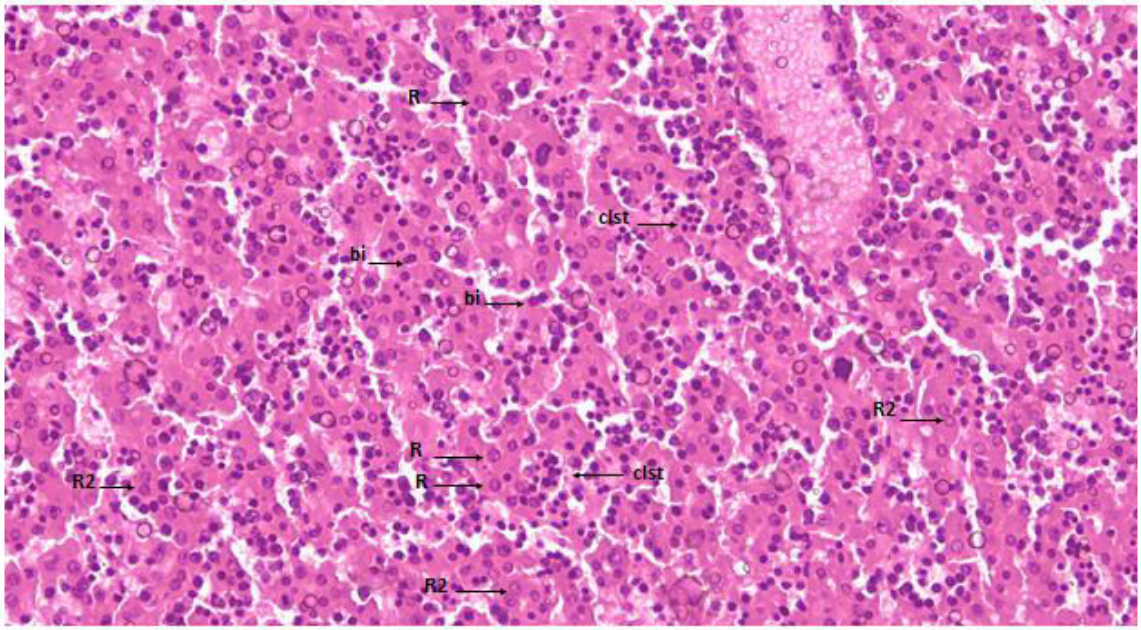

Fig.7. A magnified part of P1 thymus showing thymocytes (clst) separated by many rTECs(R). Thymocytes are binucleated (bi). Reticular TECs with large vesicular and many of them are binucleated (R2).(H&E ×200)

