

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "

Hanaa Kareem Ali

Department of Anatomy- Histology and Embryology -College of veterinary Medicine - University of
Diyala - Iraq.

hkareem1976@yahoo.com

Received: 17 December 2016

Accepted: 18 April 2017

Abstract

A total number of ten healthy normal chickens from the local breed of both sexes and different ages were used to show the distinctive histological and morphological architecture of the thymus, the morphological study included the location, shape, boundaries and color, for this purpose ten samples of the chicken thymus were used after the birds were killed either by halal method or by euthanasia then thymic samples were treated with the routine histological technique. Morphologically the thymus gland was a paired gland, consisted of two halves, each half was situated along either side of the neck till the entrance of thoracic region in parallel to the common carotid artery, jugular vein, trachea and esophagus and each half contained a number of lobes ranged between 6-8 of flattened shape, while histologically the gland enclosed by a thin connective tissue capsule from which numerous fine septa of connective tissue originated and divided it into moderately separated lobules, the septa contained blood vessels, each lobule had an outer cortex and central medulla without any mark of isolation. Cortex contained a densely packed lymphocytes of small and medium size making it give the impression of appearing deeply basophilic in contrast to the pale eosinophilic medulla which showing reticuloepithelial cells with smaller amount of lymphocytes and acidophilic cytoplasm. The reticuloepithelial cells were lesser in the cortex

and numerous in the medulla, which aggregate together thus creating the formation of Hassall's corpuscles.

Key words: Chicken thymus, histology of thymus, morphology of thymus.

دراسة نسيجية شكلية للغدة الزعترية في الدجاج المحلي

هناة كريم علي

فرع التشريخ والانسجة - كلية الطب البيطري - جامعة ديالى - العراق

الخلاصة

استخدمت مجموعة من عشرة طيور دجاج طبيعية صحية من السلالة المحلية من كلا الجنسين ومختلف الأعمار لإظهار التركيب النسيجي والبنية الشكلية المميزة للغدة الزعترية. شملت الدراسة الشكلية دراسة الشكل العام والموقع التشريحي وحدود الغدة ولونها ولهذا الغرض استخدمت عشر عينات من الغدة الزعترية بعد قتل الطيور أما بالذبح الحلال أو بطريقة القتل الرحيم ، ثم اخذت العينات التي جمعت وتمت معاملتها بأساليب التقطيع والصبغ النسيجي الروتينية. بينت الدراسة الشكلية بأن الغدة الزعترية في الدجاج المحلي غدة مزدوجة تتألف من نصفين ، كل نصف يقع على طول أحد جانبي الرقبة حتى مدخل منطقة الصدر بالتوازي مع الشريان السباتي المشترك والوريد الوداجي والقصبه الهوائية والمريء ، يحتوي كل نصف على عدد من الفصوص تراوحت بين 6-8 فصوص ذات شكل مفلطح. أما نسيجيا فكانت الغدة مغلفة بمحفظة رقيقة من النسيج الضام تنشأ منها عدة حواجز دقيقة تدخل الى متن الغدة وتقسّمها الى عدة فصيصات صغيرة مفصولة عن بعضها ، وهذه الحواجز تحوي على عدد من الأوعية الدموية ، يتكون كل فصيص من قشره خارجيه داكنة اللون ولب أو نخاع داخلي فاتح اللون دون أي علامة لانعزال القشرة من اللب. تحوي القشرة على كثافة من الخلايا الليمفاوية الصغيرة والمتوسطة الحجم مما يعطي انطبعا لضهورها قاعدية داكنة اللون على النقيض من النخاع الفاتح اللون ذو الصبغة الحامضية الذي يظهر وجود الخلايا الطلائية الشبكية مع كمية أصغر من الخلايا الليمفاوية ذات السيتوبلازم ذو الصبغة الحامضية. توجد خلايا طلائية شبكية قليلة في القشرة وكثيره في النخاع وهذه الخلايا تتجمع مع بعضها مما يهيئ لتكوين حويصلات هاسلس.

الكلمات المفتاحية: الغدة الزعترية بالدواجن ، التركيب النسيجي للغدة الزعترية، دراسة شكلية للغدة الزعترية.

Introduction

The immunity of the body has been connected to activities associated to the lymphomyeloid organs and their cells, the lymphatic system is compounded by a network of diffuse defense, birds have discrete lymphoid tissues and absence of lymph nodes [1].

