HISTOMORPHOLOGICAL AND IMMUNEHISTOCHEMICAL 
STUDY OF POSTNATAL DEVELOPMENT OF THYMUS IN 
TURKEY (Meleagris Galloppavo )

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ABSTRACT

Forty normal turkeys divided into four age groups (1 week, 1 month, 2 months and 7 months) were included. The thymus gland were collected and prepared for histological and immunohistochemical studies. Anatomically thymus gland in turkey has (4-5) thymic lobes in left side and (5) in the right, extended from the level of 7th cervical vertebra to the level of the thoracic inlet. Results of present study appeared thymus at one week as a mass of lobes in adipose tissue and gradually developed to be two chains of lobes on both sides of the neck, the parenchyma is undistinguished to cortex and medulla, no Hassall's corpuscles were noticed. At one month there are clear lobulation by fine septae, parenchyma is differentiated into cortex and medulla. Hassall's corpuscles were appeared. At two months there are very clear lobulation, well-developed septae and Hassall's corpuscles were more aqurence, and presence of erythrocytes. The histological architecture of 7th months old is similar to that of two months but the Hassall's corpuscles were more obvious. The immunhistochemical examination by using antibody (CD268) revealed presence of B lymphocytes within the parenchyma of thymus at one month, two months and seven months turkey. The current study concluded that the thymus well developed at one month and had a role in humeral immunity.
INTRODUCTION

Turkey (Meleagris Gallopavo) is a large gallinaceous bird of the family Meleagridae that is native of North America, domesticated in Europe and partially, has attended in Iraq poultry industry later. It is important source of meat in many parts of the world next to chicken and duck. Turkey is tolerant to many diseases such as Marek’s disease and infectious bronchitis as a compared to chicken (1). Birds and reptiles have special form of thymus gland as a series of large nodes along the neck, and regarded as immunologically as a principal or central lymphoid organ and its presence are vital for the development of peripheral lymphoid tissues (2). Thymus gland is important for immune response as well as antibody formation (3). It is the site for development of T cell immunological function also it generate, differentiate and mature T and B lymphocytes respectively (4, 5). It has important role in the development of marginal lymphoid tissues and cell mediated immunity (6). It is paired organs consist of numerous separated ovoid lobes closely related to vagus nerve and jugular vein, it seem to be active in young individuals (5). Thymus is extending from the 3rd cervical vertebra to the first thoracic segments (7). The number of thymus lobes is ranging from 7-9 on right side and 6-8 on left side of neck. The color of each lobe is ranging from pale white to yellowish white with elongated and flattened shape (6.) It was located in the sub dermal connective tissue at both sides of neck region. There are typically five lobes of different sizes and shape. The color of the thymus of local ducklings is pale white to yellowish white elongated and flattened (8). Thymus cortex is composed of darkly stained densely packed lymphocytes (9). In chicken embryos the thymus gland makeup of peripheral cortex and medulla centrally located, also the thymus exposed a capsular line of connective tissue, which gives rise to the septa dividing the cortex in lobes rich in thymocytes (10). As thymus increase age it suffered from increase number of Hassall’s corpuscles and regression of thymic lobules (6). Medulla is located in the center of the gland and operates less number of thymocytes which appear as clusters of vacuolated, dark and light cells (10). This study aimed to provide good informations of the developmental changes and distribution of lymphocytes in the lymphatic organs in the important periods of turkey life that allow to determination of the character and range of changes in lymphatic organs to increase the immunity and
served in planning and reprogrammed the time and mode of vaccination in Iraqi turkey farms.

**MATERIALS AND METHODS**

Fourty healthy turkeys of both sexes at four ages (1 week, 1 month, 2 months and 7 months) were used in the current study. They were selected from local farm in Diyala city, Iraq. The birds were housed in clean cages under strict hygienic conditions. Histotechniques were done at the laboratory of veterinary medicine, University of Diyala, Iraq. The thymus gland was dissected and immediately fixed in Bouin’s and neutral buffer formalin solution, Paraffin-embedding and sectioning 6-7 μm by rotary microtome. The sections were stained with Hematoxylin and Eosin (H&E) for routine histological examination and Masson trichrom stain for connective tissue of capsule and septae (12). Other sections were used for Immunohistochemical studies using polyclonal antibody to B-cell activation factor receptor (BAFFR) (CD268) produced in rabbit (cloud-clone corp. USA).

