An insight into the structure of the thymus in human foetus - a histological approach.

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Abstract :

In the present study, seventy foetuses ranging from 9th to 38th gestational week were studied to find out the histological changes that occurred at different stages of the foetal growth period. After proper fixation, alternate sections of the thymus tissues stained with Haematoxylin and Eosin, Masson's Trichrome and Leishman's stains were examined histologically under light microscope. Lobulation and differentiation of cortex and medulla of the thymus were observed at 9th week. Lobulation was completed by 12th week. The differentiation of cortex and medulla was completed between 12th and 14th weeks. The Hassall's Corpuscles were observed at 15th week and its number and size increased during 17th -24th weeks. Macrophage cells could be observed at 12th week. The study revealed that significant structural changes of human thymus such as lobulation, differentiation of cortex and medulla, appearance of Hassall's Corpuscles occurred mostly within 17th gestational week.

Keywords: Hassall's Corpuscles, lobulation, lymphocytes and gestational ages.

Introduction:

The thymus is a primary central lymphoid organ and a key regulator of the immune system, and is responsible for cellular immunity of the body. It is a bilobed structure divided into lobules by the connective tissue septae. Each lobule is consisted of a cortex and a medulla. At 8th gestational week, the two advancing lobes were united at midline; and the basophilic stem cell and thymocytes came to lie between the epitheliocytes that were visibly differentiated. Thymus was covered with a thin connective tissue capsule that was composed of collageneous connective tissue fibers (Williams et al.1995). According to Hamilton and Mossman (1976), the epithelial cells of the developing thymus became more loosely arranged to form a reticulum in which small lymphocytes soon appeared at about 9th week. The vascular mesodermal tissue invaded the gland in such a way as to produce its lobulation.

The lobulation of thymus gland were reported to occur at 10th week by Ghali et al *(1980)* and 12th week by *Harr (1974).* Different workers reported different timing for differentiation of cortex and medulla viz at 11th week *(*Ghali et al. 1980*),* about 40 mm crown rump length (CRL) *(*Hamilton and Mossman, 1976*),* 12th week (Hayward, 1972 and Muller-Hermelink et al. 1996) and 14th week *(*Harr*, 1974 and,* Lobach and Haynes*, 1987).* Various other workers reported appearance of Hassall's Corpuscles at different gestational weeks ranging from 8th to 16th week.

Thus the findings of these different workers are found to be not consistent and are contrasting in most of the cases. The present study aims to verify some of these important findings and also to study the histological changes of the thymus at different gestational weeks in human foetuses.

Materials and Methods:

Seventy human foetuses (40 males and 30 females) of different age groups ranging from 9th to 38th gestational week were procured from the Department of Obstetrics and Gynaecology of Regional Institute of Medical Sciences Hospital, Imphal for the research work with due permission of the Medical Superintendent of the mentioned hospital and respective parents. These foetuses were the products of terminated pregnancies under the Medical Termination of Pregnancy Act of India, 1971 and stillbirths. They looked fresh without any gross abnormality. Only the foetuses free from detectable abnormality belonging to the mother with normal obstetrical history were taken into consideration for the study.

The foetuses so obtained were examined for their respective crown-rump lengths, gestational ages and body weights. Thereafter, all these 70 (seventy) foetuses were fixed in 10% formalin for 10 days. Then the foetuses were subjected to dissection. The sterno-clavicular joints were disarticulated and costal cartilages were cut. Thus the entire thoracic cavity was opened and lower part of the neck was also dissected for complete exposure of the thymus gland in its natural location for proper recording.

The specimens of the human foetuses utilized in the present study were categorized into the following five groups: -

Group	*Age (weeks)*	*No. of foetuses*
Group-I:	*9th to 11th week.*	*13*
Group-II:	*12th to 14th week.*	*16*
Group-III:	*15th to 17th week.*	*20*
Group-IV:	*18th to 24th week.*	*12*
Group-V:	*25th to 38th week.*	*9*

Evaluated specimens were preserved in freshly prepared solution of 10% Formal Saline fixatives for 7 days. After proper fixation, the tissue was subjected to the standard paraffin block making procedure. Thereafter 10 micrometers thick serial sections were prepared and alternate sections were stained with Haematoxylin and Eosin, Masson's Trichrome and Leishman's stains. The stained sections were examined under light trinocular research microscope.