Lymphomyeloid organs can be categorized according to development and function into: primary organs [2], and secondary (peripheral) organs [3], and thymus gland is ranked as one of the primary lymphoid organs, it's the first organ which formed and grows instantly after birth in response to postnatal antigen stimulation and the demand for great number of mature T cells [4]. In the other hand thymus may function as a secondary lymphoid organ because it is capable of playing a direct role in the immune reactions, and the appearance of plasma cells in chicken thymus at different ages provides further support to this idea. This is a vital role in vaccination of chicken [5].

The development of primary lymphatic organs in birds, starts during embryonal life [6]. Throughout embryonic growth of chicken the various reticuloepithelial cells and humoral factors, that make up the thymic microenvironment, lead to process T-cell precursors [7].

The focus was on the thymus due to its large role in the immunological response. It considered as the initial site for development of T cell immunological function also it generate, differentiate and mature T and B lymphocytes respectively [8 ; 9], and the presence of thymus is important for the development of peripheral lymphoid tissues and responsible for cell mediated immunity and immune observation [10]; and it is necessary for normal development and function of gonads and thyroid gland as well as body growth [11].

Poultry production is an economically significant and the most rising zone around the world, but their farmers face many restrictions like diseases and many other factors like abiotic stresses that affect the immune system resulting in disorders of the histological architecture of concerned organs, immune suppression, poor production and mortalities [12].

The best understanding of the role of avian immune system in maintaining optimum health would assist us put into words effective control strategies against biotic and abiotic stresses including acute heat stress [13]. For that reason, the present study has been carried out to understand the histological architecture of the lymphoid tissues of the local chickens.

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "**Hanaa Kareem Ali****Materials and Methods**

Ten healthy normal chickens from the local breed chicken of both sexes and different ages were used for this study. The birds were killed by two ways either by halal method or be euthanized with an overdose of xylazine intravenous injection in the wing vein (1 mg /Kg. B.W), then gross morphological study was done immediately, which includes the shape, color and anatomical location. Later thymus was collected by careful ventro-lateral neck dissection. Each thymic sample which collected was prepared to the routine histological technique by washing with normal saline after making sure that these samples were devoid of any gross pathological lesions. Thymus tissues which obtained from the chickens were marked and fixed in (10%) neutral buffered formaldehyde solution and left for 72 hours. Tissues then were dehydrated in the series of ascending grade of alcohol, this process followed by clearing in three changes in xylene, then infiltrated with different grades of melted paraffin by using the oven. The tissues then embedded in paraffin and lastly were cut at 6 μ thickness by means of a rotary microtome. After cutting, the tissues were floated on warm water bath at 37C for extending and then fixed on clean slides using an adhesive like Egg albumins and dried on a slide warmer at 37C [10; 14]. Finally the sections were stained using routine stain Hematoxylin and Eosin (H & E) and Masson Trichrome stain [15]. The histological structures of the thymus gland tissues were examined by light microscope under (4x; 10x; 20x; 40x) magnifications.

Results and Discussion

Thymus gland of the local chickens was a paired gland, consisted of two halves, one half of which was situated along either side of the neck extended from the beginning of cervical region till the entrance of thoracic region in parallel to the common carotid artery, jugular vein, trachea and esophagus, (Fig. 1), so, our results are in agreement with those from [16; 17] in fowls, [18] in broiler chicken.

Each half contained a number of lobes ranged between six to eight forming along chain of continuous lobes which sometimes embedded in the surrounding adipose connective tissue (Fig. 1,2), these findings are similar to that described by [19] in the chicken; [14] in the duck; [20] in fowls of Red Bro Cou Nu lineage; [10] in the Aseel chicken. The last lobe of thymus located near to the thyroid gland in the anterior thoracic region (Fig. 2), the same findings previously visible by [21; 22] in turkey. The color of chicken's thymic lobes was varied from whitish to pinkish color, the shape of the lobes was flattened with varying in size and each lobe wasn't well separated from the other, lying in the sub-

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "**Hanaa Kareem Ali**

dermal connective tissue of the neck, (Fig. 1,2) parallel to its shape and color in other domestic fowls mentioned previously by other researchers like: [23; 24] in broiler chickens.

