

Full Length Research Paper

Histological, histochemical and immunohistochemical studies on thymus of chicken

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The thymus is the major site of T cell production and an important organ of the immune system, due to lack of some information on presence of the plasma cells in thymus we study it. Thirty six apparently normal chicken which differ in age and gender were included. The tissue samples were prepared for histological, histochemical and immunohistochemical studies. The appearance of plasma cells began at the 4th week of age, in the medulla, and their number increased along the thymus development. In concomitant with the appearance of blood capillaries at the corticomedullary junction by 3th and 4th weeks of age and a few Hassall's corpuscles in the medulla at 4th weeks of age. These corpuscles were constantly increased in number with age, and that was demonstrated in the 21, 30 weeks-old. We concluded that, the appearance of plasma cells in chicken thymus at different ages provides further support to the idea that the thymus may function as a secondary lymphoid organ, and it is capable of playing a direct role in the immunereactions. This is important role in vaccination of chicken and in future research works.

Key words: Plasma cell, thymus, Chicken, histochemical, Immunohistochemical.

INTRODUCTION

The central lymphoid organs in birds include the thymus and the cloacal bursa of Fabricius, these are places where lymphocytes develop, and the T-cell and B-cell receptor genes rearrange. Then lymphocytes migrate to peripheral lymphoid organs such as the spleen, the caecal tonsil and the lymph nodes (Glick, 1956).

It is well established that the thymus plays an essential role in the development and maturation of immunological competent cells in chicken (Warner, 1964). The first stem cells of the lymphoid system are generated within the embryonic mesenchyme. Then the developed cells migrate into the yolk sac and then to the thymus and the bursa at days 6 and 8 respectively (Jotereau and Houssaint, 1977; Tuboly, 1984). Mature B-lymphocytes are then seeded to other lymphoid organs (Toivanen and Toivanen, 1973).

The thymus is a primary lymphoid organ which is the first of the lymphoid organs to be formed and grows immediately after birth in response to postnatal antigen

stimulation and the demand for large numbers of mature T cells (Suster and Rosai, 1992). It is the initial site for development of T cell immunological function. It is histologically most consistent across species (Haley, 2003).

The thymus of chicken is a paired organ, each part consists of seven distinct lobes, connected by a connective tissue isthmus. Thin connective tissue capsule surrounds each lobe and gives rise to septae that partially subdivide the thymus into interconnecting lobules of variable size (Terszowski et al., 2006).

The bulk of supporting framework in thymus is composed of network of epithelial reticular cells. Epithelial reticular cells differ in antigenic expression, ultrastructural characteristics and their capacity to synthesize the thymic hormones; thymulin and thymosin (Banks, 1993).

Each lobule is divided into a morphologically distinct cortex and medulla, separated by a vascular corticomedullary zone. The cortex is darkly stained and heavily populated by developing small immature T cells which overshadow sparse epithelial cell population. Medulla is paler stained, less densely cellular than the cortex and contains larger, more mature T cells, prominent

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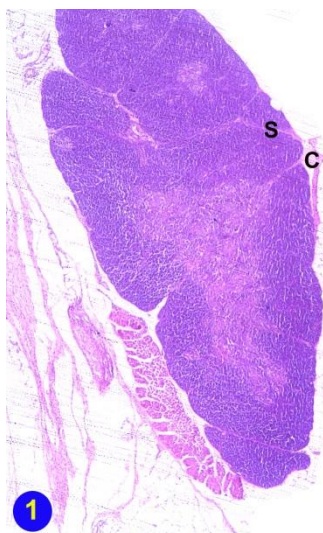


Figure 1. Showing multiple thymic lobules of one day old chicken surrounded by connective tissue capsule (C) and separated from each other by connective tissue septa (S) (H& E, X 25).

epithelial cells, Hassall's corpuscles, macrophages and dendritic cells. The medulla is continuous between adjacent lobules (Haley,2003).

Corticomedullary zone contains a plenty of vessels, mainly arterioles with scant perivascular C.T., immature T lymphocytes and dendritic cells. These arterioles ramify into capillaries that extend into the cortex and medulla . Meanwhile in the cortex , they form complex of capillary arcades which together with the perivascular lymphocytes, macrophages and epithelial reticular cells forming the blood- thymus barrier (Banks,1993).