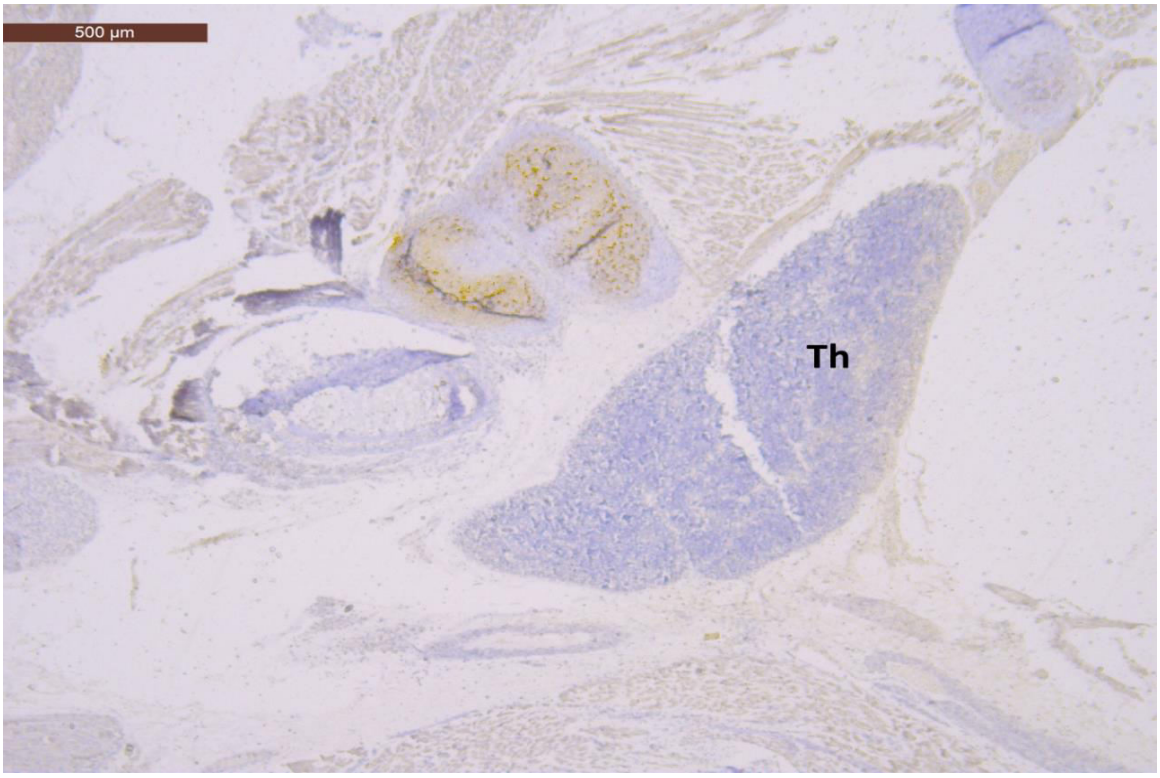

Fig. 8: Newborn TH of mouse stained by immune stain. TH appears highly cellular and lobulated.CK 18 X40)

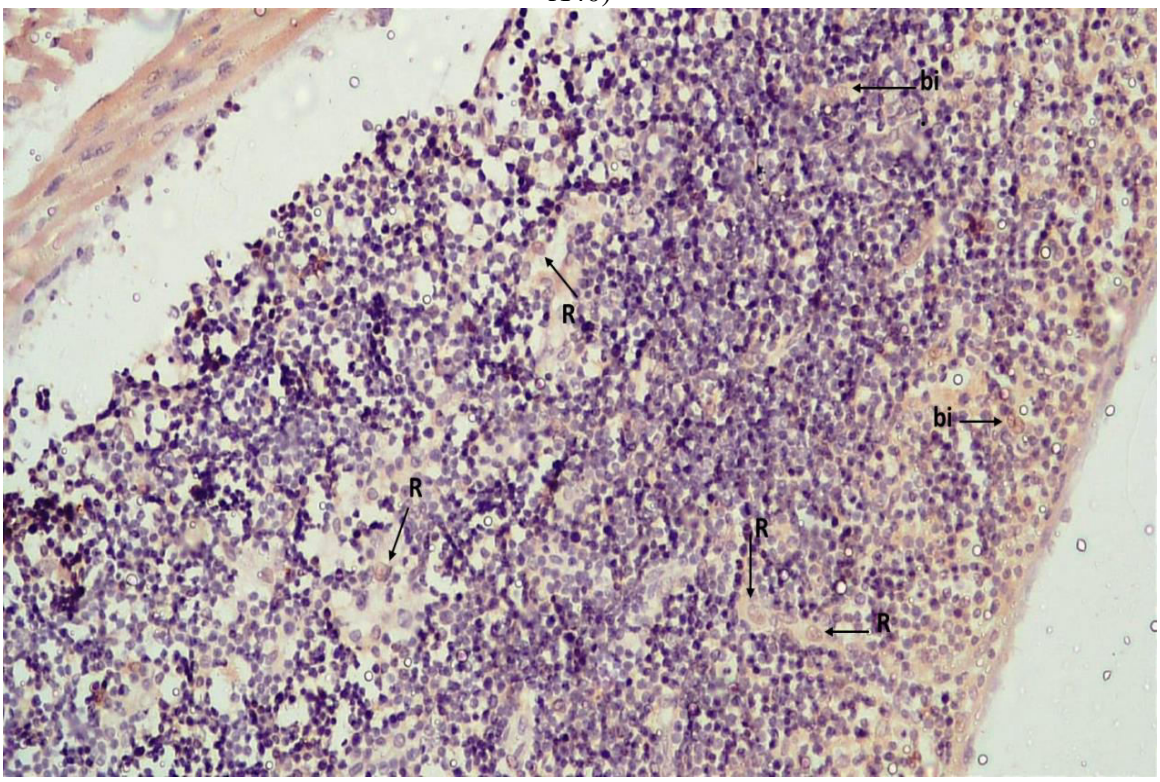

Fig.9: Magnified part of newborn mouse TH showing immune positive rTECs (R), they are large with abundant cytoplasm, stained light brown and some of them are binucleated (bi).(CK 18 X 200)

Postnatal 1st week age (P7)

TH of P7 mouse showed thymic lobulation, each lobule has an outer dark cortex and an inner light medulla. It was noticed that both cortex and medulla have blood capillaries which were relatively fewer and smaller than the previous age. The outer dark cortex which appeared highly cellular,

their main cells were thymocytes. Thymocytes appeared in large clusters. The inner light medulla appeared less cellular and thymocytes in the medulla arranged in cords rather than clusters with spaces between them. Spaces showed few rTECs (**Figs.10&11**). In IHC stained sections, TH of P7 mouse showed immune positive rTECs. They appeared brown stained, stellate in shape and relatively smaller than the previous age (**Figs.11&12**).