The histological architecture revealed that thymus gland in the local chicken was enclosed by a thin capsule of connective tissue contain collagen fibers and fine reticular fibers (Fig. 3).

From the capsule numerous fine septa of connective tissue originated containing thymic blood vessels and these septa divided the organ into moderately separated lobules (Fig. 4), similar to that mentioned by [25] in ostrich and [16] in fowls. Each lobule contained an outer cortex which located in the marginal part of the thymic lobules, and a central medulla which seemed light staining and situated in the core of the lobule without any mark of isolation between the two parts, thymic cortex was more deeply basophilic stained than that of medulla and appear denser rather than that of the medulla (Fig. 5), these results were parallel to others clarified in ducklings and broiler chickens by [14; 24] respectively. The parenchyma of the gland mainly consisted of small lymphocytes and reticuloepithelial cells which were small in number in the cortex and numerous in the medulla, the reticuloepithelial cells were large cells with central nuclei and basophilic cytoplasm (Fig. 6), such description has been reported previously by [8] in fowls; [23] in broiler chickens. The lymphocytes inside the cortex was of small size with darkly basophilic stained and centrally located nuclei, making the cortex densely packed and leading to give the impression of appearing deeply basophilic in contrast to the pale medulla. While medulla which consist of large size reticuloepithelial cells and smaller amount of lymphocytes, thus, lymphocytes inside the medulla had centrally located nuclei and acidophilic cytoplasm (Fig. 6,7,8), these results were similar to that reported by [26] in Giriraja birds (*Gallus domesticus*); [27] in Nandanam Chicken. Inside the medulla of thymic lobules the reticuloepithelial cells begin to aggregate together (Fig.8), thus creating the formation of eosinophilic masses of various sizes which started as small spherical vacuoles then filled with different amounts of homogenous eosinophilic substance, this indicated to the changing in the reticular cells into vacuoles (Fig.9), the same statement was described previously by [28] in the domestic fowls ; [29] in ostrich. These vacuoles considered sometimes the first step of the formation of Hassall's corpuscles, which are a characteristic feature of this region, although their function is unknown, they were a pale stained diffuse masses consisting of vacuolated, squamous or flattened reticular cells, resulting from a degenerating reticular cells arranged in a concentric arrangement, (Fig. 6,7,9) this histological architecture was similar to the previous findings of [30] in guinea fowl; [19] in Vencobb broiler chickens; [5] in Hubbard chickens; [31] in quail, chicken and duck.

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "

Hanaa Kareem Ali

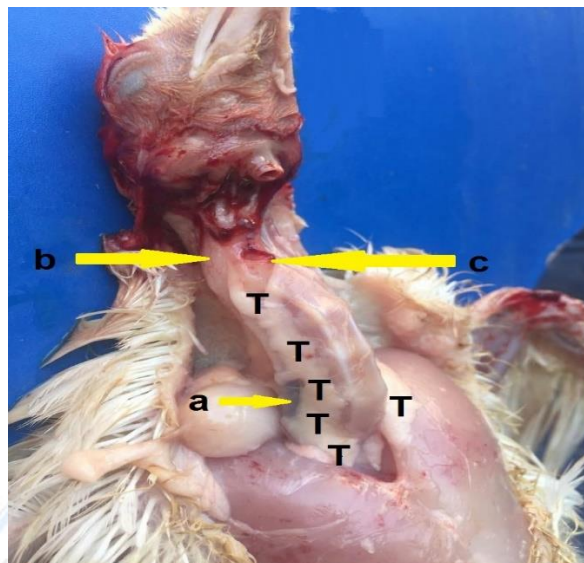


Fig. (1): Macroscopic figure showed: Thymus gland of the local chickens (T): number of thymic lobes of whitish to pinkish color and flattened shape, forming a long chain of continuous lobes situated along either side of the neck in parallel to the jugular vein (a), esophagus (b) and trachea (c).