Capillaries in the cortex are rarely fenestrated to restrict access of the circulating antigenic molecules to the cortical lymphocytes,although the medullary capillaries are fenestrated and freely permeable to circulating antigens (Kuper et al., 2002).

It has been found that thymus cells are not capable of producing antibody following administration of antigens. This failure has been usually attributed to the lack of germinal centers and plasma cells (Askonas and white, 1956; Dixon et al., 1957).

Understanding normal morphological features of the thymus provides a cornerstone to assessing immune system function.

The aim of the present study to detect presence of plasma cells and ability of the thymus to produce antibodies .

MATERIAL AND METHOD

Thirty six apparently normal and clinically healthy Hubbard chickens of both sexes at different ages were used in the present study. They were collected from "The broiler breeding center- Tarhona – Libya ". The birds

were housed in clean cages under strict hygienic conditions. They were supplied by water and food. Histotechniques were done at the laboratory of department of histology and medical genetics, faculty of medicine, University of Tripoli, Libya.

The thymus glands were dissected and immediately fixed in 10% formalin saline. then, processed for paraffin embedding , then serial sections were cut (6 µm) and stained by the following stains: -

Hematoxylin and Eosin (H&E) for routine histological examination (**Bancroft,2007**). Methyl Green Pyronine [MGP] stain for demonstration of Plasma cells (Sigma-aldrich, Germany). Adjacent sections were used for immunohistochemical studies using Anti-SDC1 antibody (CD138) produced in rabbit (Sigma-aldrich, Germany).

RESULT

Light microscopic examination of serial sections of chicken thymus showed the following:

Histological staining: H & E stained sections of the thymus of one day old chickens showed that it was formed of lobes and lobules. The thymus lobes were surrounded by connective tissue capsule. Connective tissue septa extend from the capsule to divide the thymus into lobules. These capsule and septa contain thymic blood vessels. Each thymic lobule was divided into two parts, outer darkly stained cortex, and inner lightly stained medulla (Figure 1).

At the age of oneweek, the thymus showed more lobulation with outer dark cortex, filled with numerous lymphocytes (thymocytes)and inner light medulla was formed of large lymphocytes with central nuclei and acidophilic cytoplasm (Figure 2).

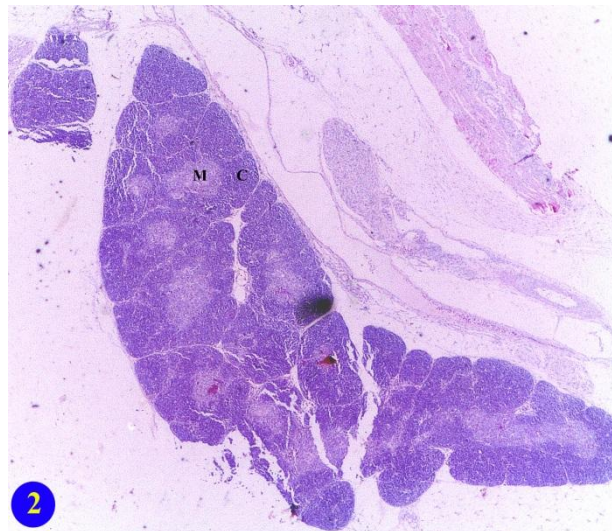


Figure 2. Showing a thymic lobule of one week old chicken. Notice the outer darkly stained cortex (C) and pale stained central medulla (M) (H&E, X 25).