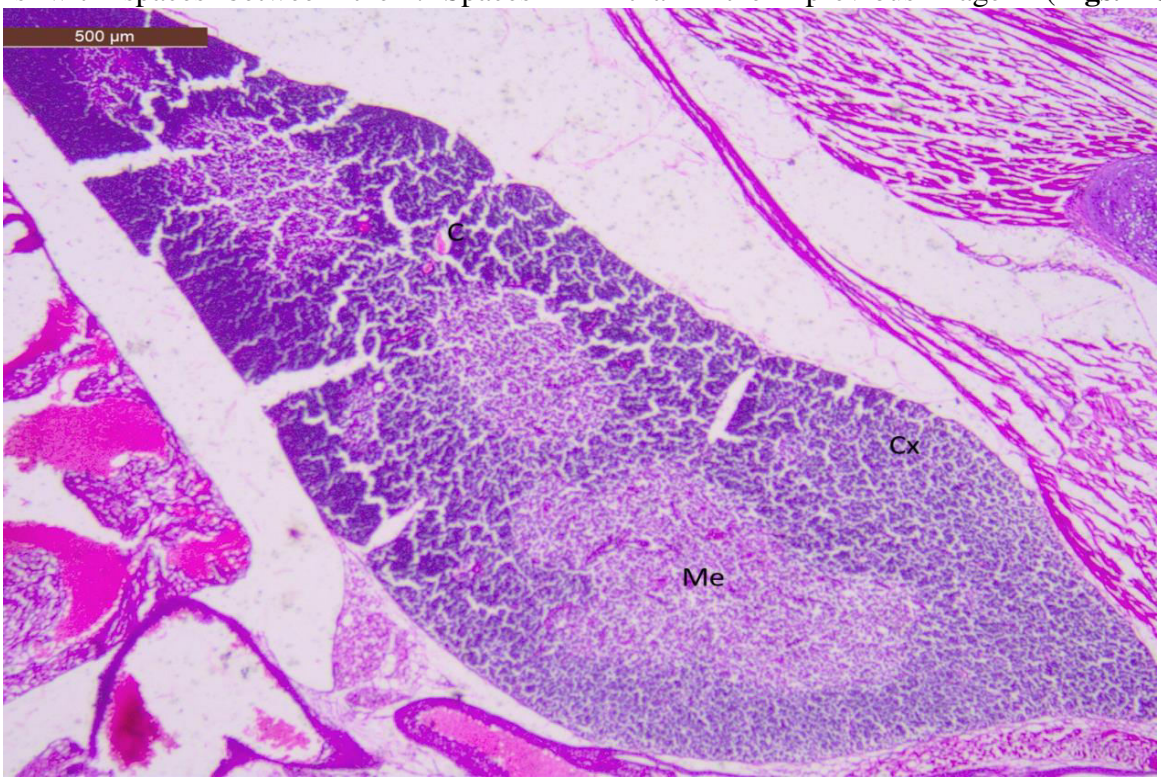

Fig.10: Thymus of P7 mouse showing thymic lobulation, each lobule has an outer dark cortex (CX) and an inner light medulla (Me). Blood capillaries (C) could be seen. (H&E x40)