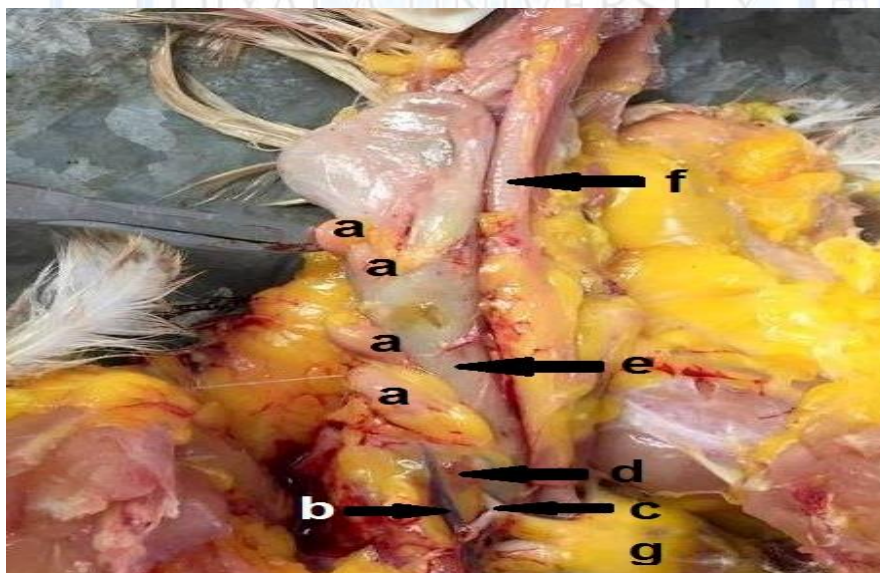


Fig. (2): Macroscopic figure showed: (a) number of thymic lobes of whitish to pinkish color forming a long chain of continuous lobes embedded in the adipose tissue, located near to (b) jugular vein, (c) common carotid artery, (d) thyroid gland, (e) esophagus, (f) trachea and (g) heart.

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "

Hanaa Kareem Ali

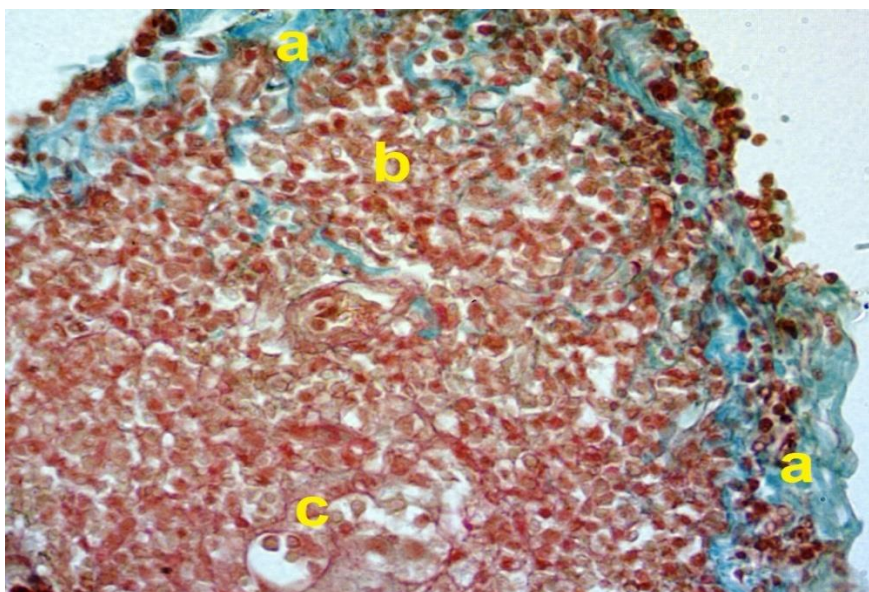


Fig.3: Histological section showing the thymus gland enclosed by a thin capsule of connective tissue (a) contain collagen fibers and fine reticular fibers, (b) cortex and (c) medulla, (Masson Trichrome stain, 40 X).