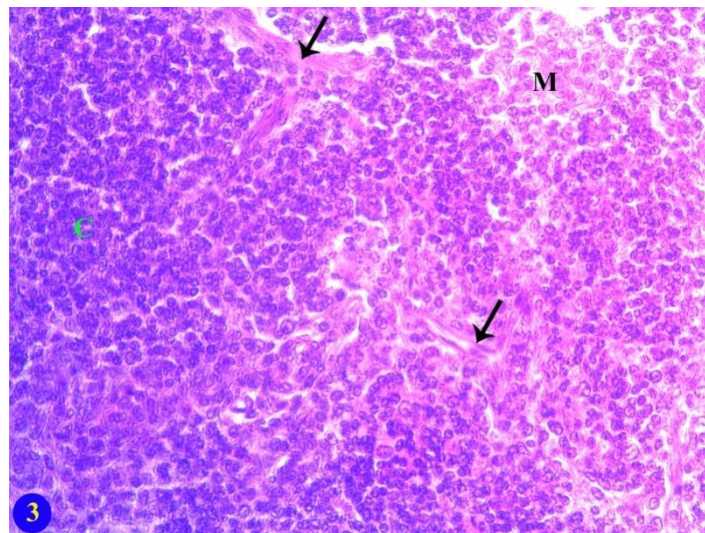


Figure 3. Showing a thymic lobule of 3 weeks old chicken . Notice the endothelial capillary present at the corticomedullary junction (Arrow), (C) cortex and (M) medulla (H&E , X400).

At the age of 3 weeks thymic lobules showed similar structure to that of one week old chickens. In addition to appearance of blood capillaries at the corticomedullary junction (Figure 3).

At the age of 4 weeks the same results were found also in the thymic lobules , in addition to few Hassall's corpuscles which started to appear in the medulla at this age (Figure 4,a). The Hassall's corpuscles were having structureless hyalinized center and peripheral concentrically arranged epithelial reticular cells, in addition to interseptal position of aggregation of fat cells (Figure 4,b).

At the age of 6 and 8 weeks , the thymus showed an increase in the appearance of Hassall's corpuscles in thymic medulla (Figure 5).

At the age of 21, 24 and 30 weeks the thymic lobules showed similar findings to that of 4, 6, and 8 weeks old, further increase in Hassall's corpuscles compared to that of the other previously younger ages (Figure 6).

A continuation of marked interseptal aggregation of fat cells were observed by 24th weeks old age (Figure 7).

Histochemical staining: At age of one day, one week and 3 weeks old chickens, the medulla of thymic lobule showed thymocytes and no plasma cells were observed.

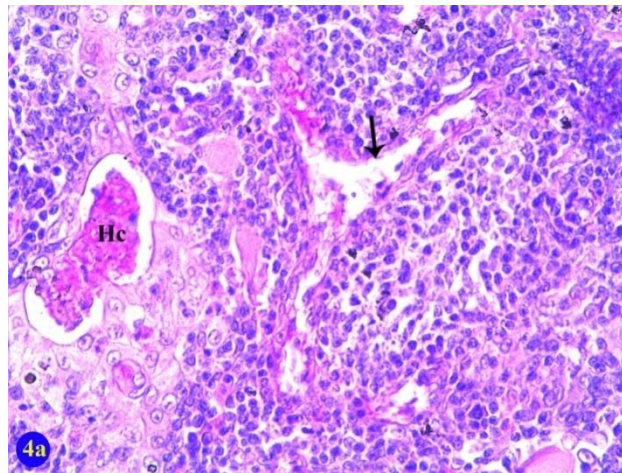


Figure (4a). Showing a thymic lobule of 4 weeks old chicken . Notice the endothelial capillary present at the corticomedullary junction (Arrow) and the presence of some Hassell's corpuscles in thymic medulla (Hc) (H&E, X400).

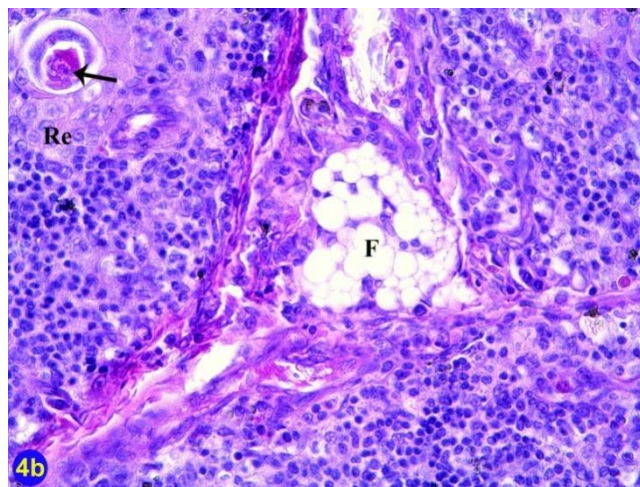


Figure (4b). A thymic lobule of 4 weeks old chicken. Showing central hyalinization (Arrow) and eccentric arrangement of reticuloendothelial cell (Re) of Hassell's corpuscles in thymic medulla and interseptal aggregation of fat cells (F) (H&E, X400).

