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International Journal of Current Research
Vol. 10, Issue, 03, pp.66538-66541, March, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IMMUNOHISTOCHEMISTRY OF PERSISTENT ACTIVE THYMUS IN HUMAN CADAVERS

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ARTICLE INFO

Article History:
Received 06th December, 2017
Received in revised form
02nd January, 2018
Accepted 17th February, 2018
Published online 28th March, 2018

Key words:
T cell, thymus,
Immunohistochemistry, Cytokeratin,
Epithelial Cell.

ABSTRACT

Introduction: Histologically, each lobe of the thymus can be divided into a morphologically distinct cortex and medulla separated by a vascular cortico-medullary zone.
Material and Methods: Out of 15 cadaver's two males and one female cadaver showed persistent thymus. Initial sections were stained with Haematoxylin–Eosin methods. Additional sections were used for immunohistochemistry and stained for the expression of high molecular weight cytokeratin, CD3.
Result: Immunohistochemistry profile had shown intensely positive immunoreaction for CD3 to lymphocytes
Discussion: The involution process of thymus begins at puberty, but it is now known that the relative volume of the thymus decreases even in the mid-childhood. In our study adequate number of cortical T cells with epithelial cells were noted. Moderate affinity of high molecular weight cytokeratin to epithelial cell is seen which is suggestive of persistent of active thymus. The immunohistological findings of persistent active thymus are not only important for the better understanding of the a etiology of thymic disorders but also beneficial for better patient management and outcome.

Citation: Naina S Wakode, Santosh L Wakode, Babita Kujur, Swagatika Samal, Praveen Kumar and Manisha Gaikwad. 2018. "Immunohistochemistry of persistent active thymus in human cadavers", *International Journal of Current Research*, 10, (03), 66538-66541.

INTRODUCTION

The thymus is a specialized primary lymphoid organ of the immune system where T cells mature. The thymus consists of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum. Histologically, each lobe of the thymus can be divided into a morphologically distinct cortex and medulla separated by a vascular cortico-medullary zone. The epithelial cells form an open framework containing predominantly T lymphocytes, smaller populations of B lymphocytes and plasma cells and scattered populations of other cells such as neuroendocrine cells (Standring, 2008). Epithelial cells has a capacity to synthesize the thymic hormones thymulin, thymosin, thymopoetin and thymic humoral factor. Immunohistochemically, epithelial cells can be divided into four distinct subtypes: sub capsular cortical, inner cortical, medullary and Hassall's corpuscles. A decrease in the number of different antigenic epithelial populations occurs with age (Krishnamurthy *et al*., 2012). T cell plays a central role in immunity.

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The thymus is the primary organ responsible for genesis of immunocompetent T cells with a distinct reserve of antigen-recognition. With the ageing thymus undergoes involution, the cortex of specific lobules becomes thinner and parenchyma is gradually replaced with adipose tissue. This process termed as age-related thymic involution. The main components of the thymus include thymocytes of hematopoietic origin and thymic epithelial cells (TECS) of non-hematopoietic origin (Shanley *et al*., 2009). Age associated changes of the thymus are said to be reversible (Krishnamurthy, 2012). In the early stage, there is significant decrease in the number of T cells of the cortex, without significant changes in prominent epithelial cells. In progressive stage of involution there is a remarkable decrease in the number of epithelial and T cells of the cortex, and the parenchyma is gradually replaced by adipose tissue (Gill *et al*., 2003). The thymic epithelium starts to decrease and it is a main feature of age-related thymic involution (Taub, 2005). Involution of the thymus is never complete, because small islands of thymus tissue may be found even in individuals over 80 years old (Gill *et al*., 2003). Apart from the previously mentioned reports on the embryonic and fetal development of the cervical thymus in literature, the present study is presenting detailed histological and immunohistological analysis of the superficial mediastinal persistent thymus in the old age.

MATERIALS AND METHODS

The present study was carried out at All India institute of medical sciences Bhubaneswar. After institutional ethics committee approval, fifteen cadavers (14 male and 1female) aged between 55 years and 75years were dissected for the presence of persistent thymus. Out of 15 cadaver's two males and one female cadaver showed persistent thymus. The persistent thymus specimen was removed, fixed in 10% buffered formalin and then paraffin embedding done. Initial sections were stained with Haematoxylin–Eosin methods. Additional sections were used for immunohistochemistry and stained for the expression of high molecular weight cytokeratin, CD3. The working system was LSAB2 for all antibodies, and the final reaction product was visualized with 3, 3'-diaminobenzidine as chromogen in brown. Nuclei were stained with Lillie's modified haematoxylin. After dehydration and clarification, sections were mounted with permanent medium. Examined under DSS Magnus multi head microscope and images were captured.