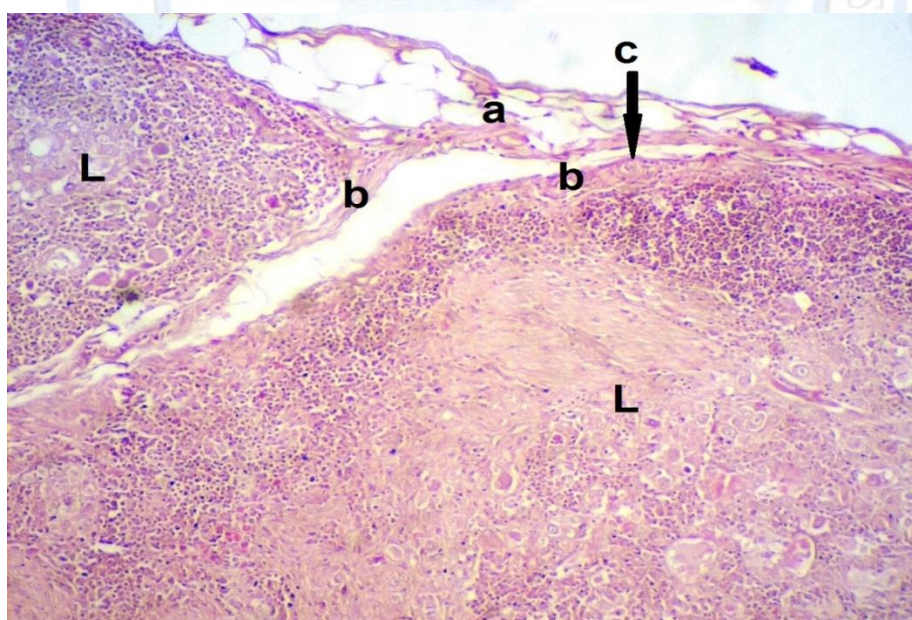


Fig.4: Histological section of thymus showing connective tissue capsule (a) from which numerous fine septa originated (b) containing blood vessels (c) and divided the gland into lobules (L) (H & E stain, 10 X).

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "

Hanaa Kareem Ali

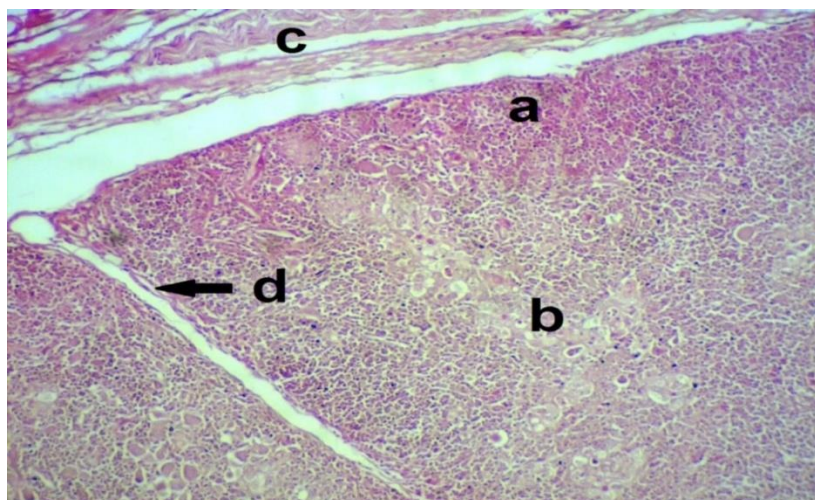


Fig.5: Histological section of thymus showing the dark cortical zone (a) and the light medullary zone (b), capsule (c), septa (d), (H & E stain, 10 X).

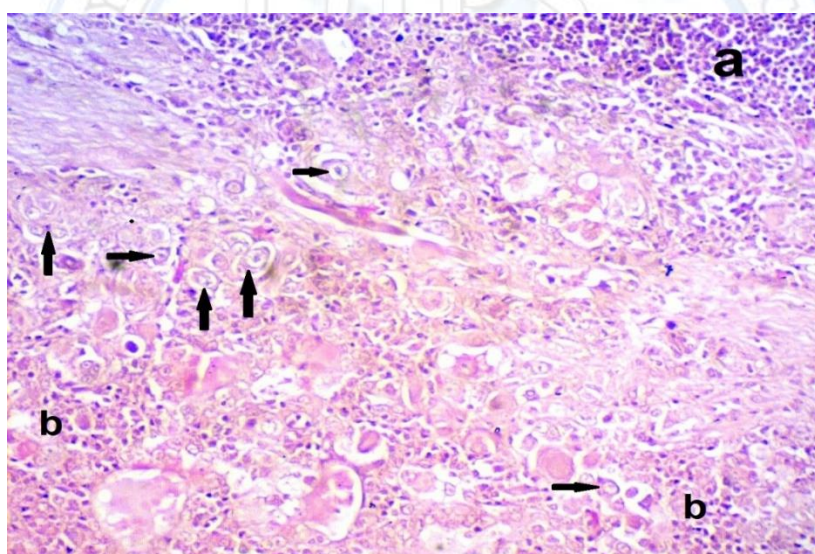


Fig.6: Histological section show: (a) cortex contains small lymphocytes, (b) medulla contains lymphocytes as well as great number of epithelial reticular cells with large and light-stained nuclei and basophilic cytoplasm (arrowheads), (H & E stain, 20 X).