Observations:

Observations made on the morphological and structural changes were as follows: -

Morphological observations:

Appearance:

The foetal thymuses were soft, friable, lobulated and light pink in colour. The two lobes were seen interconnected and fused from 9th week onward.

Location:

The gland was seen to be located in superior mediastinum and upper part of the anterior inferior mediastinum extending from the root of the neck to the level of the third costal cartilages. However, in one normal case of 24th week foetus, it was found to extend from the lower border (base) of the thyroid gland to the anterior mediastinum.

Histological observations:

On light microscopy, the observations at different gestational weeks were as follows: -

Group-I (9-11 weeks):

The gland was seen to be composed of lymphocytes with a delicate capsule. The lobulation and development of cortex and medulla had started at this stage. However, the medulla was of very small size (Fig. 1). Trabeculae associated with blood vessels had also been observed. Red Blood Corpuscles and spindle shaped epithelial cells started appearing at 9th week. Some of these Red Blood Corpuscles were nucleated (Fig. 2). No Hassall's Corpuscles was observed.

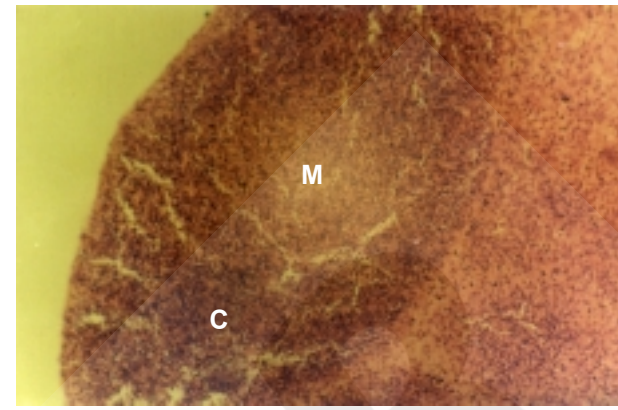

Fig. 1: Human Foetal thymus (9th g.w.) stained with Haematoxylin & Eosin and examined under light microscope showing lobulation and development of Cortex (C) and Medulla (M) (x 10).

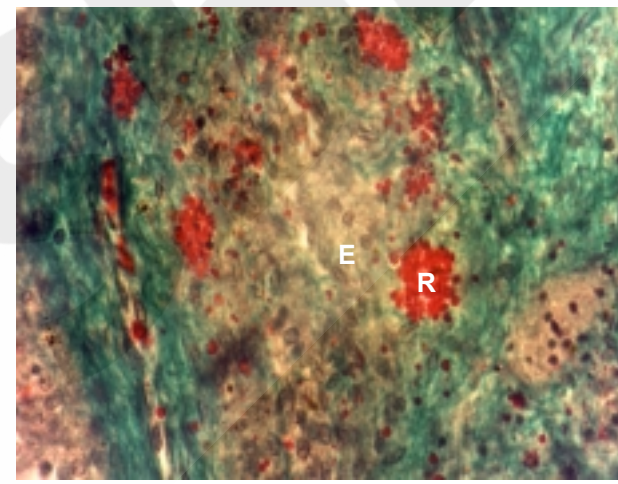

Fig. 2: Human Foetal thymus (9th g.w.) stained with Masson's Trichrome and examined under light microscope showing RBCs (R) Epithelial Cells (E) (x 40).

Group-II (12-14 weeks):

The lobulation of the thymus gland was still continuing at this stage with the developing connective tissue trabeculae between the lobules. The lobules were found to possess blood vessels. They had a recognizable cortex and medulla (Fig. 3). Till 14th week, no Hassall's Corpuscles could be seen. Monocytes and macrophages were also seen at this stage (Fig. 4).

Group-III (15-17 weeks):

The number of lobules had increased further. By 15th week, in some of the sections, the Hassall's Corpuscles were clearly visible in the medulla (Fig. 5). The Hassall's Corpuscles were present in all sections from 16th week and it increased in number and size with the increase of gestational age.

