

Morphology and Histogenesis of Thymus as a Tool for Estimating Foetal Age: A Cross-sectional Study

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ABSTRACT

Introduction: The thymus is located in the superior mediastinum and anterior part of the inferior mediastinum. It plays a crucial role in the development, differentiation, and clonal expansion of T lymphocytes. In forensic cases involving foetal death, accurate estimation of foetal age is essential. When the foetus is extensively damaged, other organs can be used to estimate age. The thymus, being retrosternal and well-protected, is easily accessible during dissection. Therefore, studying the gross features and histogenesis of the thymus can be helpful in estimating gestational age in cases of severely mutilated foetal specimens.

Aim: To examine the morphological features and histogenesis of the thymus in aborted and stillborn foetuses from normal pregnancies.

Materials and Methods: A pilot study was conducted using a cross-sectional design at the Department of Anatomy, BLDEDU's Shri BM Patil Medical College, Vijayapura, Karnataka, India, from May 2016 to April 2018. The dimensions and histogenesis of the thymus were studied in a total of 50 human foetal thymus specimens, collected from normal pregnancies across different gestational age groups ranging from 12 to 40 weeks. The specimens were obtained from the Department of Pathology and

the Department of Obstetrics and Gynaecology. The dimensions and weight of the thymus were recorded during autopsy. Slides were prepared, stained with Haematoxylin and Eosin (H&E), and examined under a compound microscope. The histogenesis of the thymus was studied to observe its maturation.

Results: Foetal weight increased from 134.6±58.3 g at 12 weeks to 1942±598 g at 40 weeks. The weight of the thymus ranged from 0.2 g at 12 weeks to 5.9 g at 40 weeks. Crown rump length varied from 10.7 cm to 29.9 cm. The length of the thymus measured between 0.5 cm and 3.3 cm, breadth between 0.4 cm and 2.3 cm, and height between 0.2 cm and 0.9 cm from 12 to 40 weeks of gestation. Microscopic features observed in the present study at different gestational ages included a well-formed thick capsule at 17 weeks, lobulation starting at 19 weeks, cortico-medullary differentiation beginning at 17 weeks, appearance of epithelial cells around 19 weeks, and initial appearance of Hassall's corpuscles at 17 weeks, with more numbers observed later on.

Conclusion: The thymus can be used in forensic investigations to estimate the gestational age of mutilated foetuses. It exhibits varying features at different gestational ages, which can aid in thymectomy procedures. Additionally, these findings can be considered during prenatal imaging studies.

Keywords: Epithelial reticular cells, Gestational age, Hassall's corpuscles, Medicolegal cases

INTRODUCTION

The Greek word "warty excrescence" originates from the word thymus, which resembles the flowers of the thyme plant [1]. The thymus is known as the primary lymphoid organ. It is located in the superior mediastinum and anterior part of the inferior mediastinum. Its function is to develop T lymphocytes, differentiate them, and assist in the clonal expansion of lymphocytes. The size of the thymus grows until puberty, reaching its maximum at puberty, and then degenerates. However, it may persist in old age as a fibrofatty organ [2]. In cases of foetal death, accurate estimation of foetal age is crucial, especially when the foetus is extensively mutilated. In such cases, other organs can be used to estimate the age. The thymus, being a retrosternal and well-protected organ, can be utilised for foetal age determination, highlighting its importance in accurately estimating foetal age and its significance in legal, ethical, and clinical implications [3].

The thymus develops as a bilobed structure from the endoderm of the third pharyngeal pouch during the 6th week of embryonic life. This endoderm interacts with neural crest cells, which become encapsulated within a capsule [4]. While previous studies have focussed on the histogenesis of the thymus, less emphasis has been placed on its morphology along with histogenesis. Therefore, the gross features and histogenesis of the thymus can be used to

estimate the gestational age of foetuses in cases of grossly mutilated foetal deaths. Several authors have conducted studies on the histogenesis of the thymus in various regions of Tamil Nadu, Andhra Pradesh, and North India, with only a few reported in Karnataka. Thus, the present study aimed to examine the morphological features and histogenesis of the thymus in aborted and stillborn foetuses from normal pregnant women.