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "

Hanaa Kareem Ali

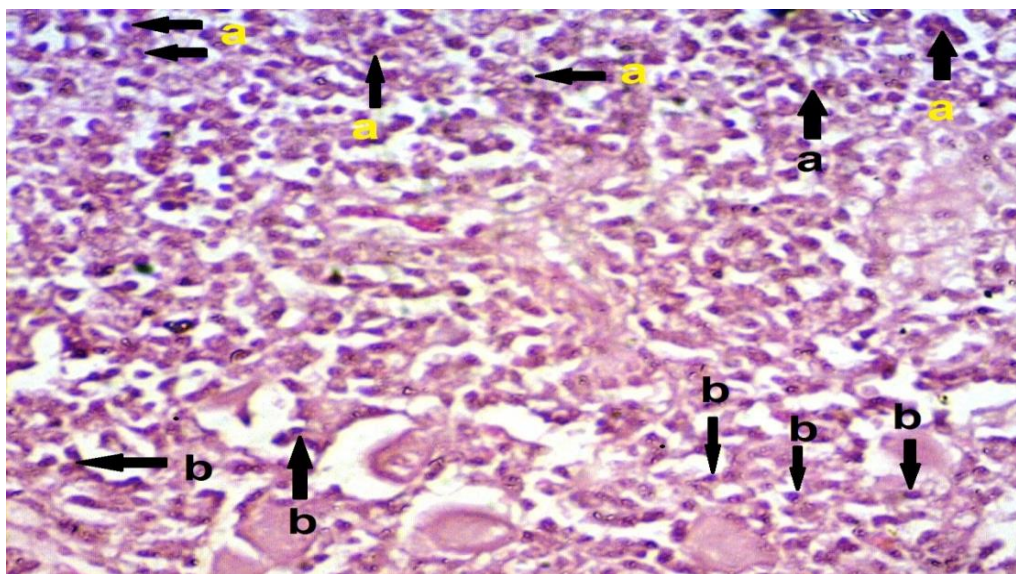


Fig.7: Histological section showing: (a) The lymphocytes inside the cortex was of small size with darkly basophilic cytoplasm and centrally located nuclei, (b) lymphocytes inside the medulla were centrally located nuclei and acidophilic cytoplasm (H & E stain, 40 X).

