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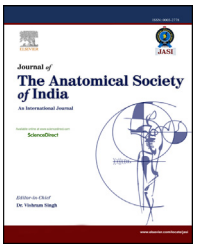

Original Article

Estimation of gestational age from histogenesis of the thymus in human fetuses

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ABSTRACT

Introduction: Stages in development and maturation of various organs and tissues may help in fetal gestational age estimation. The present study was undertaken to study the maturation of fetal thymic tissue.

Methods: The study was conducted on 30 human fetuses of gestational age ranging from 9th to 38th weeks were used for the study. Gestational age estimation was done by the crown rump length followed by distribution of fetuses into five groups. Thymus was dissected out from each fetus followed by tissue processing. Sections of 4–5 μm were cut and stained with Haematoxylin and Eosin stain. All slides were observed under the low and high powers of the light microscope.

Results: Lymphocytes first appeared in the thymus at the 9th week, trabeculae developed from the 9th week onwards, lobulation started to develop at the 9th week and continued till the 12th week, corticomedullary differentiation was apparent during the period of 9th–14th week, Hassall's corpuscles first appeared at the 15th week. Other developmental features continued to appear till the 38th week.

Discussion: The time of appearance of the various features in the developing thymus may help in rough estimation of the gestational age from minimal fetal thymic tissue remains especially during the early gestational weeks.

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1. Introduction

Estimation of the gestational age of a dead or aborted foetus is a specialized task in the medicine. Techniques for determining the gestational age of fetal or perinatal remains are mainly aimed at calculating the time since conception and at determining if a specific disease could be the cause of fetal loss. The whole skeletal length, long bone length (femur length), foetal ultrasonography etc have been used routinely for age estimation. As several organs show major changes in developmental patterns throughout fetal development,

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maturation of fetal tissues and organs has also been proposed for gestational age estimation. Study of development of tissues for age estimation becomes even more important if only a few foetal remains are available. Depending on the general condition of fetal remains, there might be difficulties in age estimation.[1] Tissues from several organs like skin, lungs, thyroid gland, kidneys, adrenal glands, and central nervous system (each with unique but distinguishable stages of development) have been found to be useful for a more accurate assessment of gestational age when integrated with long bones measurements.[2]

The thymus is a lymphoepithelial organ and the key regulator of the immune system. It is responsible for cellular immunity of the body. Its location in the anterior mediastinum behind the shield of the sternum during most part of the fetal life not only saves the thymus from destruction even in grossly mutilated specimens, but also makes it an easily accessible organ which can be easily dissected out due to its superficial location. These features suggest that the thymus can serve as an important organ for forensic examination in the determination of fetal gestational age.

The size of the thymus is largest during embryonic life. It develops as two separate primordia one on each side of the midline. The thymus is divided into a number of macroscopic lobules, varying from 0.5 to 2 mm in diameter. The lobules are separated from one another by connective tissue and are divided into a darkly staining, peripheral (cortical) area and a lighter staining, inner (medullary) portion.[3] Maturation of the fetal thymus and the time of appearance of various histological features may help in estimation of gestational age.

2. Materials and methods

The study was conducted in the Department of Anatomy, Himalayan Institute of Medical Sciences, HIHT University, Dehradun. 30 human fetuses of gestational age ranging from 9th to 38th weeks were obtained from the Department of Obstetrics and Gynaecology of the same institute after taking approval from the Institutional Ethics Committee and written informed consent from the concerned families. The fetuses were either products of pregnancy terminated under Medical Termination of Pregnancy Act of India, 1971 or were stillbirths. Only those fetuses which were apparently free from any gross anatomical or congenital abnormality were selected.

The fetuses were fixed in 10% neutral buffered formalin for two weeks. Age estimation for all fetuses was done from the Crown Rump Length (CRL) measured by Vernier Calipers from the vertex of the skull to the midpoint between the apices of buttocks and from the available history. The fetuses were categorized into the 5 groups arbitrarily as shown in Table 1.