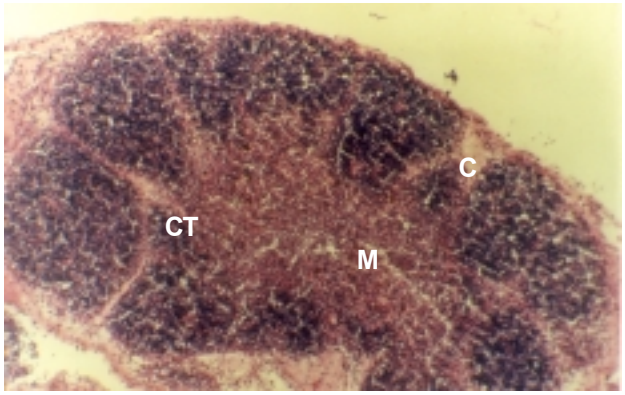

Fig. 3: Human Foetal thymus (12th g.w.) stained with Haematoxylin & Eosin and examined under light microscope showing development of well differentiated connective tissue septa (CT), Cortex(C) and Medulla (M) (x 10).

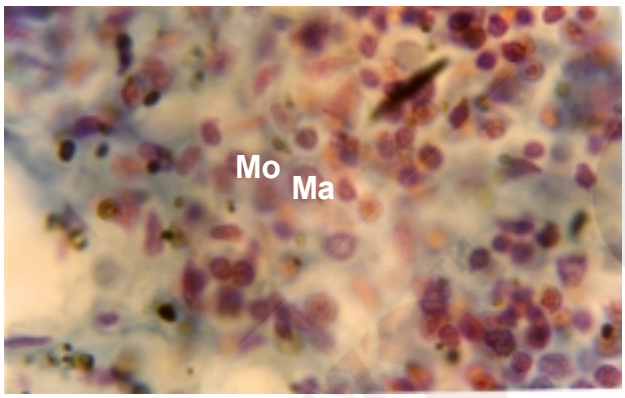

Fig. 4: Human Foetal thymus (12th g.w.) stained with Leishman's and examined under light microscope showing Monocytes (Mo) and Macrophages (Ma) (x 100).

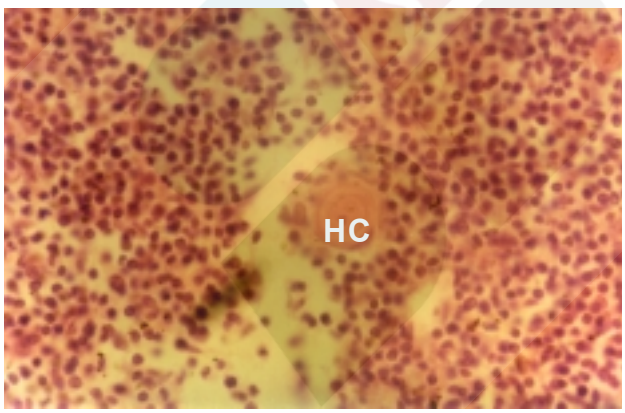

Fig. 5: Human Foetal thymus (15th g.w.) stained with Haematoxylin & Eosin and examined under light microscope showing appearance of Hassall's Corpuscles (HC) (x 40).

Group-IV (18-24 weeks):

Blood vessels and connective tissues of its capsule and trabeculae became more extensive at this stage. Number and size of Hassall's Corpuscles as well as number of lobules also increased (**Fig. 6**).

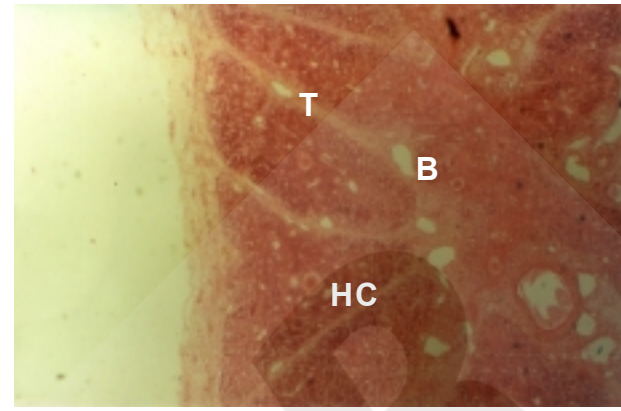

Fig. 6: Human Foetal thymus (24th g.w.) stained with Haematoxylin & Eosin and examined under light microscope showing Trabecular Framework (T) and increase Blood Vessels (B) and well developed Hassall's Corpuscles (HC) (x 5).