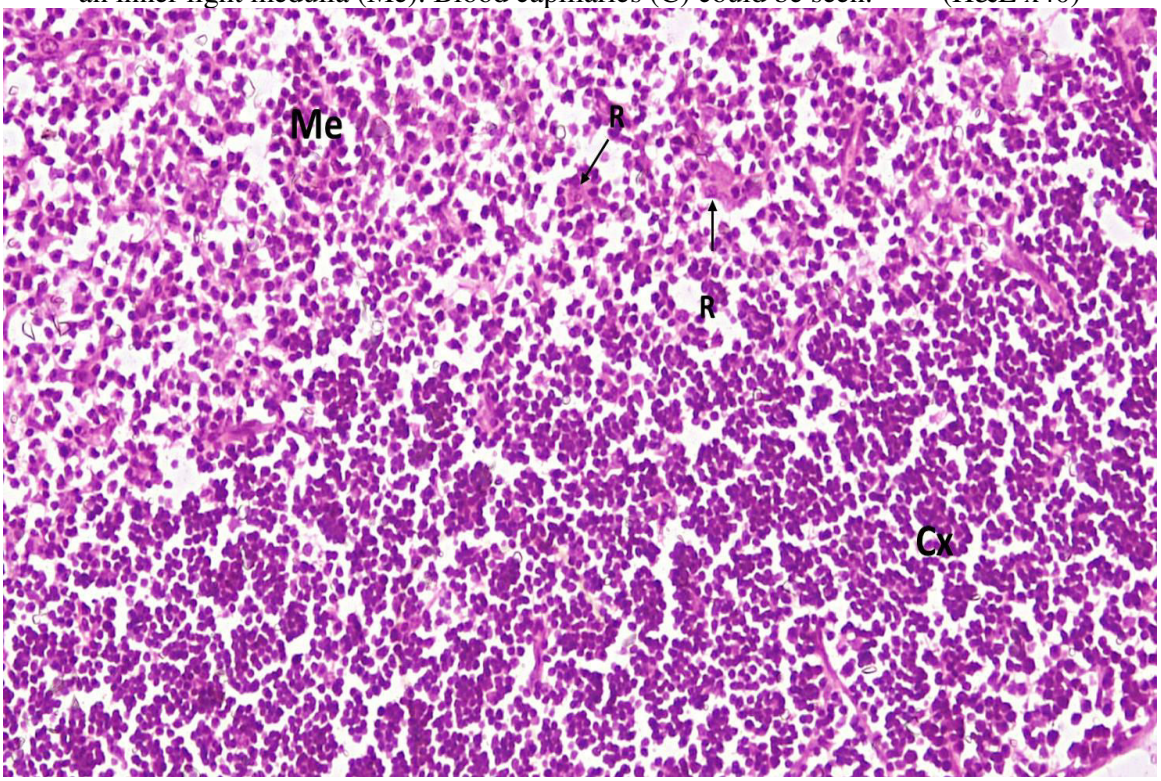

Fig. 11:A magnified part of P7 mouse TH showing the outer dark cortex (CX). The inner light medulla (Me) appears less cellular. Spaces show few rTECs (R). (H&E X200)