The thymus was dissected out from each fetus and fixed in 5% neutral buffered formalin for histological study. Tissue processing was done. Sections of 3–4 μ thickness were cut followed by staining with Haematoxylin and Eosin stain. All slides were viewed under the low and high powers of the light microscope. All tissue remains were packed in yellow bags and disposed off as per the provisions for disposal of biomedical waste.

Table 1 – Distribution of fetuses in different groups according to gestational age.

Groups	Age (weeks)	Number of fetuses
Group I	9–11	5
Group II	12–14	5
Group III	15–17	6
Group IV	18–24	8
Group IV	25–38	6

3. Results

The histological findings in the various groups were as follows:

3.1. *Group 1 (9th–11th week)*

A thin capsule was visualized. Trabeculae were seen arising from the capsule and extending into the parenchyma. These trabeculae were having blood vessels lined by flattened endothelial cells. Mesenchymal cells were loosely arranged. The parenchyma started dividing into lobules but the cortex and medulla were not differentiated. Hassall's corpuscles were not visualized at this stage (Fig. 1). Two types of lymphocytes were visualized, few with small nucleus and plenty cytoplasm and others with large nucleus and less cytoplasm.

3.2. *Group 2 (12th–14th week)*

The capsule increased in thickness as compared to group 1. The trabeculae were well defined. The mesenchyma appeared to be denser. The lobulation of the parenchyma appeared to be completed. Each lobule had a recognizable cortex and medulla. Till the 14th week, no Hassall's corpuscle was seen. Lymphocytes were more numerous in number (Fig. 2).

3.3. *Group 3 (15th–17th week)*

The capsule became well defined. The trabeculae did not show any significant change. Mesenchyme became denser. The

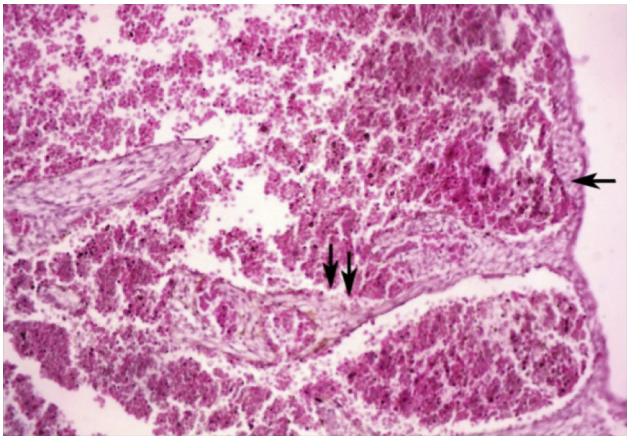

Fig. 1 – Photomicrograph of thymus of group I fetus showing capsule (arrow) and trabeculae (double arrow) (H&E X200).

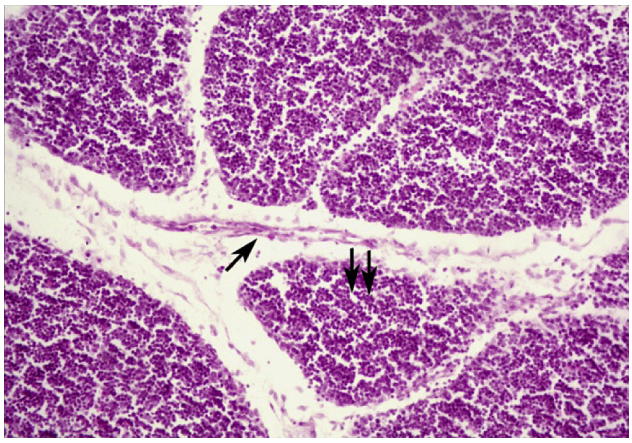

Fig. 2 – Photomicrograph of thymus of group II fetus showing expansion of trabeculae (arrow) and lobulation of the gland (double arrow) (H&E X200).

number of lobules increased further. It was at the age of 15 weeks that the Hassall's corpuscles first appeared in the medulla. The Hassall's corpuscles were present in all sections and increased in number and size with further increase in the gestational age (Figs. 3 and 4).