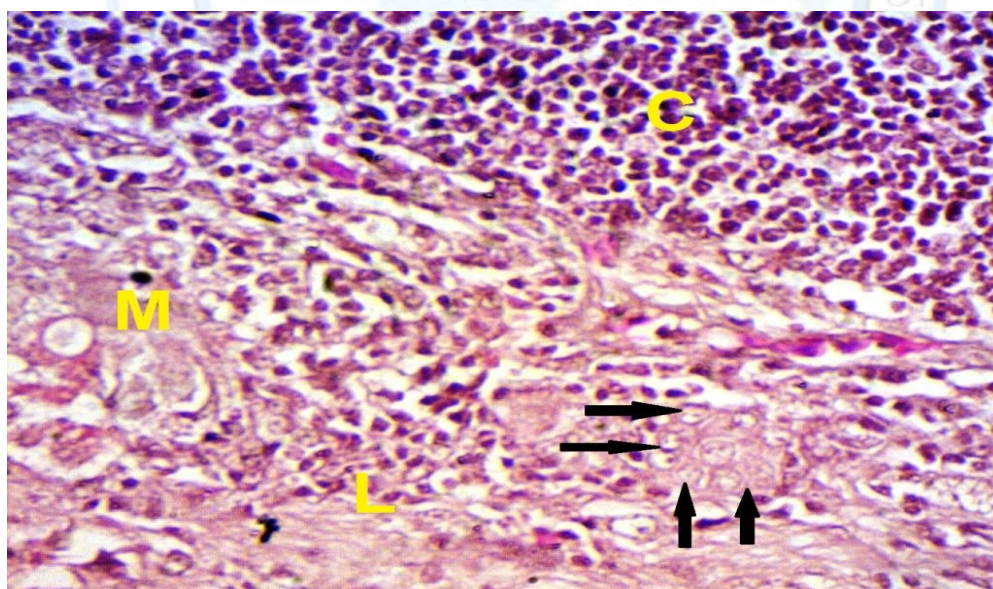


Fig.8: Histological section showing: (C) cortex (M) medulla containing lymphocytes with acidophilic cytoplasm (L) and reticuloepithelial cells which begin to aggregate together (arrowheads), (H & E stain, 20 X).

Histomorphological Study of Thymus in Local Chicken " *Gallus gallus domesticus* "

Hanaa Kareem Ali



Fig.9: Histological section (a), capsule (b), cortical zone, identified by its dark staining (c), medullary zone, identified by its lighter staining (d), Hassall corpuscles (e) the reticular cells changing into vacuoles filled with homogenous substance (H & E stain, 10 X).

References

1. Rose, E. (1979). The immune system in birds. Journal of the Royal Society of Medicine, 72, (9) : 701-705.
2. Szenberg, A. (1976). Ontogenesis of the immune system in birds. In: MARCHABRIS, J. J. (Ed.) Comparative immunology. Oxford: Blackwell Scientific Publications, p. 419-431.
3. Glick, B. (1978). The immune response in the chicken: Lymphoid development of the bursa of Fabricius and thymus and an immune response role for the gland of Harder. Poultry Science, 57, (5) : 1441-1444.
4. Suster, S. and Rosai, J. (1992). Thymus. In Histology for Pathologists (S. Sternberg, ed.) Raven Press, New York : 261-75.

5. **Treesh S.A., A.O. Buker and N.S. Khair. (2014).** Histological, histochemical and immunohistochemical studies on thymus of chicken. International Journal of Histology and Cytology 1(11): 103-111.
6. **Glick, B. (1994).** The Bursa of Fabricius: the evolution of a discovery. Poultry Sci.;73: 979-83.
7. **Aita, M. and N. Romano. (2006).** Effects of partial decerebration and hypophyseal allograft in the thymus of chicken embryos: thymostimulin localization and enzymatic activities. European Journal of Histochemistry. 50 (1) :69-78.
8. **Haley, P. J. (2003).** Species differences in the structure and function of the immune system. Toxicology 188: 49- 71.
9. **Hui S, Kemei P, Shenghe LI, Yan W, Lan W et al. (2012).** Morphological characterization of the immune organs in ostrich chicks. Turk. J. Vet. Anim. Sci. 36(2): 89-100.
10. **Haseeb, A., M.G. Shah, J.A. Gandahi, G.M. Lochi, M. S. Khan, M. Faisal, F.A. Kiani, R.A. mangi and S.K. Oad (2014).** Histo-morphological Study on Thymus of Aseel chicken. J. Agric. Food. Tech., 4(2):1-5.
11. **Azab, M.E; Fathalla, S.I and Emara, S.A (2004):** Effect of thymectomy on some physiological aspect in Japanese quails (*Coturnix, coturnix japonica*). Egypt.J.Basic Appl. Physiol., 3(1): 121-135.
12. **Hussan MT, Khan MZI, Lucky NS (2009).** Immunohistochemical study of the postnatal development of lymphoid tissues and mucosa of broilers. Bangl J Vet Med 7(1): 253-258.
13. **Davison F., Kaspers B. and Schat K. A. (2008).** Avian Immunology. 1st edition. Elsevier Ltd., 84 Theobald's Road, London WC1X 8RR, UK, pp.1-13.
14. **Sultana, N., Khan, M. Z. I., Wares, M. A. and Masum, M. A. (2011).** Histomorphological study of the major lymphoid tissues in indigenous ducklings of Bangladesh. Bangl. J.Vet. Med. 9(1): 53 – 58.