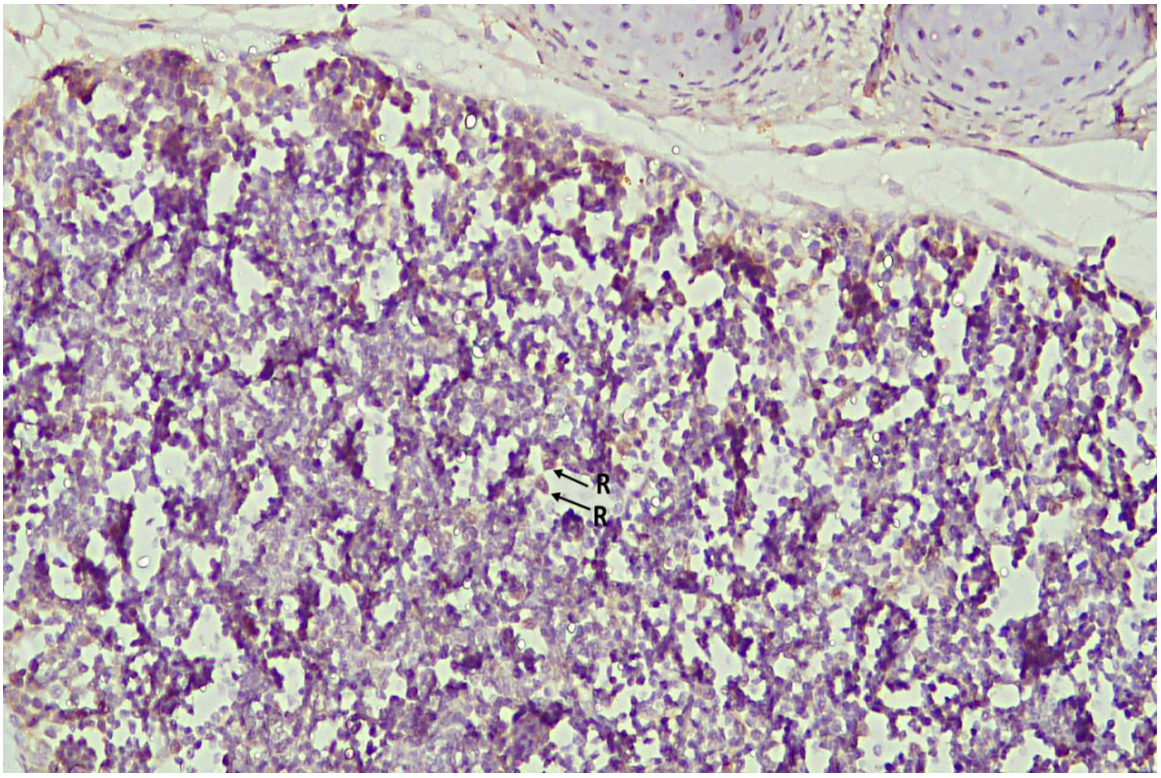

Fig.12: TH of P7 mouse stained by immune staining shows immune positive rTECs (R).(CK18 X 200).

Postnatal 6th weeks age (P42)

TH of P42 mouse was lobulated; lobules were large with fusion between adjacent lobules. Each thymic lobule showed an outer dark cortex and a wide inner medulla, the medulla seemed wider than the previous age. Capillaries could be detected. Thymocytes were arranged in large clusters. The medulla showed that thymocytes arranged in cords with intervening rTECs. Some rTECs appeared flattened with flattened nuclei, sign of beginning of degeneration. Hassle's corpuscles could be detected (**Figs.13&14**). P42 with immune stain showed that the TH was formed of cortex and medulla in which rTECs stained golden brown (immune positive) could be observed. Most rTECs were stellate in shape and some of them were flattened. It was noticeable that rTECs were fewer in the cortex than in the medulla (**Fig.15**).

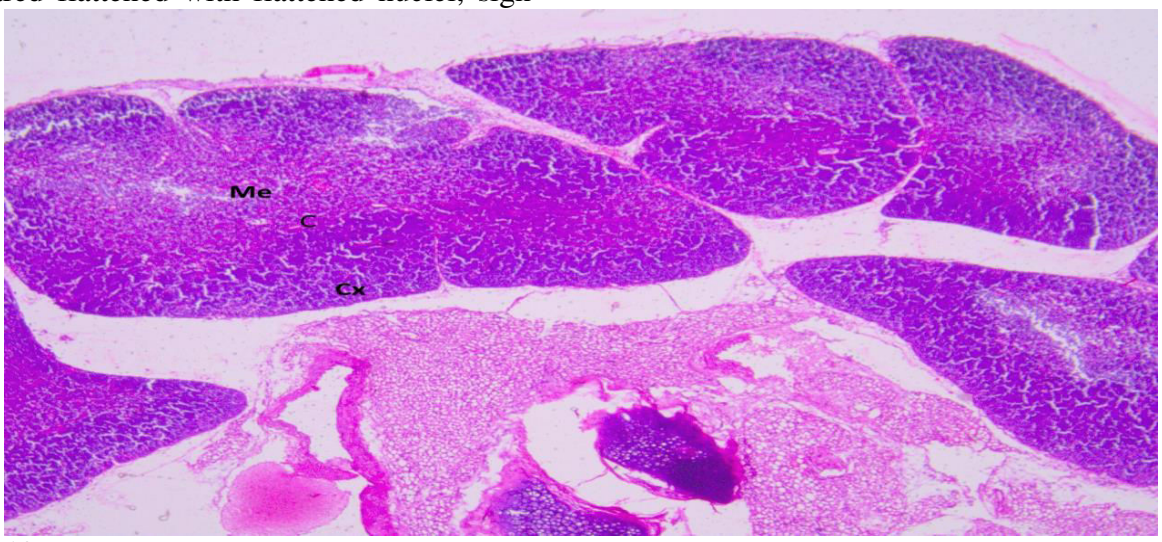

Fig.13:TH of P42 mouse shows an outer dark cortex (CX) and a wide inner medulla (Me). Capillaries could be detected (C). (H&E X40)