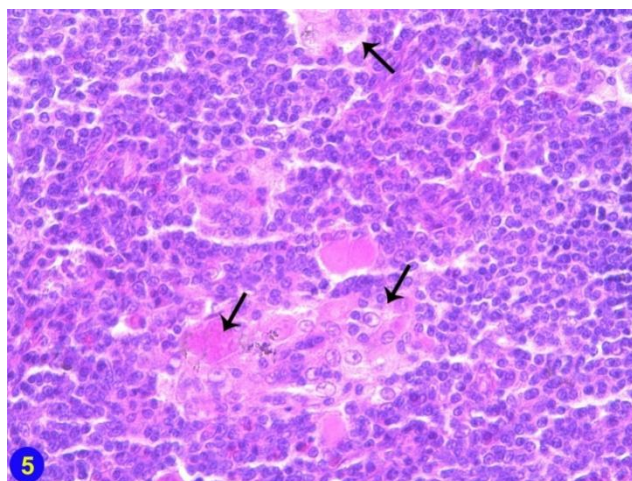


Figure 5. Showing a thymic lobule of 6 weeks old chicken. Notice the increased number of Hassell's corpuscles (Arrows)(H&E , X400).

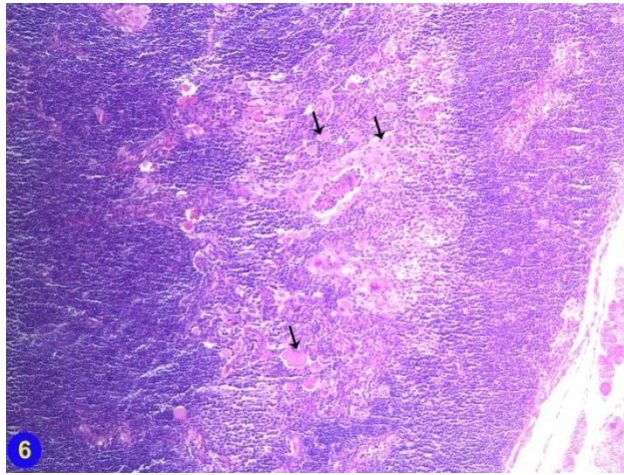


Figure 6. Showing a thymic lobule of 21 weeks old chicken. Notice the numerous Hassell's corpuscles in thymic medulla (Arrows) (H&E , X200).

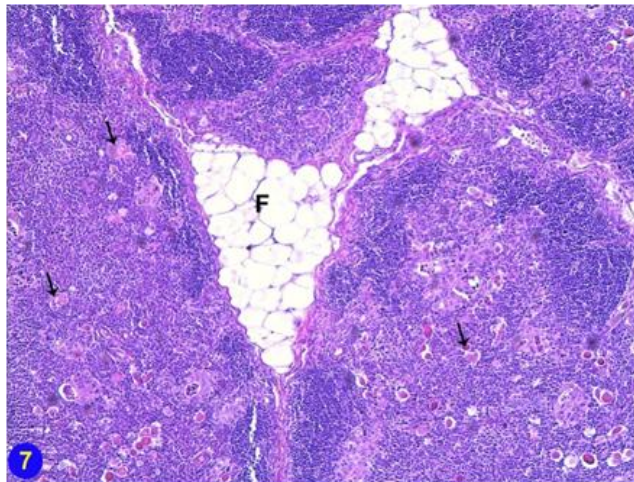


Figure 7. A thymic lobule of 24 weeks old chicken. Showing numerous Hassell's corpuscles in thymic medulla (Arrows) and interseptal aggregation of fat cells (F) (H&E, X200).

