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HISTOGENESIS OF MESODERMAL COMPONENTS OF HUMAN FETAL THYMUS

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ABSTRACT

The present study is conducted to observe the early cellular events and histological maturity of mesodermal component of thymus. After ethical approval from the institutional ethics committee 53 human fetuses were obtained from department of Obstetrics and Gynecology with permission of head of department and written informed consent from respective parents. The gestational age was determined by menstrual history and Crown Rump length (CRL). The specimen were dissected through parasternal incision and processed in paraffin. The sections were taken by rotary microtome. The slides were stained by Haematoxylin & eosin, Massons trichrome and Periodic acid-Schiff (PAS) stains. Differentiation of thymus started at 9th week and all significant structural changes such as lobulation, appearance of blood vessels and corticomedullary differentiation were occured within 17th week and thereafter thymus showed microscopic growth and maturity. The findings of the present study are comparable with the findings of standard text books and previous workers.

KEYWORDS: Thymus, Thymocytes, Lobulation, Corticomedullary differentiation

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INTRODUCTION

It seems hard now to believe that much of the 20th century's physicians and scientists believed that the thymus, at best was an endocrine organ and at worst, was an evolutionary remnant with no function at all. Hence it was generally assumed as not essential for life.[1] During the ensuing decades, the myriad complexities of the thymus and the thymocytes development have been intensively investigated and it is now accepted as a key stone of the defense mechanism of the body.[2] However details of microscopic development of the thymus are not studied to great extent in the human fetuses and most of our knowledge regarding early fetal histology of this organ is based on the studies in different animals. The findings of different workers are also not consistent and are contrasting in the most cases. Secondly, the histological details of the different cellular components of the gland in the early developmental stages can be observed to a better advantage than in the adults, due to less crowding of the lymphocytes and no infiltration of fat. The present study, therefore, has been undertaken to observe the early cellular events in the different stages of human fetal period, and an attempt has been made to note the degree of histological and functional maturity attained by mesoderm of this important gland of immune system.

MATERIALS AND METHODS

The study was conducted in department of anatomy, Government medical college, Aurangabad during 2006 to 2009. After ethical approval from institutional ethics committee, 53 human fetuses (24 male and 29 female) of different age groups ranging from 9th to 40th weeks of gestation were procured from the department of Obstetrics and Gynecology of Government medical college and hospital, Aurangabad for research work with due permission from the Professor and Head of the department and consent from respective parents. Only fetuses free from detectable abnormality belonging to the mother with normal obstetrical history were taken into the study. These specimens included the spontaneous abortuses, still born and terminated fetuses under the Medical Termination of Pregnancy Act of India1971. Twins and fetuses with gross anomalies were omitted. Fetuses were obtained within 4-5 hrs of birth to avoid post-mortem changes and immediately fixed in 10% formalin.

Gestational age of fetus was calculated from first day of last menstrual period (LMP). Fertilization age was obtained by subtracting two weeks from gestational age. Fertilization age was also determined from Crown Rump Length of fetus and using table in the Moore and Persaud. [3] The sternoclavicular joints were disarticulated and costal cartilages were cut. Thus the entire thoracic cavity was open and lower part of neck was also dissected for complete exposure of thymus in its natural location. The tissue sample was fixed in Bouins's fluid, processed to prepare paraffin embedded blocks and 4-5 micron thick sections were cut. The slides were stained with Haematoxylin & Eosin (H &E), Masson's Trichrome (MT) and Periodic acid-Schiff (PAS) stains and were studied under light microscope.