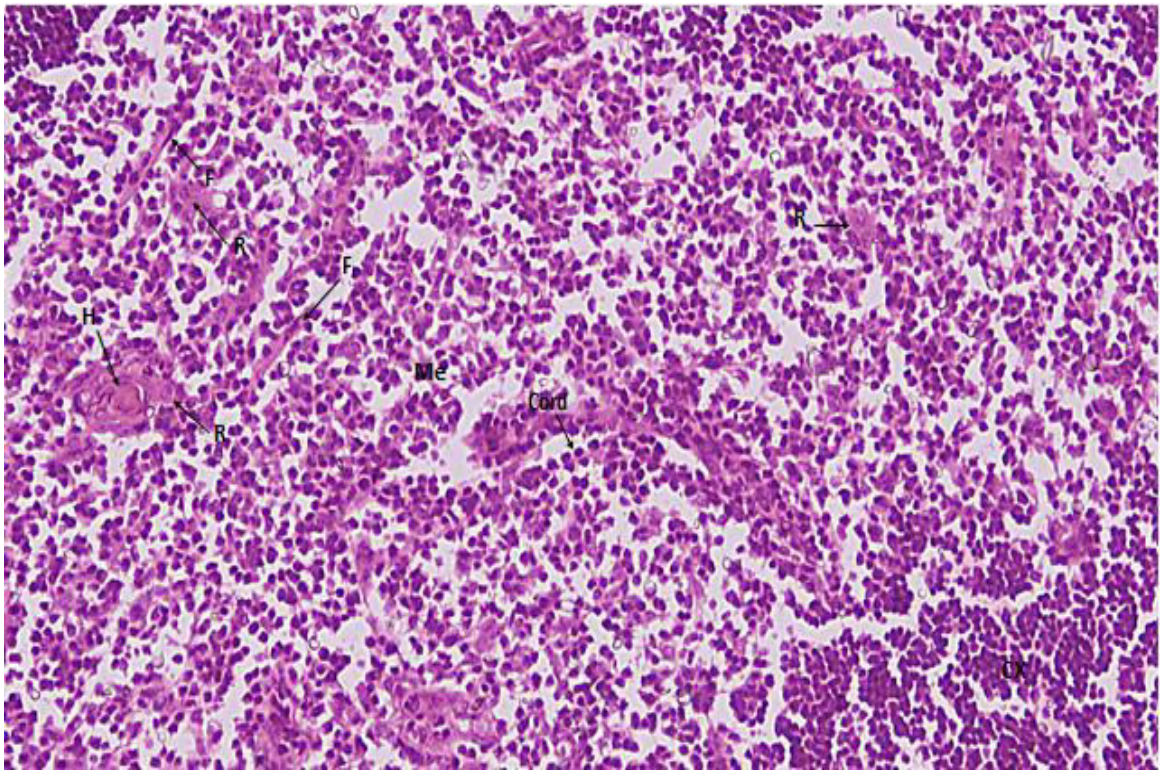

Fig.14.A magnified part of P42 mouse TH shows small part of the cortex (CX). The medulla (Me) having thymocytes arranged in cords (cord) with intervening rTECs (R). rTECs with flattened nuclei (F)and Hassall's corpuscles (H) could be detected. (H&E X 200)