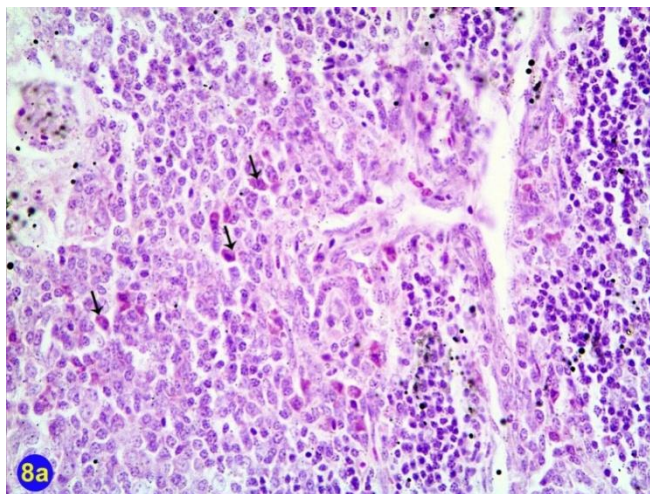


Figure 8a. Showing a thymic lobule of 4 weeks old chicken. Notice the plasma cells (Arrows) near blood vessel (Methyl green pyronin, X400).

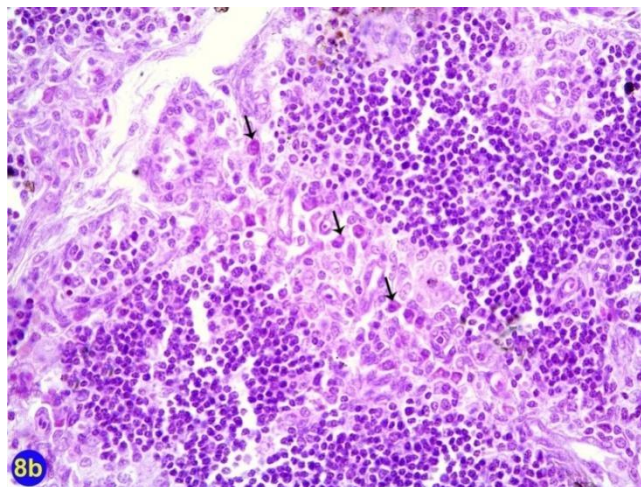


Figure 8b. Showing a thymic lobule of 4 weeks old chicken . Notice the presence of some plasma cells within the medulla (Arrows) (Methyl green pyronin , X400).

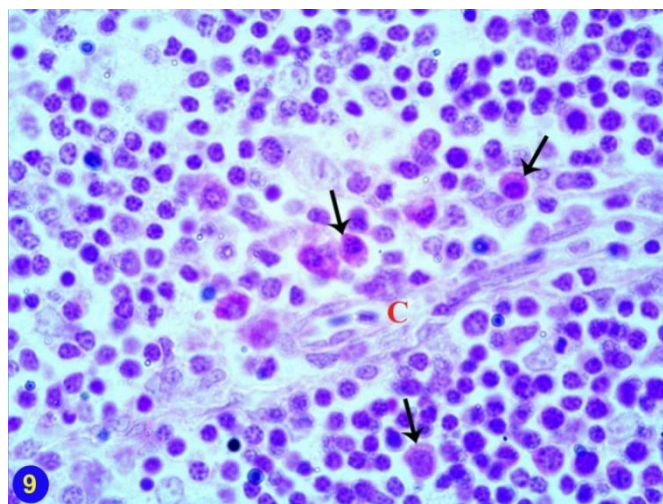


Figure 9. Showing a thymic lobule of 6 weeks old chicken. Notice the plasma cells (Arrows) surrounding the blood capillary (C) (Methyl green pyronin , X1000).

At age of 4, 6, and 8 weeks old chickens thymic lobules showed the beginning of appearance of groups of plasma cells stained red with methyl green-pyronin were located in medulla, especially around of blood vessels, near epithelial structures (Figures 8a, b and 9).

A similar result was also found in chickens of 21, 24 and 30 weeks old (Figure 10).

Immunohistochemical staining: CD138 is a marker of plasma cell differentiation. Its reactivity was observed for plasma cells which occurred in scattered and or perivascular distribution. All plasma cells seemed to be positive and other cellular elements are non-reactive. Staining intensity showed variability ranged from weak, moderate to strong. Positivity was characterized by a membranous staining pattern which observed in plasma cells and also in thymic Hassall's corpuscles.