Group-V (25-38 weeks):

The trabecular framework and blood vessels had become distinct. The gland during this stage had an internal architecture similar to that seen in the adults viz. a cortex composed of a dense population of lymphocytes and a medulla commonly possessing Hassall's Corpuscles in a graded series of increasing maturity and having fewer lymphocyte cells than that in the cortex.

Discusson:

Different findings have since been reported by various workers as regards the structures of the thymus in human foetuses. While reviewing the findings of various workers in the field, the present study focused upon the following sub-headings:

Location:

Hollinshead (1971) reported that the upper pole of one or both lobes of the thymus extended upward in the neck to or beyond the level of the thyroid gland, such an extension presumably represented failure of the thymus to complete its migration into the thorax. Williams et al. (1995) also reported that sometimes the upper pole of the thymus reached the inferior pole of the thyroid gland. Similarly in the present study, in one of the cases of 24th week (normal foetus), the upper pole of the thymus reached the lower pole of the thyroid gland. Otherwise, in the remaining cases the positions of the thymus were located in the same positions as described by the other authors.

Lymphocytes:

There were different observations regarding the time at which the lymphocytes were present in the thymus such as from 8th week *(*Williams et al.1995*)*, at 9th week *(*Harr, 1974; Hamilton and Mossman*,* 1976;

Von Gaudecker, 1991 and, Ritter and Lampert, 1992). The present study observed the presence of the lymphocytes from 9th week onwards covered (Fig.1). Since the present study examined foetuses from 9th week onwards, it could not be ascertained whether lymphocytic infiltration occurs from 8th week onwards.

Blood Vessels:

Ghali et al (1980) reported that thymus was vascular at 11th week of gestation. Harr (1974) and Hamilton and Mossman *(1976)* reported that extra-thymic blood vessels associated with connective tissue fibers and mesenchymal cells surrounding the thymus were present at 9th week. Williams et al (1995) mentioned of the developing erythroblastic cell by 10th week old thymic tissue. In the present study the mesenchymal tissue with blood vessels invaded thymus from 9th week and nucleated Red Blood Corpuscles were also observed (Fig. 2).

Lobulation:

Ghali et al (1980) and Harr (1974) reported lobulation of thymus gland at 10th and 12th week respectively. Whereas, in the present study formation of lobules had started at 9th week and distinct formation of lobules were observed at 12th week (Fig. 3).

Cortex & Medulla:

There were different opinions about the time of differentiation of the cortex and medulla of the thymus viz. at 11th week Ghali et al (1980), in embryos of about 40 mm crown-rump length Hamilton and Mossman, (1976), by about 12th week Hayward, (1972) and Muller-Hermelink et al (1996), 14th week Harr, (1974) and; Lobach and Haynes, (1987), between 12th and 14th week Von Gaudecker and Muller-Hermelink, (1980). Whereas the present study indicated that the differentiation of the cortex and the medulla had started from 9th week (Fig. 1) and became well distinguished from 12th to 14th week stage (Fig 3).

Epithelium:

Williams et al *(1995),* Hamilton and Mossman *(1976)* described the presence of epithelial cells from 8th week. Hayward *(1972)* reported that the epithelial component of the thymus was recognizable at 10th week. Von Gaudecker and Muller-Hermelink (1980) reported that at 8th week, the primordium of the thymus contained almost exclusively undifferentiated epithelial cells. At 10th week, the epithelial cells in the central part were spindle-shaped. As the present study examined from 9th week onward only, the presence of epithelial cells were seen from this week onwards (Fig. 2). Since foetus prior to 9th week were not examined in the present study, it could not be ascertained whether epithelial cells were present at the earlier stages.

Macrophages:

Haynes (1990) reported that macrophage cells were present in the human foetal thymus at 10th week. But, in the present study macrophage cells could be observed from 12th week (Fig. 4).