RESULTS

Histological results

Case one (Female cadaveric specimens): Section showed large lobulated thymus gland located in superior mediastinum around the great vessels and consisted of numerous lobes and were covered by connective tissue capsule composed mainly of collagen and fine reticular fibers. Numerous incomplete connective tissue septa extended from the capsule and divided it into lobules, these septa contained the thymic blood vessels (Figure 1).The parenchyma of cortex was enriched with densely packed lymphocytes which were small in size with darkly stained central basophilic nuclei (Figure 1).

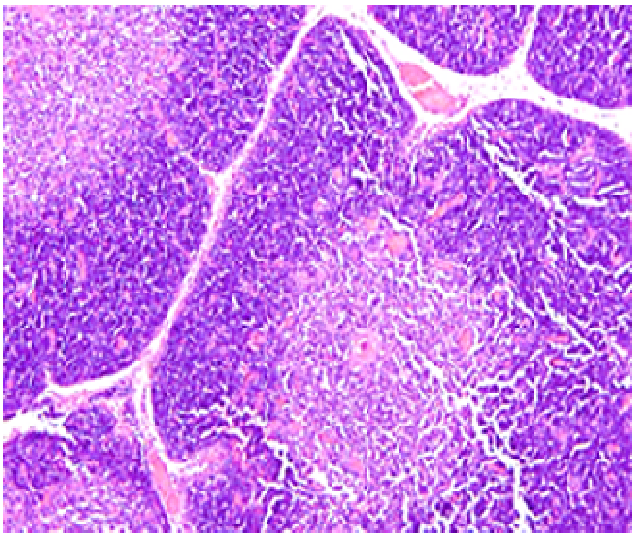

Figure 1. Darker staining cortex is due to numerous small lymphocytes with light stain medulla consisting of epithelial cell with pink colored stain Hassall's corpuscle (Haematoxylin & Eosin stain)

The medulla was typically consisted of lightly stained epithelial cells with by large size lymphocytes with centrally located nuclei and acidophilic cytoplasm (Figure 1).The Hassall's corpuscles seen were characterized by degenerated structure less, hyalinised centre and concentrically arranged peripheral epithelial-reticular cells.

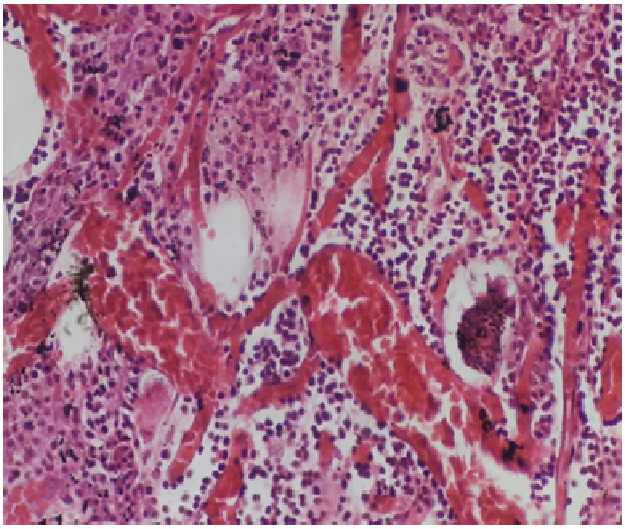

Figure 2. Perivascular epithelial cell with darkly stain lymphocytes and degenerated Hassall's corpuscles. (Haematoxylin & Eosin Stain)

Case two: Male cadaveric specimen showed thymic lobule and consisted of the framework of scattered epithelial reticular cells. The epithelial reticular cells were characterized by distinct cell boundaries and faint basophilic cytoplasm and nuclei. Few Small lymphocytes were found in medulla as compare to the cortex (Figure 2).

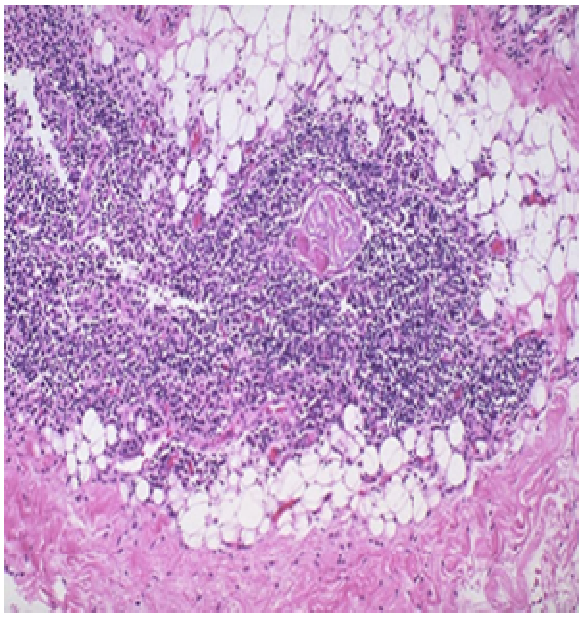

Figure 3. Darkly stain cortex contain lymphocytes, lighter stain medulla and periphery placed adipocytes (Haematoxylin & Eosin stain)