At one day, one week and 3 weeks old chickens showed -ve reaction for plasma cells. Most of the cells were

lymphocytes while some were macrophages (Figure 11). At 4 weeks old chickens revealed beginning of appearance variable degree of +ve reaction for plasma cells (brown staining) (Figure 12) . At age of 6 and 8 weeks old chickens showed +ve reaction, there was an apparent increase in the number of plasma cells compared to chickens of 4 weeks old (Figure 13). The degree of staining was variable within sections of the same age . Also there was variability in staining among sections of chickens of different ages. At age of 21 weeks old revealed slightly +ve reaction for plasma cells compared to different ages of other chicken (Figure 14). At age of 24 and 30 weeks old chickens showed +ve reaction in plasma cells. The degree of stain ability was varying from moderate to intense (Figure 15). Furthermore, there was an apparent increase in the number of plasma cells in this group compared to the previous ages.

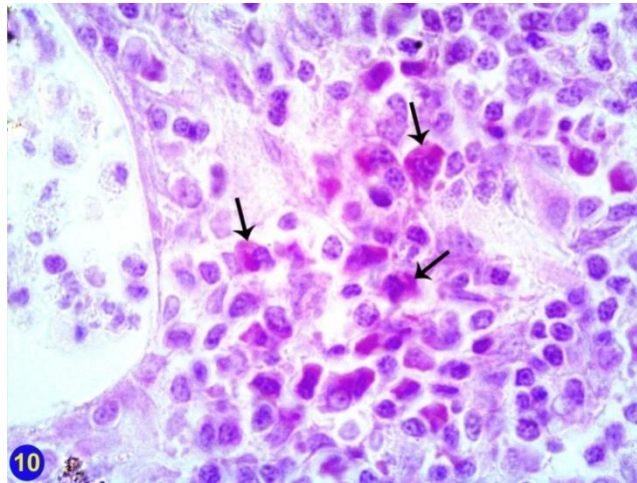


Figure 10. Showing a thymic lobule of 30 weeks old chicken. Notice the plasma cells (Arrows) (Methyl green pyronin , X1000).

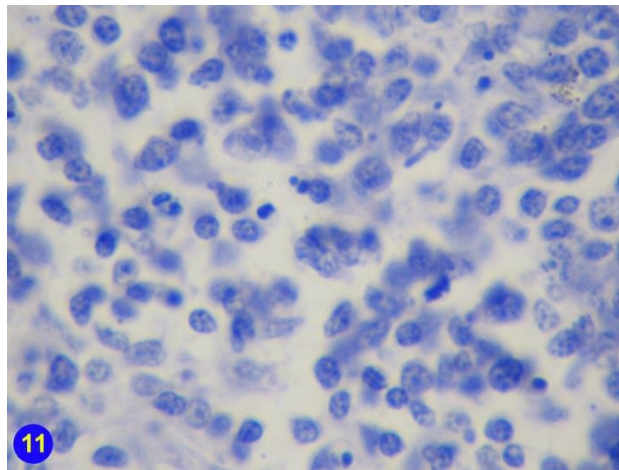


Figure 11. Immunostaining of a section of the thymus of one day old chicken showing –ve reaction for plasma cells. Notice most of the cells were lymphocytes , some were macrophages (Immunoperoxidase,x1000).

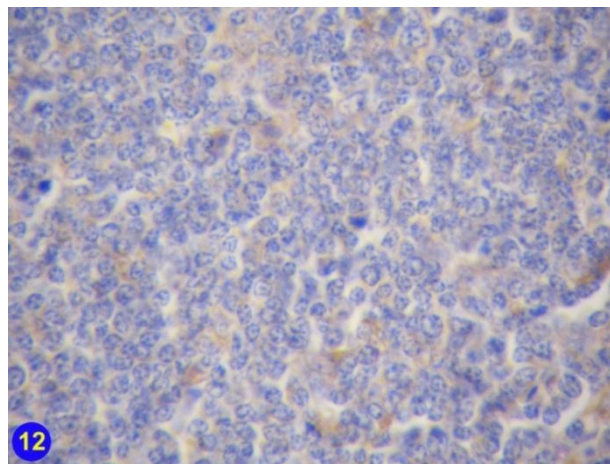


Figure 12. immunostaining of a section of the thymus of 4weeks old chicken showing +ve reaction for plasma cells with variable degree of stain ability. (Immunoperoxidase,x1000).