MATERIALS AND METHODS

A cross-sectional pilot study was conducted at the Department of Anatomy, BLDEDU's Shri BM Patil Medical College, Vijayapura, Karnataka, India, from May 2016 to April 2018. The study received approval from the Ethical Committee of the Institution (IEC no:169/2016-17), and written consent was obtained from the parents. All foetuses that appeared grossly normal were included in the study. A total of 50 human aborted and stillborn foetuses were obtained from the study centre.

Inclusion criteria: All aborted and stillborn foetuses that appeared grossly normal and were aged between 12 to 40 weeks of normal pregnancy were included in the study (n=10 in each group) [Table/Fig-1]. This time period was selected because organogenesis is completed by 12 weeks of gestation.

Exclusion criteria: Foetuses with anomalies were excluded from the study. The foetuses were obtained from the Department of

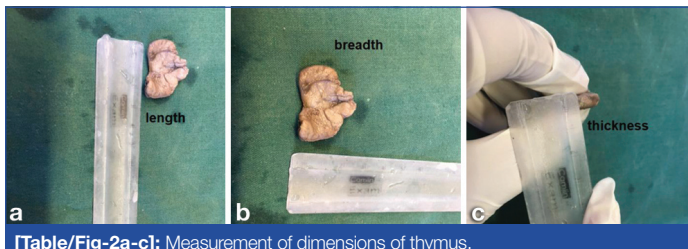
Number	Group	Age of foetus
1	I	12-18 weeks
2	II	18.1-24 weeks
3	III	24.1-30 weeks
4	IV	30.1-36 weeks
5	V	36.1-40 weeks

[Table/Fig-1]: Gestational ages were categorised, and ten fetuses in each age group were studied.

Obstetrics and Gynaecology, immediately fixed in 10% formalin, and sent to the Department of Pathology for examination.

Study Procedure

The age of the foetus was determined by measuring the crown-rump length and foetal weight. The fetuses were dissected using the standard protocol described by Romanes GJ to obtain the thymus [5]. The weight of the thymus was recorded using a weighing machine. The dimensions of the thymus, including length, breadth, and thickness, were measured using a measuring scale. Thin slices, as small as 5 mm, were cut from the entire thymus [Table/Fig-2a-c]. The tissue was processed, slides were prepared, and stained with H&E. The slides were observed under a compound microscope at magnifications of 40, 100, 400, and 1000 times for interpretation.



[Table/Fig-2a-c]: Measurement of dimensions of thymus.

STATISTICAL ANALYSIS

Descriptive statistics, including the Number (N), mean, and Standard Deviation (SD), were used for summarising the characteristics. The

Gestational weeks	12-18 weeks		18-24 weeks		24-30 weeks		30-36 weeks		36-40 weeks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Foetal weight (grams)	134.6	58.3	539	389.4	590	278.7	1563	556.3	1942	598.5
Thymus weight (grams)	0.2	0.1	1.1	1.3	1.5	1.3	4.6	1.6	5.9	1.4
CRL (cm)	10.7	2.8	18.1	5.3	19.5	3.5	28.5	6.1	29.9	2.6
Length of thymus (cm)	0.5	0.5	1.6	0.7	1.7	1	3.2	0.9	3.3	1.1
Breadth of thymus (cm)	0.4	0.2	1.1	0.6	1.2	0.7	2.2	0.7	2.3	1.1
Thickness (cm)	0.2	0	0.4	0.1	0.8	0.3	0.9	0.2	0.9	0.3

[Table/Fig-3]: Foetal weight and gross parameters of the foetal thymus.

CRL: Crown-rump length

statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 20.0.