RESULTS

Group-I (9-12 weeks)

At 9th week, an incomplete fibrous capsule is observed around the parenchyma which consists of undifferentiated mesenchymal cells and few lymphocytes. Mesenchymal cells are spindle shaped with basophilic oval nuclei and delicate branching cytoplasmic extensions. Lymphocytes have round purple nuclei with basophilic cytoplasm. At 10th week, incomplete connective tissue septa (trabeculae) extend from capsule into the parenchyma, giving it lobulated appearance. At 12th week, distinct lobules are seen, which are small and roughly polyhedral in shape. At the periphery of lobule, lymphocytes are numerous and densely packed forming darkly stained cortex. At the centre, lymphocytes are fewer and form lightly stained medulla. Mitotic figures are visible in cortex. Surrounding connective tissue contain small blood vessels and small lymphocytes in groups. (Figure-1)

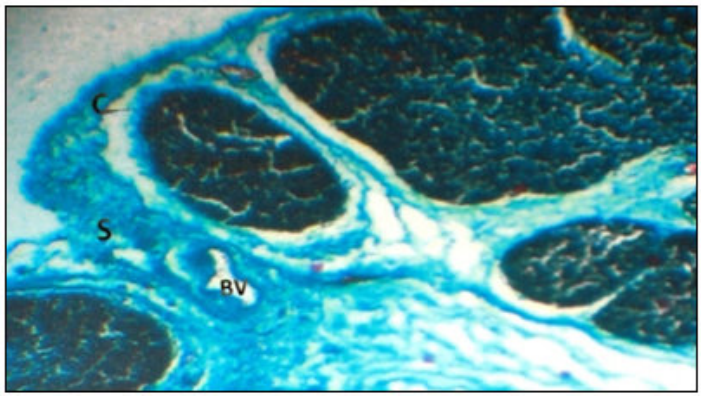

Figure 1
Microphotograph of thymus (12 weeks) stained with Masson's trichrome showing capsule (C), interlobular septa (S) and blood vessel (BV).

Group-II (13-15 weeks)
The lobulation is still continuing at this stage with developing connective tissue trabeculae in between the lobules. Although development of cortex and medulla starts at 12th week, they become more distinct at 14th week with darkly stained, more cellular, outer cortex and lightly stained, less cellular, inner medulla. Larger blood vessels are seen in the capsule and septa. Some blood vessels are also visible at medullary region. The capsule and interlobular septa contain occasional macrophages and granulocytes especially eosinophils along with lymphocytes. (Figure-2).

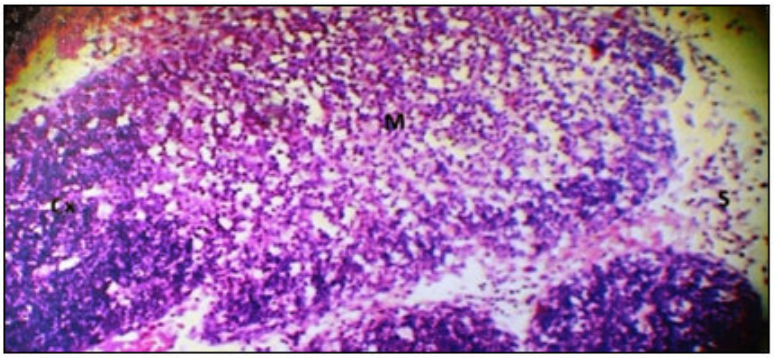

Figure 2
Microphotograph of thymus (14 weeks) stained with Haematoxlin and eosin showing cortex (Cx) and medulla (M).

Group III (16-18 weeks)
The gland gradually increases in size and shows secondary and tertiary lobulations with deeper penetration of connective tissue septa. The corticomedullary differentiation is complete at this stage. Medulla is continuous from one lobule to another. The surrounding connective tissue capsule, trabeculae and parenchyma contain numerous blood vessels. These vessels increase in size and number with increase in gestational age. Macrophages and eosinophils are visible in the medulla especially around blood vessels. (Figure-3)

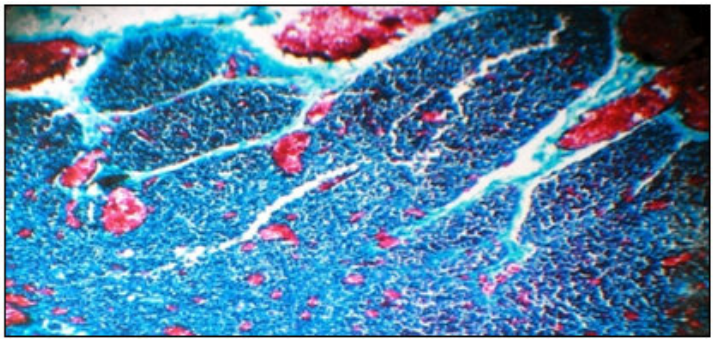

Figure 3
Microphotograph of thymus (18 weeks) stained with Masson's trichrome. Note red stained red blood corpuscles in blood vessels.