Case three: Male cadaveric specimen showed capsular septa dividing the thymic parenchyma into incomplete lobules with darkly stain cortex and lightly stain medulla. Lightly stain epithelial cell with dark stain lymphocytes were visible in the parenchyma. At the periphery of cortex adipocyte were visible. Hassal's corpuscles seen were characterized by degenerated structurless, hyalinised centre (Figure 3). Immunohisto chemistry profile of persistent thymus epithelial cell showed high affinity for moderate molecular weight cytokeratin (Figure 4). The immunoreaction for CD3 was positive but number of positive cells in both cortex and medulla was reduced (Figure 5).

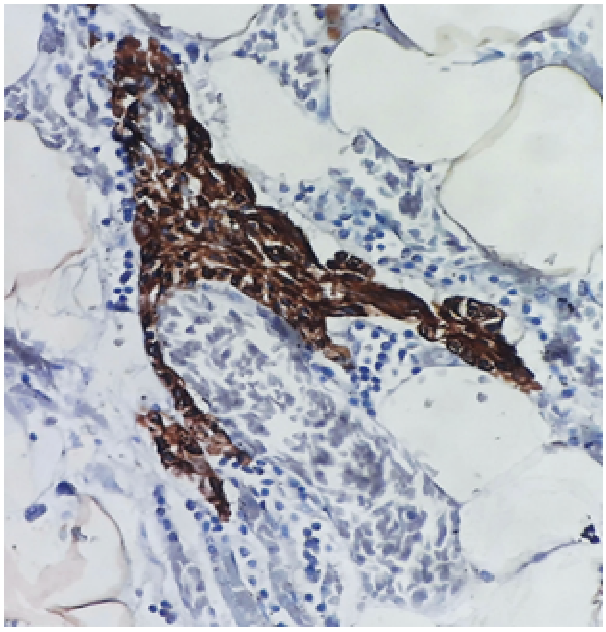

Figure 4. Expression of cytokeratin in epithelial cells of thymus

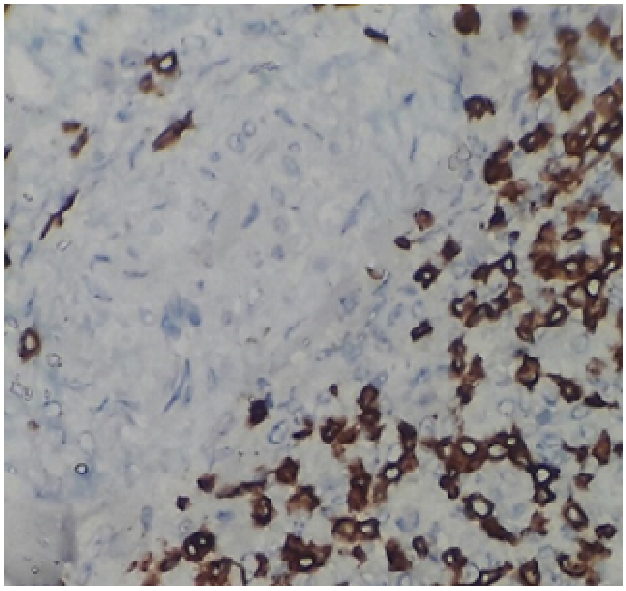

Figure 5. CD3 positive cells with sparse distribution of lymphocytes

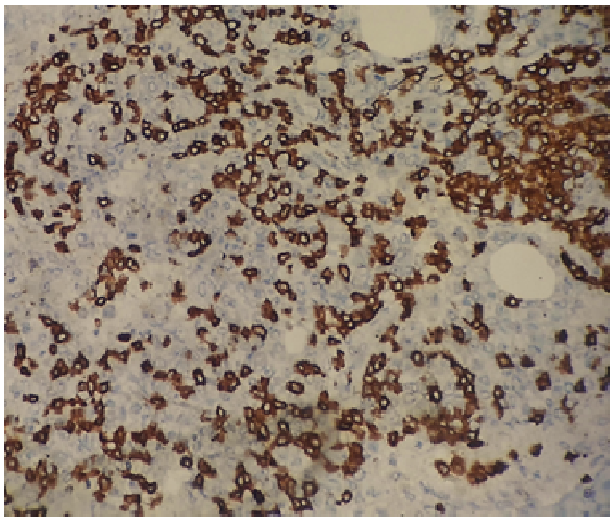

Figure 6. CD3 strongly positive lymphocytes in the cortex

While in another specimen, immunohistochemistry profile had shown intensely positive immunoreaction for CD3 on the lymphocytes and numbers of T lymphocytes were significant both in cortex and medulla (Figure 6).