15. Bancroft, J. D., Suvarna, S. K. and Layton, C. (2013). Bancroft's Theory and Practice of Histological Techniques. 7th Edition. Churchill Livingstone Elsevier Limited. British Library Cataloguing in Publication Data Pp:106 – 118.
16. Hodges, R. D. (1974). The Histology of The Fowl. Academic Press INC. New York. U.S.A. PP: 440-458.
17. King, A. S. and J. McLelland. (1984). Birds: Their Structure and Function. 2nd edition. Bailliere Tindall. Philadelphia. 1st Anne's Road, Eastbourne, England. PP: 200-208.
18. Tarek, K., M. Mohamed, B. Omar and B. Hassina (2012). Morpho-Histological Study of the Thymus of Broiler Chickens During Post-Hashing Age. International Journal of Poultry Science. 11 (1): 78-80.
19. Karim, M. R., Khan, M. Z. I. and Haque, Z. (2005). Morphometrical analysis of major lymphoid organs of chemotherapy treated chickens. Bangl. J. Vet. Med. 3(2):106-109.
20. Vianna, A.R.D.C.B., F.A.C. DeMelo, M.I.S. Santana, H.G. Benedicto, F.O.C. Silva, R.S. Severino, and E.M.M. De Lima. (2012). Macroscopic morphology and arterial vascular supply of thymus in fowls (*Gallus gallus domesticus*) of Red Bro Cou Nu lineage. Acta. Scientiarum. Biological Sciences Maringá. 34(4): 457-462.
21. Muthukumaran, C., A. Kumaravel, K. Balasundaram and S. Paramasivan. (2011). Gross Anatomical Studies on the Thymus Gland in Turkeys (*Meleagris gallopavo*). Tamilnadu J. Veterinary and Animal Sciences. 7 (1): 6-11.
22. Ali, H. K. (2016). Anatomical and Histological study of thymus gland in the local breed of turkey "*Meleagris gallopavo*" in Iraq. 3rd Scientific Conference - College of Veterinary Medicine - University of Tikrit. 2,3 May 2016. P: 93-97.
23. Akter, S. H., Khan, M. Z. I., Jahan, M. R., Karim M. R. and M. R. Islam. (2006). Histomorphological Study of the Lymphoid Tissues of Broiler Chickens. Bangl. J. Vet. Med. 4 (2): 87–92.
24. Islam Khan, M.Z., Masum, M. Khan, MD. Z.I., Aziz, A.R.B. Nasrin, M., Siddique, M.N.H. & Arshad, M.M.B. (2014). Histomorphology of the Lymphoid

- Tissues of Broiler Chickens in Kelantan, Malaysia Sains Malaysiana. 43(8): 1175–1179.
25. Elewa, Y.H.A. (2005). Some morphological studies on immunological organs in ostrich. M.V.Sc. Thesis. Fac. Of Vet. Med. Zagazig. Univ.
 26. Leena C., R.V. Prasad, K. Kakade and K.V. Jamuna. (2008). HISTOLOGY AND AGE RELATED INVOLUTARY CHANGES OF THE THYMUS OF GIRIRAJA BIRDS (*Gallus domesticus*). J. Vet. Anim.Sci. 39: 40-43.
 27. Kannan, T.A., G. Ramesh, S. Ushakumari, S. Venkatesan, and S. Vairamuthu. (2015). Light and electronmicroscopic details of Thymic Reticuloepithelial cells in Nandanam Chicken (*Gallus domesticus*). International Journal of Advanced Research, 3(9): 841 – 846.
 28. Payne, L.N. (1971). the lymphoid system. In'' Physiology and biochemistry of domestic fowl''. 2nd Ed. Edited by Bell, D.J and Freeman, B.M. Academic press.London.PP.985-1037.
 29. El-Zoghby, I.M.A. and H.F. Attia (2007). Fine structure of the thymus gland in ostrich (*Struthio camelus* L). The 31th Scientific Conference of the Egyptian Society of Histology and Cytology : 1-16.
 30. Onyeausi, B.I, J.C. Onyeausi, A.N. Emma, C.D. Ezeokoli (1994). The thymus of the guinea fowl from the eighteenth day of incubation until maturity. Anat. Histol. Embryol. 23(4):320-329.
 31. Senapati, M.R., P.C. Behera, , A. Maity, and A.K. Mandal (2015). Comparative Histomorphological Study on the Thymus with Reference to its Immunological Importance in Quail, Chicken and Duck. Explor. Anim. Med. Res. 5(1): 73-77.