Group IV (19-24 weeks)
The thymus appears to be fully differentiated at this stage with mature histological picture. The lobules increase in size. The medulla contains small lymphocytes while large lymphocytes are present in cortex. Blood vessels and connective tissue of capsule and trabeculae become more extensive. All grades of blood vessels are present in the medulla. (Figure-4).

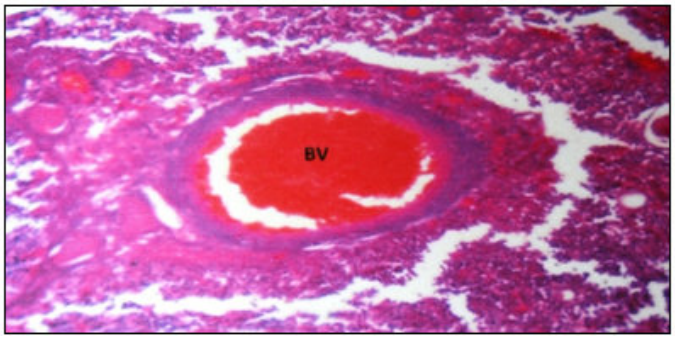

Figure 4
Microphotograph of thymus (24 weeks) stained with Haematoxlin and eosin showing blood vessel (BV).

Group V (25- 40 weeks)
At this stage gland shows microscopic growth and maturity with distinct blood vessels and trabecular framework . Connective tissue septa surround the blood vessels forming perivascular space which contain macrophages, granulocytes and scattered nuclei of connective tissue cells. The cortex consists of densely packed large lymphocytes and medulla contains fewer small lymphocytes. (Figure 5).

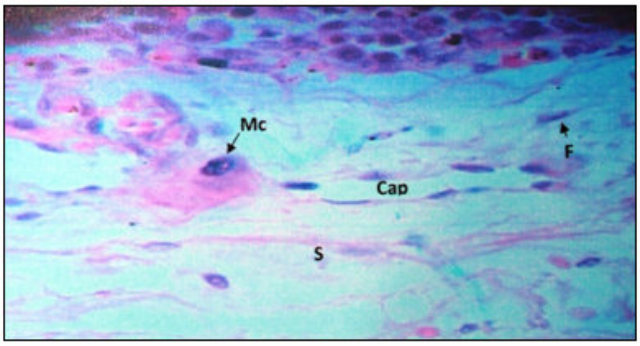

Figure 5
Microphotograph of thymus (40 weeks) stained with Haematoxlin and eosin showing connective tissue septa (S) fibroblast (F) capillary (C) and macrophage (Mc).

DISCUSSIONS

In the present study, the development of human fetal thymus in antenatal period is studied with following histological parameters.

1) Lymphocytes

The lymphocytes originate in the embryo from mesenchymal cells in the yolk sac initially, and later in the liver and spleen. These primitive stem cells subsequently reside in the bone marrow, which becomes the only site of stem cell proliferation after birth. When these stem cells divide, they give rise to further stem cells and to lymphoblasts which continue to divide, eventually becoming small lymphocytes. Some of these cells pass in the blood circulation to thymus at 8th week, where they migrate into its cortex and divide repeatedly.[4] In the present study, the lymphocytes are observed at 9th week which have round purple nuclei with basophilic cytoplasm. They are randomly scattered in the parenchyma. This study confirms the findings of Haar[5], Hamilton and Mossman [6] , Von Gaudecker[7] and Ritter and Lampart[8] who reported the lymphocytes at 9th week. Standring S et al.[4] and Romanes GJ [9] mentioned the presence of lymphocytes at 8th week.