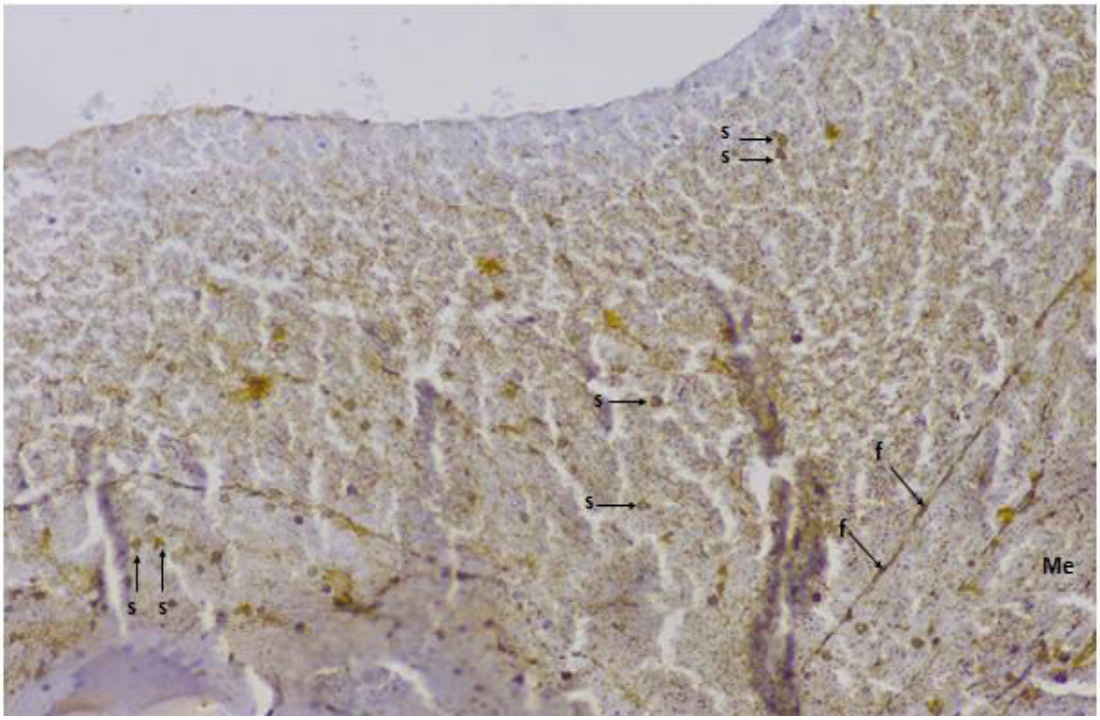

Fig.15: TH of P42 stained by immune stain showing the cortex (CX) and part of the medulla (Me),rTECs stained golden brown (immune positive). Most rTECs are stellate (s) in shape and some of them are flattened (f).(CK 18 X200)

Configurational comparison of CK-18 positivity

We counted the number of CK-18 positive thymic epithelial cells. As shown in (**Table 1 and Fig.16**), the rate of CK-18positive cells

increased to reach its maximum at the end of the 1st postnatal week where it recorded a mean value of 9.8 x10^6 . However, the number of CK-18 positive cells gradually decreased from the 2nd postnatal week (8.43 x10^6) to reach a lower level at the 6th postnatal week where it recorded a mean value of 6.71 x10^6.

Table1.Comparison among the studied groups as regard CK-18 for thymic epithelial cells.

CK-18 (x10^6)	Group A (n = 9)	Group B (n = 9)	Group C (n = 9)	Group D (n = 9)	Group E (n = 10)	F	*P*
Range	2.01 –3.39	4.61 –6.56	7.61 – 11.88	7.24 – 9.96	6.04 –7.48	92.268	<0.001*
Mean±SD	2.61 ±0.46	5.52 ±0.71	9.8 ±1.4	8.43 ±0.94	6.71 ±0.49		

F:one-way ANOVA t-test, *p:p*value for comparing between different categories and *:Statistically significant at p≤0.05

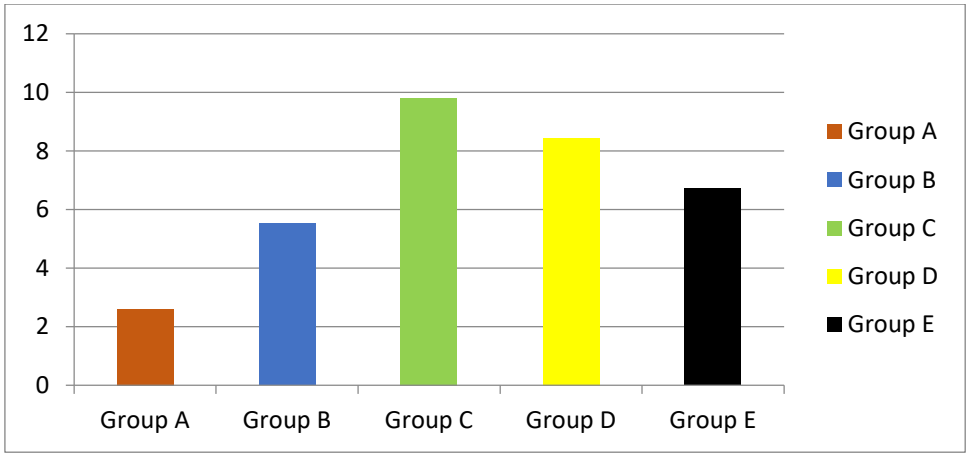

Fig.16.Comparison among the studied groups as regardCK-18 for thymic epithelial cells**.**

Discussion

In the present study we focused on TH anatomical and histological changes since a fetus's thymus, which predicts its maturity, can be a valuable adjuvant in recognizing a variety of illnesses. **(Richards et al., 2021).**

In the present study, five groups were designed: two prenatal age groups (E1 and E2), two postnatal age groups (P1 and P2) and one adult group (P42). This is to evaluate the anatomical and histological structures of the TH. In accordance with our study **Cao et al.,(2017);Kendall and Clarke, (2000)**suggested that TH remains active throughout pregnancy, which is also, in agree with the findings of **Kuper et al., (2013),** who reported that as pregnancy progresses and the cortex begins to involute, the microenvironment of the TH becomes significantly different from those discovered in virgins who were age-matched, with evidence of higher selective activity in both the cortex and medulla.