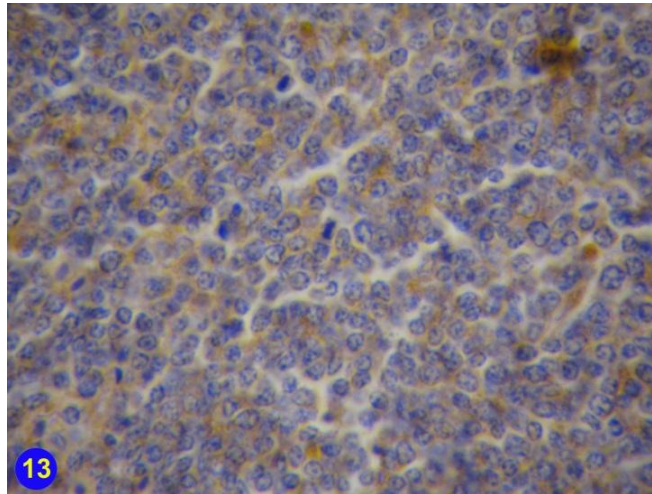


Figure 13. Immunostaining of a section of the thymus of 6 weeks old chicken showing +ve reaction for plasma cells with varying degrees of stainability. (Immunoperoxidase, x 1000).

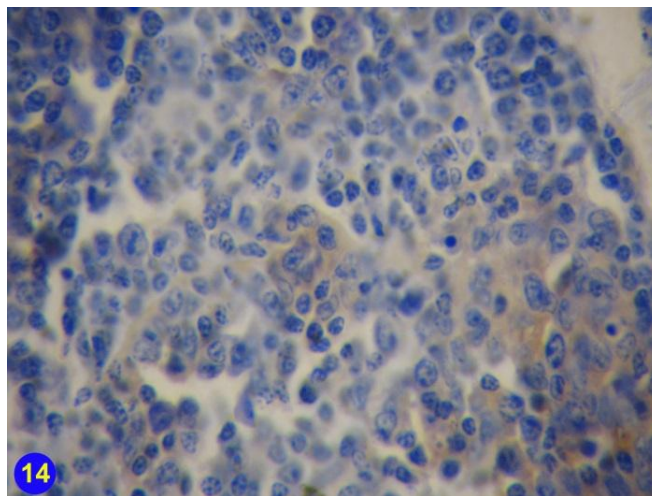


Figure 14. Immunostaining of a section of the thymus of 21 week old chicken showing slight +ve reaction for plasma cells. (Immunoperoxidase, x1000).

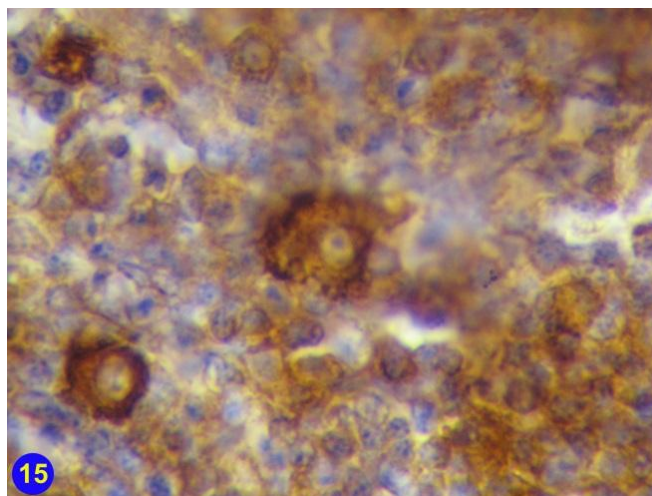


Figure 15. Immunostaining of a section of the thymus of 30 week old chicken , showing strong +ve reaction for plasma cells. (Immunoperoxidase,x1400).

DISCUSSION

Although the previous studies consider thymus as a primary lymphoid organ, through which bone marrow derived progenitor cells undergo differentiation, maturation within the thymic microenvironment to form the functional T cells. The growth of the thymus in chickens was reported by (Dieter and Breitenbach, 1968). The thymic lobes reach a maximum size of 6-12 mm in diameter by 3-4 months of age, before physiological involution begins (Ciriaco et al., 2003).

Our finding reported that, in one day and one week old chicken, the thymic lobes formed of several lobules. Each thymic lobule divided into two parts, outer darkly stained cortex, and inner lightly stained medulla, Its cortex consisted of densely packed small and medium sized lymphocytes making it to appear deeply basophilic.