2) Blood vessels

Vascularization of the thymus begins with its descent in the thorax. Major blood vessels enter the gland at corticomedullary junction and pass within each lobe giving off small capillaries to cortex and larger vessels to medulla. By the 12th week, blood vessels reach the medulla.[4] Vascularization of the thymus started at 9th week with small blood vessels observed in connective tissue. Medullary vessels are observed at 12th week. Later on the vasculature become more extensive and larger with increase in age. These findings are in accordance with Haar[5], Hamilton and Mossman[6] and Ajita et al.[10], who observed the blood vessels associated with connective tissue fibres and mesenchymal cells at 9th week.

3) Red blood corpuscles

Haemopoiesis commences at 3rd week of gestation in the mesenchymal blood islands of the yolk sac, where only giant nucleated erythroid cells (megaloblasts) are formed. These are soon replaced by nucleated erythroblasts and these in turn are replaced by moderately anucleated, biconcave fetal erythrocytes by about 4th month of gestation.[4] Nucleated red blood corpuscles are observed at 10th week in the blood vessels of connective tissue. The study confirms the finding of Standring S et al. who mentioned the developing erythroblasts at 10th week.[4] Aita et al found nucleated red blood corpuscles at 9th week.[10]

4) Lobulation

The epithelial cells are enveloped by mesenchyme. They grow as thumblike protrusions into a mass of mesenchyme which later forms a thin capsule around the organ. In the region between epithelial

protrusions, the mesenchyme remains and forms thin incomplete septa. Hence the gland appears lobulated.[2] The elongated series of lobules are connected with a central parenchymatous cord along which they are arranged in irregular necklace fashion. The lobules are subdivided into secondary lobules by delicate areolar tissue.[9] In the present study lobulation is started at 10th weeks. Ghali et al. observed the lobulation at 10th week.[11] Haar reported the lobulation at 12th week. [5] Ajita et al. found that formation of lobules started at 9th week and distinct lobules were seen at 12th week.[10]

5) Cortex and medulla formation

The cells of lymphatic series are more concentrated towards those borders of each lobule that abut on the capsule or interlobular septa. Hence at the periphery of the lobule, lymphocytes are numerous and densely packed forming darkly stained cortex. At the centre, lymphocytes are fewer and form lightly stained medulla.[2] In the present study at 12th week, cells of lymphatic series are more concentrated towards those borders of each lobule that abut on the capsule or interlobular septa, whereas at the centre, they are fewer and dispersed. This arrangement becomes more distinct at 14th week.

There are different opinions about the time of differentiation of the cortex and medulla of the thymus.

- In the embryos of 40 mm crown-rump length by Hamilton and Mossman [6]
- At 11 weeks by Ghali et al.[11]
-At 12 week by Arey [12], Hayward[13] and Muller-Hermelink et al[14]
- At 14th week by Haar[4] and Lobach and Haynes [15]
- Between 12th and 14th weeks by Von Gaudecker and Muller-Hermelink[7]

Ajita et al.reported that differentiation of cortex and medulla started at 9th week and it become more distinct at 12th to 14th week.[10]

6) Macrophage lineage cells

These are found as monocytes at corticomedullary junction, as mature macrophages in the cortex and as interdigitating cells in the medulla.[4] Phogocytosis is one of the mechanism used for trapping the alien elements and subjecting them to immune reactions.[16] In the present study, the monocytes and macrophages are observed at 14th week. Haynes reported that the macrophages were present at 10th week.[17] Ajita et al observed these cells at 12th week.[10] Standring S et al mentioned the presence of macrophages at 14th week.[4] Milicevic NM et al in his electron microscopic study on thymus, demonstrated polysaccharide content of macrophages by thiocarbohydrazide-silver proteinate method.[18] In the present study, some macrophages appeared Periodic acid-Schiff (PAS) positive. Macrophages appear PAS positive when they engulf carbohydrate contents.