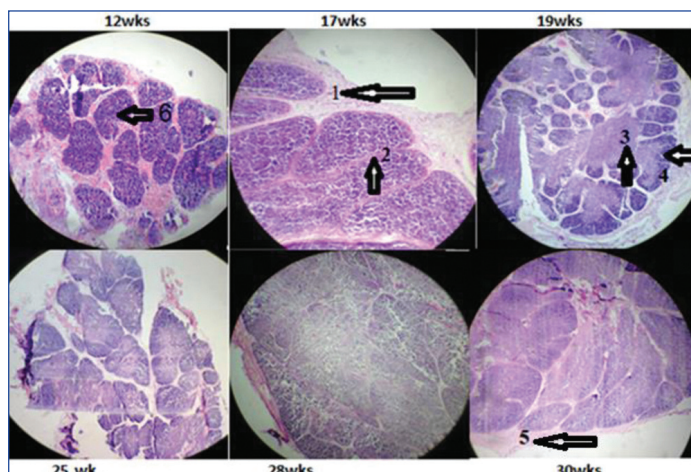
RESULTS

Foetal weight, thymus weight, crown-rump length, thymus length, breadth, and thickness increased from 12 weeks to 36 weeks [Table/Fig-3]. In terms of histogenesis, the following parameters were included: formation of the capsule, lobulation and cortico-medullary differentiation, description of the stroma including blood vessels, epithelial reticular cells, and morphology of Hassall's corpuscles.

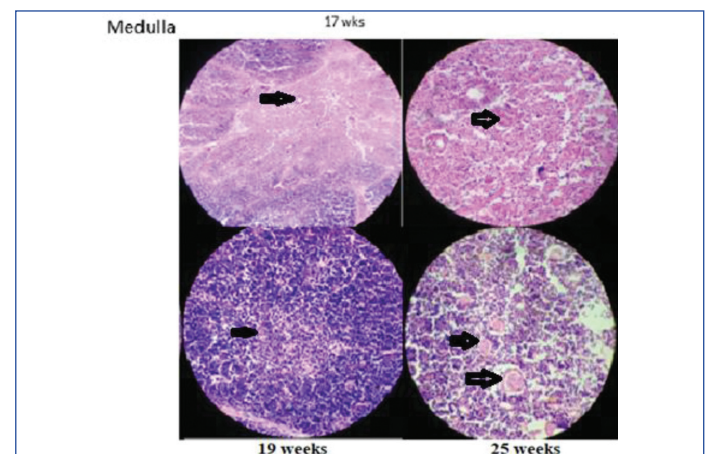
As shown in [Table/Fig-4], the thymus exhibited the formation of a well-formed thick capsule (arrow 1) at 17 weeks of gestation. The capsule consisted of mucoid connective tissue with collagen fibres, abundant ground substance, and few fibroblasts. Interlobular septa were observed (arrow 2). Lobulation appeared at 19 weeks, and cortico-medullary differentiation started at 19 weeks, with well-formed trabeculae observed. The cortex of the thymus exhibited well-formed lymphocytes at 12 weeks (arrow 6), which were scattered at this gestational age.

These lymphocytes moved towards the periphery of the lobules at 17-19 weeks (as indicated by arrows 3 and 4). In later stages of gestation, such as 25-30 weeks onwards, fully formed lobules were observed (arrow 5). Blood vessels began to appear at 19 weeks. At 32 to 36 weeks, the connective tissue septa contained more blood vessels in the intralobular and interlobular regions.

As observed in [Table/Fig-5,6], the medulla was distinctly visible at 17 weeks. The medulla of adjacent lobules fused together. Hassall's corpuscles (indicated by black arrows) appeared at 17 weeks at the cortico-medullary junction, and their numbers gradually increased with increasing gestational age, both in the medulla and at the cortico-medullary junction. Different types of Hassall's corpuscles were observed. Epithelial cells were seen at 19 weeks. Blood vessels were also observed (yellow arrow)