At age of 3 weeks old chickens, thymic lobules showed similar structure to that of one week old. In addition, blood capillaries appeared at the corticomedullary junction. The same results were found also in the thymic lobules of 4th weeks old chickens, in addition to few Hassall's corpuscles which started to appear in the medulla at this age. The Hassall's corpuscles were having structure less hyalinized center and peripheral concentrically arranged epithelial reticular cells. Interseptal fat cells well observed at this age.

At age of 6 and 8 weeks old chickens the presence of groups of plasma cells in thymic medulla was observed. In addition, some Hassell's corpuscles were also found in thymic medulla.

At age of 21, 24 and 30 weeks old chickens showed similar findings to that of 6th and 8th weeks old. On the other hand, Hassall's corpuscles were increased further compared to that of the other groups. In addition to aggregation of interseptal fat cells.

A continuous layer of reticuloepithelial cells was noted around the blood vessels. These cells are a component of blood thymic barrier, which has three components namely the capillary with its basement membrane, the perivascular space and reticuloepithelial cell with its basement membrane (Leena et al., 2008). This protective blood thymic barrier prevented the occurrence of plasma cells in the thymus (Ham and Cormack, 1979).

The principal findings shown by the present study is the presence of plasma cells in the thymus of some chickens during the course of normal growth as normal feature, this finding was clear from 4th weeks old. The density of plasma cells was low in the 4th weeks of age and then increased, however at 21th weeks old the density decreased again, these chickens may have exposed to antigens normally occurring in their environment. This indicates that the thymus participate directly in immune responses. The appearance of a few plasma cells of the thymus was also found in chickens older than 8th weeks of age (Awaya et al., 1973).

Wolfe et al. (1962) reported that, there was continuous increase in weight of thymus up to 12th weeks of age. The

maximum weight of the thymus was at 17th weeks of age. (Dieter and Breitenbach, 1968) indicated that by 15th week age, involution and regression had begun due to depletion of lymphocyte series.

The thymus cells are not capable of producing antibody following systemic administration of a variety of antigens (ASKONAS and WHITE, 1956; DIXON et al., 1957).

Many different opinions have been put forward about the existence and location of plasma cells. Some investigators (Bradley et al., 1960; Yilmaz et al., 1996) have stated that these cells present mostly in cortex, (Özcan, 1984) in agreement with our study they present in medulla, while (Thorbecke et al., 1957) has reported that they present only in outer areas of medulla. Others observed plasma cells both in medulla and in cortex in geese (Gulmez and Aslan, 1999). While our observation showed that the plasma cells were mostly found in the medulla near to corticomedullary junction.

(Toivanen and Toivanen, 1987) stated that the blood thymic barrier was present in the cortex and not in the medulla. In our study the presence of Blood capillaries was in connective tissue capsule and septa at one day, one week old chickens and in corticomedullary junction in 3th week old. Our data agree with that the barrier is not absolute in the thymus.

(Nomura, 1973) suggested that the thymus in certain circumstances responds to direct contact with antigen with resultant production of plasma cells. However, (Awaya et al., 1965) shown that plasma cells had been reported in thymus of guinea pigs, following direct injection of antigens into the thymus. Such findings have not been observed following systemic administration of antigen. These suggested the presence of thymic barrier, preventing the entrance of antigen into thymic parenchyma.

(Sainte-Marie, 1963) however showed that soluble antigen or trypan blue injected intramediastinally or subcutaneously penetrates into thymic parenchyma. Other authors confirmed that there is no absolute barrier in the thymus (Raviola and Karnovsky, 1972).

Hassall's corpuscles in chicken were described by Frazier et al. (1973) and Gilmore and Bridges (1974) they showed that their number increased with age. They may play a role in disposing of exhausted materials including cell debris.

CONCLUSION

We concluded that, the presence of plasma cell in thymic lobules in different ages of chickens during the course of normal growth as normal feature, this begins from 4th weeks and increased with age and the blood barrier in thymus is not absolute, as well as this study provided further support to the idea that the thymus in addition to its role as a primary lymphoid tissue may func-

tion as a secondary lymphoid organ and it is capable of playing a direct part in immune reactions.

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