7) Completion of differentiation

The differentiation implies to increase in the structural complexities. Tissue differentiation culminates in the assumption of coordinated functional activities.[12] The total process by which cells differentiate into distinctive kinds and assume specific tissue characters is known as histogenesis.[12]

In the present study, all significant structural changes occur in thymus within 17th week of gestation. The organization of the capsule and trabeculae from the surrounding mesenchyme formed the lobules. Infiltration of the lymphocytes leads to formation of cortex and medulla. Vessels were present in the capsule and trabeculae. They reached to the medulla allowing entry of macrophage lineage cells. Granulopoiesis occured in the perivascular space. Standring S et al.[4] and Ajita et al[10] mentioned that thymus appeared fully differentiated at 17th week. Sawant SP[19] reported that differentiation was completed at 18th week.

CONCLUSION

The present study concludes that during development of human fetal thymus, invasion of blood vessels and lymphocytic and other haemopoietic cells is followed by lobular organization. The differentiation of human thymus starts at 9th week and all significant structural changes of thymus such as

lobulation and corticomedullary differentiation occur within 17th week of gestation and thereafter thymus shows microscopic growth and maturity in the form of increase in size of lobules and blood vessels. The findings of the present study are comparable with the findings of standard text books and previous workers.

Conflict of Interest

Conflict of interest declared none.

REFERENCES

1. Savino w,Lemos G, Histophysiology of thymic epithelial reticular cells. Arch. histol. Jap, 45 (2): 139-144, (1982).
2. Ham AW. Histology, 7th edn, JB Lippincott company publisher Philadelphia and Toronto: 326-35, (1974).
3. Moore KL, Persaud TVN, The developing human, 8th edn, Saunders Elsevier publisher Philadelphia: 96-167, (2008).
4. Standring S, Borely NR, Collins P, Crossman AR, Gatzzoulis MA, Healy JC, Johnson D, Mahadevan V, Newell RLM, Wigley CB, Gray's Anatomy, 40th edn. Chrchill Livingstone Elsevier publisher: 945-49, (2008).
5. Haar JL, Light and electron microscopy of the human fetal thymus, Anat. Rec, 179: 463-467, (1974).
6. Hamilton WJ and Mossman HW, Hamilton, Boyd and Mossman's Human embryology, 4th edn, The Macmillan Press Ltd publisher London: 291-376, (1976).
7. Von Gaudecker B, Muller-Hermelink HK, Ontogeny and organization of the stationary non-lymphoid cells in the human thymus, Cell Tissue Res, 207(2):287-306, (1980).
8. Ritter MA and Lampart IA, Oxford text book of pathology, Oxford university press publisher Oxford: 1807-1821, (1992).
9. Romanes GJ, Cunningham's text book of anatomy, 11th edn, Oxford University press publisher London: 568-572, (1972).
10. Ajita RK, Singh TN, Singh YI and Singh LC, An insight into the structure of the thymus in human foetus - a histological approach, Journal of Anatomical society of India 55(1): 45-49, (2006).
11. Ghali WM, Abdel-Rahman S, Nagib M and Mahran ZY, Intrinsic innervations and vasculature of pre-and post natal human thymus, Acta Anat, 108:115-23, (1980).
12. Arey LB, Developmental anatomy, 6th edn. WB Saunders company publisher Philadelphia and London: 234-236, (1956).
13. Hayward AR, Myoid cells in the human fetal thymus, J. Path, 106: 45-48, (1972).
14. Muller-Hermelink HK, Marx A and Kirchner, Anderson's Pathology, 10th edn. Mosby st. Luis publisher: 1218-1243, (1996).
15. Lobach DF and Haynes BF, Ontogeny of the human thymus during fetal development, Journal of clinical immunol, (7): 81-97, (1987).
16. B. Deivasigamani, Role of the immune organ (thymus) in Mystus Gulio, International journal of pharma and biosciences, 2 (1): (2011)
17. Haynes BF, Human thymic epithelium and T cell development, Current issues and future directions, 16:143-157, (1990).
18. Milicevic NM, Milicevic Z, Colic M and Mujovic S, Ultrastructural study of macrophages in the rat thymus with special reference to the cortico-medullary zone, J Anat, 150: 89-98, (1987).
19. Sawant SP, Development of thymus, Journal Of Anatomical society of India, 52 (1): (2003).