**RESULTS**

**Morphological Findings**

At one week old: the thymus gland is composed of accumulation of mass of lobes at the thoracic inlet on the margin of clavicle bone (fig.1). At one month old thymus arranged in two chains of oval lobes on each side of the neck. It is consists of 5 lobes in the right side and (4-5) lobes on the left side. The color of the thymic lobes was white to creamy (fig.2). At two and seven months old the turkey poult the number, shape and color of thymic lobes are constant (fig.3, 4).

**Histological Findings**

At one week old the thymus surrounded by fibrous capsule with fine and delicate septae. Thymus parenchyma began to no differentiate into cortex and medulla, no Hassall's corpuscles seen (fig.5). The thymic lobules were not completely separated. Erythrocytes are present among the lymphocytes (fig.6, 10). At one month old the parenchyma easily defined as cortex and medulla. Well-developed septae are seen (fig.7, 11). At age of two months, the thymus gland consists of cortex and medulla. The number of Hassall's corpuscles are increased in thymic medulla. An increase of
Lymphocytes and plasma cells number within cortex and medulla are noticed. Also erythrocytes are present (fig.8, 12). At seven months age the same picture of thymic lobule at two months lobules further increase in Hassall's corpuscles compared to that of the other previously younger ages are clearly seen (Fig.9, 13).

**Immunehistochemical finding**

CD268 is a marker of choice for identification of mature B cells distributions within the thymus parenchyma. All B cells seemed to be slightly or strongly positive expression dependent on the age of development while other cellular elements are non-reactive. Positivity was characterized by brown staining of B cells and also in thymic Hassall's corpuscles. Mature B cells showed –ve expressions at the first week of age (Fig.14). At one month old turkey showed variable degree of +ve expression for B cells (brown staining) in medulla and around blood vessels (Fig.15). At two months old turkey showed +ve expression in medulla with little expression in cortex (fig.16). At age of seven months old turkeys there are equal areas of ++ve expression in cortex and medulla (fig.17).

**DISCUSSION**

The morphological observation:

In turkey the thymus gland is extended from the level of seventh thoracic vertebra to the level of the thoracic inlet. This result also explained by (7) referred to the Thymus extending from the 3rd cervical vertebra to the first thoracic segments. Thymus was embedded in connective tissue on each sides of neck superficial to the jugular vein and vagus nerve. (5, 2) also mentioned "the thyroid lobes are closely related to vagus nerve and jugular vein". At one week old; the thymus gland is composed of accumulation of undifferentiated mass of lymphoid tissue. (13) studied the thymus of deshi chicken and in Kelanta referred to this fact. At one month the shape of thymus differentiate to two chains of irregular with rough surfaces lobes on each side of the neck. Due to the growth of bird the neck length is increase therefore thymus gland is drawn into two chains (left and right) and the distance between lobs disappeared, this was as what mentioned earlier by (14) in white pekin ducks, the irregularity could be caused due to the rapid growth of the thymic lobes which faster were than the growth
of the bird neck which cause crowded and the lobes compressed and lost their oval shape. Thymus gland consists of 5 lobes in the right side and (4-5) lobes on the left side. The lobes number was (5-6) in white pekin ducks (14), in native geese (15), counted (6-9) lobes at the right side of the neck and (5-9) in the left. At two and seven months old the turkey poult the number of thymic lobes are constant but the weight and size are increase due to the rapid growth of the bird. The thymic lobes number was constant in the individual's birds with age. The number of thymic lobes in the current study was similar to the number which recorded by (13) in deshi chicken, (16) in Broiler chicken and (17) in Giriraja birds where they found that the number of thymic lobes were (5-6) in the right side and (7-8) in the left side while in Assel chick the number of thymic lobes were (7-9) in the right side of the neck and the left thymic chain contained (6-8) lobe (5). The color of the thymic lobes was white to creamy. This result was in compatible with results of (18) who studies the thymus of guinea fowl and the thymus appeared as series of pink thymic lobes.