Hassall's Corpuscles:

There were different reports on the time of appearance of Hassall's Corpuscles as follows: as early as 8th week Fawcett, (1994), from 9th week Gilhus et al. (1985), at 10th week Williams et al, (1995), at 11th week Ghali et al. (1980), between 15th and 16th week Lobach and Haynes, (1987). It was reported that development of the first Hassall's Corpuscles occurred during the second part of the third intrauterine lunar month in human foetuses but the greatest development of the Hassall's Corpuscles was observed between 6th and 10th lunar months in humans Bodey and Kaiser, (1997). In the present study, the presence of Hassall's Corpuscles was observed only from 15th week of gestation (Fig. 5).

Liberti et al (1994) reported that the mean areas of the Hassall's Corpuscles increased with the foetal age, with the greatest difference observed between 16th–19th week and 20th–23rd week foetal age groups. The present study revealed that Hassall's Corpuscles increased in number and size during 17th to 24th week (Fig. 6).

CONCLUSION:

The finding of this study is in conformity with some of the studies and varies with some of the studies. However, the present study revealed that lobulation was completed by 12th week and, differentiation of cortex and medulla possessing blood vessels was completed between 12th and 14th week. The presence of Hassall's Corpuscles was observed in 15th week, which increased in number and size during 17th-24th week. Macrophage cells could be observed at 12th week. The study revealed that significant structural changes of human thymus such as lobulation, differentiation of cortex and medulla, appearance of Hassall's Corpuscles occurred mostly within 17th gestational week, and the invasion of the blood vessels inclusive of lymphocytic and other haemopoetic cells was followed by the lobular organization and appearance of the Hassall's Corpuscles.

References:

1. Cormack DH: Ham's Histology In: Lymphatic tissue and the immune system. 9th Edn, JB Lippincott Company, Philladelphia. pp.234-61(1987).
2. Fawcett DW: A Text Book of Histology In: Thymus. 12th Edn, Chapman and Hall: New York. pp.432-444 (1994).
3. Ghali WM, Abdel-Rahman S, Nagib M and Mahran ZY (1980): Intrinsic innervation and vasculature of pre- and post-natal human thymus. Acta Anat; 108:115-123.
4. Gilhus NE, Matre R and Tonder O (1985): Hassall's Corpuscles in the thymus of foetuses, infants and children: immunological and histochemical aspects. Thymus; 7(2): 123-135.
5. Haar JL (1974): Light and electron microscopy of the human foetal thymus. Anat. Rec; 179:463-467.
6. Hamilton WJ and Mossman HW: Hamilton, Boyd and Mossman's Human Embryology In: Alimentary and respiratory system, pleural and peritoneal cavity. 4th Edn; The Macmillan Press Ltd, London. pp.291-376 (1976).
7. Haynes BF (1990): Human thymic epithelium and T cell development: current issues and future directions. Thymus; 16: 143-157.
8. Hayward A.R. (1972): Myoid cells in the human foetal thymus. J Path; 106: 45-48.
9. Hollinshead WH: Anatomy for Surgeons: The Thorax, Abdomen and Pelvis In: The Thorax in General. 2nd Edn, Vol. 2, Medical Department, Harper & Row Publishers, New York, Evanston, San Francisco, London. pp.1-43 (1971).
10. Liberti EA, Fagundes TP, Perito MA, Matson E and Konig Junior B (1994): On the size of Hassall's Corpuscles in human foetuses. Bull Assoc Anat (Nancy); 78(242): 15-18.
11. Lobach DF and Haynes BF (1987): Ontogeny of the human thymus during foetal development. J Clin Immunol; 7:81-97.
12. Muller-Hermelink HK, Marx A and Kirchner T: Anderson's Pathology In: Thymus and Mediastinum (A-Thymus). 10th Edition, Vol-I. Mosby, St. Louis. pp.1218-1243 (1996).
13. Ritter MA and Lampert IA: Oxford Text book of Pathology In: Thymus. Vol-2b. Oxford University Press, Oxford. pp.1807-1821 (1992).
14. Von Gaudecker B and Muller-Hermelink HK (1980): Ontogeny and organization of the stationary non-lymphoid cells in the human thymus. Cell Tissue Res; 207(2): 287-306.
15. Von Gaudecker B (1991): Functional histology of the human thymus. Anat Embryol (Berl); 183(1): 1-15.
16. Williams PL, Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Ferguson MW: Haemolymphoid system In: Gray's Anatomy. 38th Edition; ELBS with Churchill Livingstone, Edinburgh, London. pp.1423-1431 (1995).