3.4. *Group 4 (18th–24th week)*

The capsule contained blood vessels and connective tissue of the capsule and trabeculae became more extensive at this stage. Number and size of Hassall's corpuscles and number of lobules increased (Fig. 5).

3.5. *Group 5 (25th–38th week)*

At this stage thymus gland appeared to be similar to that seen in the childhood and puberty. The trabecular framework and blood vessels became more distinct. Thymus at this stage, was composed of a dense population of lymphocytes and a medulla commonly possessing Hassall's corpuscles. Medulla was

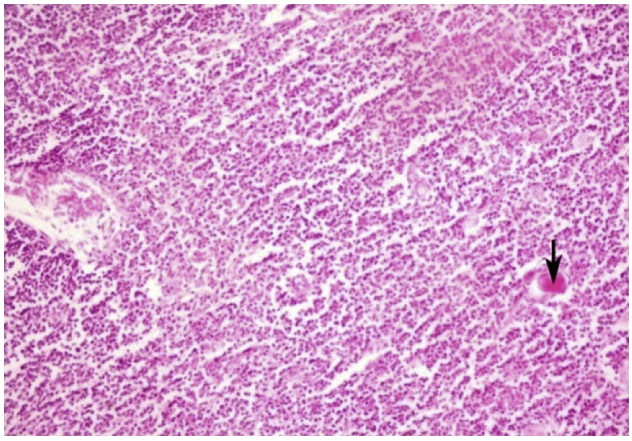

Fig. 3 – Photomicrograph of thymus of group III fetus showing appearance of Hassall's corpuscles (arrow) (H&E X200).

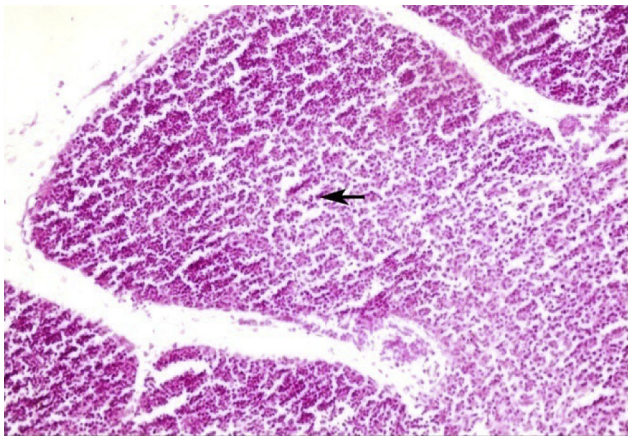

Fig. 4 – Photomicrograph of thymus of group III fetus showing cortex with distinct lobulation (arrow) (H&E X200).

having fewer lymphocyte cells as compared to the cortex (Fig. 6).

4. Discussion

Determination of the foetal age at the time of death is one of the major concerns of medicine. It is also important to find out whether the fetus was viable or not at the time of death, the threshold for viability being 20 weeks. Methods generally used for fetal age estimation are body weight, crown heel length, crown rump length, femur length, length of foot and ossified parts of other long bones.[4] These may not be very reliable, for example, the growth of long bones is affected by various conditions responsible for intrauterine growth retardation. Moreover, a complete fetus is required in these methods. For this reason, many studies have identified the need for developing new methods to measure the gestational age apart from long bones.[1] Histological examination of tissues/organs is rarely used as a method although the chronology of fetal tissue development is already well established.[5] This method

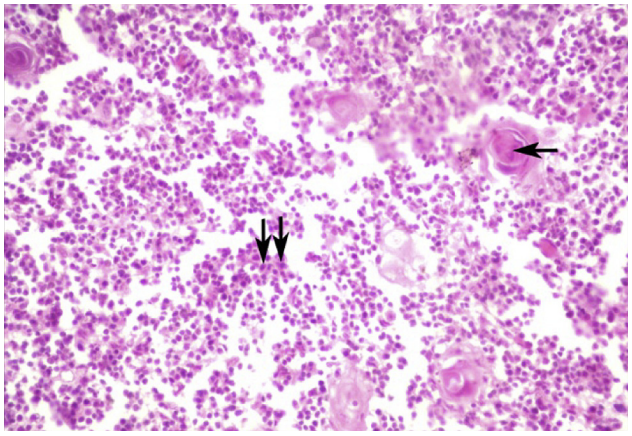

Fig. 5 – Photomicrograph of thymus of group IV fetus showing Hassall's corpuscle (arrow) and parenchyma with lymphocytes (double arrow).