In this regard Pregnancy-related atrophy was more severe than that brought on by ageing. Further evidence that crossbreeding, parity, or delivery had no impact on thymus involution came from the same thymocyte structures seen in all term pregnant females and those who died one day after giving birth. They then compared the ratios of the lymphocyte's subpopulations within the groups. Significant TH alterations may be brought on by a combination of reduced stem cell input, rapid thymic lymphocyte export brought on by pregnancy's elevated steroid levels, or both**(Cortina et al., 2012)**.

In accordance with our results, **Hamedi & Zandi, (2018)** reported that the histological structures of TH gland initiates as

a network of fibers at 10th day embryo, and then surrounded by a connective tissue capsule. They added that, Hassall's corpuscles appeared at 40th day old embryo. In this regard, **Gayathri, (2016); Gayathri et al., (2019)**declared that the first 6thweeks of a mouse's existence are considered to be the young postnatal mouse phase. The transitional stage, which corresponds to adulthood, is defined as being between 6thweeks and 12thmonths old. A baby mouse is defined as being up to two weeks old, an elderly mouse as being between 12 and 14 months old.

In accordance with our results , the study of **Aschenbrenner et al. (2007)** reported that the thymic epithelial cells were observed at 1st week. However, another study reported that the thymic epithelial appeared by 10th embryonic day **(Gayathri, 2016).**

As regard mice thymus at 7 days old an obvious demarcation appeared between the dark outer layer and light inner layer medulla. The cortex showed that most population cells were T thymocytes. Most population cells in the medulla are thymic epithelial cells. In accordance with our results, **Reda et al., (2019)**showed that TH parenchyma was composed of a core, light-stained medulla and a peripheral, dark cortex that connected to form tiny buds. These buds extended deep into the cortex and were continuous between adjacent lobules.

Our results of (P42) age showed that the TH was lobulated with dense cortex and light medulla. The medulla showed that thymocytes arranged in cords with intervening rTECs . Some rTECs appeared flattened with flattened nuclei as a sign of beginning of degeneration. Also, a lower level of CK-18 positive thymic epithelial cells was recorded in this age. This might be explained by the influence of hormonal variables on rTEC activity which in turn results in a downregulation of target cells in adult mice.**(Haydarian et al., 2015 ; Garmaeva et al., 2022).** Another postulation was reported with **Cuddihy et al., (2009)**who claimed that rTECs exhibit vascular endothelial growth factor (VEGF) synthesis. VEGF is necessary for the development of TH. This is associated with the development of newborn TH's vascular architecture. VEGF levels gradually drop from the early postnatal age until they reach nearly to zero in elder age, which reflects TH involution.

Hassall's corpuscles within the medulla were plenty in (P42) age in our study. In accordance with our results, **Reda et al., (2019)**indicated that TH had big lymphocytes with large nuclei and prominent nucleoli, Also rTEC which are characterized by their light cytoplasm and large nuclei and prominent nucleoli, but they were less stained than large lymphocytes and Hassall's corpuscles.. According to **Leposavić & Perišić, (2008),**Hassall's corpuscles appeared as a collection of degenerating epithelial cells. Such degeneration may affect the nucleus resulting in cysts with an amorphous protein in the middle. Sometimes it may appear as layers of rTECs grouped in a concentric pattern with a keratinized center.

Statistical analysis of our study as regarding CK-18 positive thymic cells showed that its number reached the maximum at the end of the 1st postnatal week whereas this number was gradually decreased from the 2nd postnatal week to reach a lower level at the 6th postnatal week. This is in agree with**(Liang et al., 2021);Liang et al., (2022)**who demonstrated that age-related rTEC degradation can start as early as 6 weeks postnatally in mice. In this regard, **Wu et al., (2018)**reported that the TH reaches its maximum at the end of 1st postnatal week and involutes as early as 6th postnatal weeks in mice and 1 year in humans. Accordingly, in such ages most other body organs do not show any signs of aging. In such situation, TH involution may be an evolutionarily conserved process. The process of TH organogenesis and involution, in which a transcription factor (Foxn1) is necessary for embryonic TH organogenesis and rTEC maintenance in adults, was thought to be governed by molecular mechanisms**(Žuklys et al., 2016;Kadouri et al., 2020).**

Conclusion

Age-related differences in the morphology and histology of the thymus

reflects the development of T cell and immunity and its crucial role in immune disorders. Mice TH gain full maturity by the end of 1st postnatal week and involution appears in the 6th postnatal week of age. The distinction between the medulla and cortex gradually disappears as people age.

Conflict of interest: no conflicts of interest.

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