DISCUSSION

The thymus is a soft, encapsulated, lymphoid organ; its two lobes are joined in the midline by means of connective tissue that merges with the capsule of each lobe. It is located in the superior mediastinum in front of the ascending aorta and below the level of left brachiocephalic vein (Standring, 2008) Thymus is derived from third pharyngeal pouch and fourth pouch (Sadler, 2002). The involution process of thymus begins at puberty, but it is now known that the relative volume of the thymus decreases even in the mid-childhood. Raica *et al* had noted large masses of adipose tissue with scattered islands of thymict issue consisting of epithelial cells and few Lymphocytes in adult (Raica *et al*., 1999-2004). In present study, three cases of thymus showed the adequate number of epithelial cells and lymphocytes. Lymphocytes were darkly stained in the cortex; similar findings were noted by Nayak *et al*. (2015). Early involution is represented by the decrease of cortical T cells without significant changes in the medulla. In late involution there is a noticeable retrogression of cortical T cell as well as epithelial cells and they are replaced with adipose cells. The remnant of the thymic parenchyma consist cords of epithelial cells, occasional lymphocytes with Hassall's corpuscles (Raica *et al*., 1999-2004). However in our study adequate number of cortical T cells with epithelial cells were noted as shown in Figure 4, moderate affinity of high molecular weight cytokeratin to epithelial cell is seen which is suggestive of persistent of active thymus.

Late involution and final derangement of the thymus may be observed in old individuals (Kraft *et al*., 1988). Kraft *et al* revealed the presence of thymic tissue in all autopsy cases with age ranged between 63 to 91 years, similar findings were seen in our study (Figure 1,2,3). Rafique *et al.* (2009) and Raut *et al.* (2013) reported that, in old age, there is definite increase in the thickness of capsule and interlobular connective tissue; also there is decrease in number of Hassall's corpuscles and there diameter is increased. Our findings were similar to the above studies wherein we found the ill-defined lobules, thickened capsule and reduced number of Hassall's corpuscles. (10, 11)Blackburn *et al* had described that the expansion of epithelial cells requires the prolonged presence of the mesenchyme; they also demonstrated the perseverance of thymic epithelial stem cells (TESC).TESC persist in the adult thymus and it is thought that they are necessary for the conservation and regeneration of the organ. Thus persistent active thymus seen in present study may suggest the role of epithelial stem cell of thymus in old age (Blackburn, 2004).Some researcher described the role of thymus in immune rebuilding in aging, after bone marrow transplantation and HIV-1 infection. Haynes *et al* believed that thymus when present optimizes the peripheral T cell reserve in older age group by increasing the peripheral T cells and thus, maintains the immune system. Thus persistent thymus can function well even in old age to optimize the functioning of immune system in humans (Haynes *et al*., 2003). While some scientists believe that thymus gland when present might give incorrect instructions to developing immune cells, ultimately resulting in autoimmunity and the production of acetylcholine receptor antibodies (Skandalakis *et al*., 2004).

Thymus appears in a variety of shape and size even in same individual due to acute shrinkage of thymus during bodily stress. During recovery period, it grows back to its original or even larger size. This phenomenon is known as thymic rebound hyperplasia. These anatomic variation and dynamic changes appears as main source of confusion to radiologist and often it is misinterpreted with pathological condition that leads to prolonged chemotherapy or radiotherapy or unnecessary biopsy (Nasseri *et al*., 2010) Thymus in adult life may be considered normal or abnormal, thus knowledge regarding morphology and immunohistochemistry of the persistent thymus in the old age is important for pathologists and surgeons. The immunohistological findings of persistent active thymus are not only important for the better understanding of the etiology of thymic disorders but also beneficial for better patient management and clinical outcome.

Conclusion

Persistent active thymus in adult life may be considered normal or abnormal. So knowledge regarding morphological and immunohistochemical investigation of the persistent thymus in the old age is important to pathologists, radiologist and it also helps the surgeons in properly planning the surgery. Thus the immunohistological findings of persistent active thymus are very useful in understanding and treating thymic disorders.

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