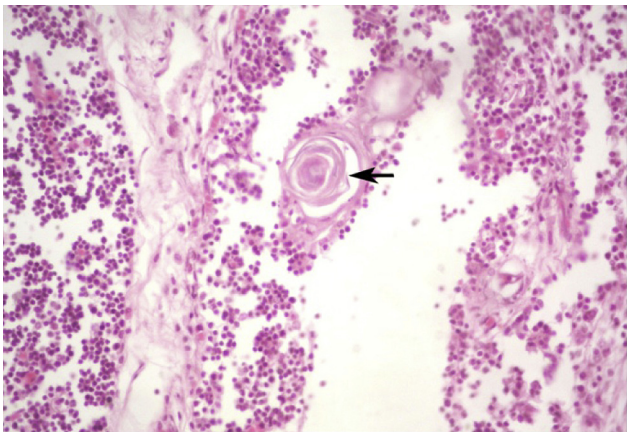

Fig. 6 – Photomicrograph of thymus of group V fetus showing a well developed Hassall's corpuscle (arrow) (H&E X400).

may prove to be of immense help in complicated circumstances. The present study may help in rough age estimation in cases where only few fetal neck or upper thoracic remains are available.

Different findings have been reported by various workers as regards the time at which the lymphocytes appeared in the thymus such as from the 8th week according to Williams et al and the 9th week according to Harr et al. In the present study we observed the presence of lymphocytes from the 9th week onwards.[6,7]

Ghali et al reported that the thymus was vascular at the 11th week of gestation. Harr, Hamilton and Mossman reported that extrathymic blood vessels associated with connective tissue fibres and mesenchymal cells surrounding the thymus were present at the 9th week. Williams et al mentioned about the presence of developing erythroblast cells in the 10th week old thymic tissue. In the present study, trabeculae started entering from the capsule into the parenchyma from the 9th week onwards with the simultaneous appearance of blood vessels lined by a thin layer of endothelial cells.[6–9]

Ghali et al and Harr reported lobulation of the thymus gland at 10th and 12th weeks respectively. Whereas, in the present study, formation of lobules started at the 9th week and lobulation was distinct at the 12th week.[7,8]

There are different opinions about the time of differentiation of the cortex and medulla in the thymus. This differentiation appeared at the 11th week according to Ghali et al, in embryos of about 40 mm CRL according to Hamilton and Mossman, by about the 12th week according to Hayward, 14th week according to Harr and between 12th and 14th week according to Von Gaudecker. The present study indicated that the differentiation of the cortex and medulla started from the 9th week and became well distinguished during the 12th to 14th week stage.[7–11]

There are different reports on the time of appearance of Hassall's corpuscles as follows: as early as the 8th week by Fawcett, from the 9th week by Gilhus et al, at the 10th week by Williams et al, at the 11th week by Ghali et al and between the 15th and 16th week by Lobach and Haynes. In the present study, till the 14th week, no Hassall's corpuscles were seen. At the 15th week, Hassall's corpuscles started appearing in the medulla.[6,8,12–14]

5. Conclusion

In the present study, development of the thymus was studied histologically in developing fetuses.

Lymphocytes first appeared in the thymus at the 9th week, trabeculae developed from the 9th week onwards, lobulation started to develop at the 9th week and continued till the 12th week, corticomedullary differentiation was apparent during the period of 9th to 14th week, Hassall's corpuscles first appeared at the 15th week. The time of appearance of the various features in the developing thymus, especially the time of appearance of Hassall's corpuscle, may help in the rough estimation of the gestational age from minimal thymic tissue remains where all other conventional methods may fail to have applicability.

Conflicts of interest

All authors have none to declare.

Acknowledgements

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