**Histological study**

At one week old turkey, the thymus gland parenchyma is began to differentiated into cortex and medulla, (10) mentioned in chicken embryos the thymus gland makeup of peripheral cortex and medulla centrally located. No hassal's corpuscle seen, no plasma cells, there are many trabecular vessels (6) mentionerd that "With increase age thymus suffered from increase number of Hassall’s corpuscles and regression of thymic lobules". The dark appearance of cortex explained by the present of high content of lymphocytes with large dark stained basophilic nucleus and thin rim of eosinophilic cytoplasm. The medulla appeared light because it consisted mainly of epithelial reticular cells with light stained nucleus and more eosinophilic cytoplasm. The thymic lobules were not completely separated which could be related to the short growing connective tissue septa, that originated from the thymus gland capsule and they extended and divided the cortex but did not reach the medullary area, this was incomputable with results of (18) who studied the development of thymus gland in Guinea fowl. The presence of erythrocytes between the lymphocytes of the medulla proved that the thymus gland was hematopoietic organ in old embryo and newborn poult. This result agrees with the result of (17) in chicken and the results of (19). The current study revealed that the thymus was histologically completed in

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turkey. These results were in agreement with the results of (14) in white Pekin duckling and the results of (20) who studied the development of thymus gland of partridge birds before and after hatching. At one month old turkey the cortex and medulla were easily defined and the cortex was stained deeply with basic dyes while the medulla was light and eosinophilic (21). The current study revealed complete separation of the lobules at one month old turkey and this was different than the results of (11), who stated that the thymic lobes in chicken were divided into complete lobules at the age of three weeks. The histological structures of the thymus and appearance of plasma cells in the thymus of one month old turkey was in parallel with results of (14) in white Pekin duckling and (18) in Guinea fowl and of (11) in chicken. At two months, the thymus gland consists of very clear cortex and medulla. Increases in the number of Hassall's corpuscles in thymic medulla are seen. The cortex and medulla were easily defined and the cortex was stained deeply with basic dyes while the medulla was light and eosinophilic (21). The current study revealed complete separation of the lobules at the two months turkey and this was different from the results of (11), who stated that the thymic lobes in chicken were divided to complete lobules. The finding changes which present in thymus gland at two month old chickens were similar to the findings of (15) in native geese and the results of (22) and (4) in ostrich and the findings of (6) and (11) in chickens. At seven months age the same picture of thymic lobule at two months lobules further increase in Hassall's corpuscles compared to that of the other previously younger ages are clearly seen. B cells showed –ve expressions at the first week of age, because of the most of the cells at this age were immature B cells. At one month, two months and seven months old respectively, there were gradual increase in number of mature B cells with increase in age therefore variable degrees of +ve expression for B cells are noticed, (11) referred to there are variable degree of expression with age.
Figure (1, 2, 3, 4) show the shape and position of thymus gland in one week (A), one month (B), two months (C) and seven months (D).

Figure (5) shows the thymus gland capsule (arrow) and parenchyma. Masson trichrom 4x.
Figure(6, 7,8,9) show the thymus gland structure in one week(A), one month(B), two months(C) and seven months(D). Thymus capsule arrow, cortex(C), medulla(m) and Lobulation(arrow). H&E stain 10X.

Figure(10,11,12,13) show central hyalinization and eccentric arrangement of reticuloendothelial cell of Hassell’s corpuscles in thymic medulla (arrows). Thymus gland in one week(A), one month(B), two months(C) and seven months(D). H&E 40x.
Figure (14, 15, 16, 17) show the Immunohistochemical staining of a section of the thymus gland in one week (A), one month (B), two months (C) and seven months (D). showing +ve reaction for mature B cells with variable degree of (brown) staining ability. (Immunoperoxidase 40x).

REFERENCES