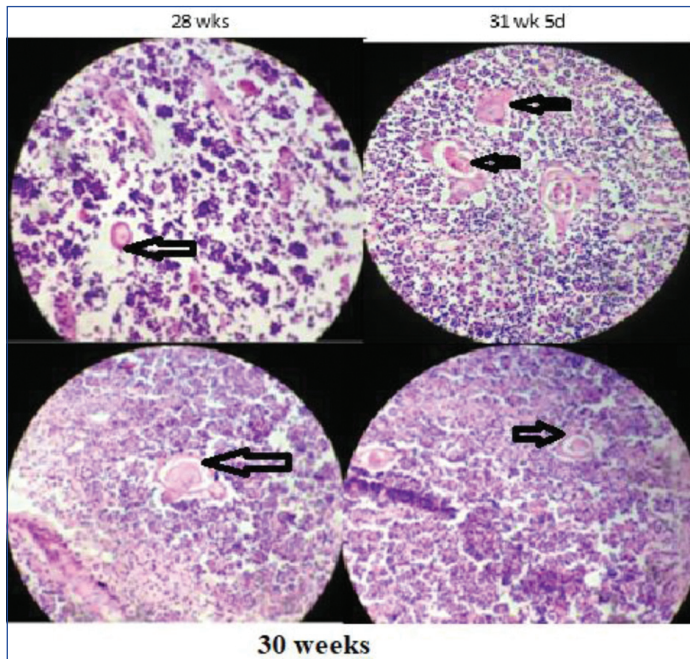


[Table/Fig-4]: Microscopic view of thymus at different gestational age stained with haematoxylin and eosin as observed under 4x objective with 40 times magnification.

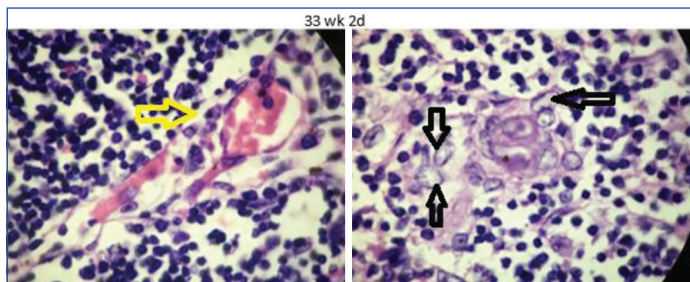


[Table/Fig-5]: Microscopic view of medulla of the thymus at different gestational age stained with haematoxylin and eosin as observed under 10x objective with 100 times magnification.

[Table/Fig-7]. More epithelial reticular cells (black arrows) were seen at 33 weeks.



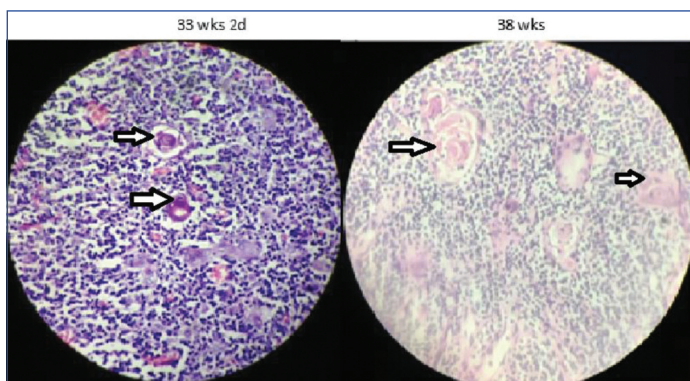
[Table/Fig-6]: Microscopic view of medulla of the thymus showing Hassall's corpuscles at different gestational age stained with haematoxylin and eosin as observed under 10x objective with 100 times magnification.



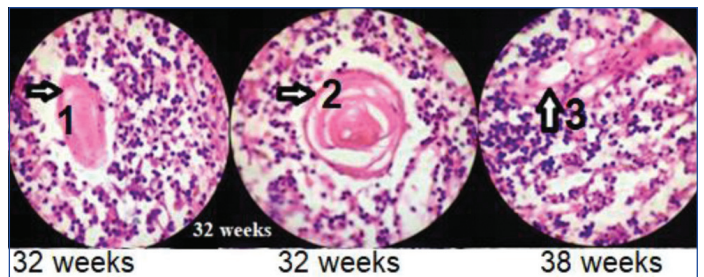
[Table/Fig-7]: Microscopic view of medulla of the thymus stained with haematoxylin and eosin showing blood vessels and epithelial reticular cells at 33 weeks of gestational age as observed under 100x objective with 1000 times magnification.

As seen in [Table/Fig-8], Hassall's corpuscles were observed. At 32 weeks [Table/Fig-9], arrow 1, primary cystic corpuscles were seen. They consisted of a hyalinised homogeneous acidophilic mass in the centre, surrounded by a cystic space encapsulated by flat epithelial cells. Arrow 2, large Hassall's corpuscles consisted of a core of central hyalinised mass with layers of eosinophilic concentrates, resembling an onion peel. At 38 weeks, arrow 3, blood vessels were closely observed near Hassall's corpuscles.

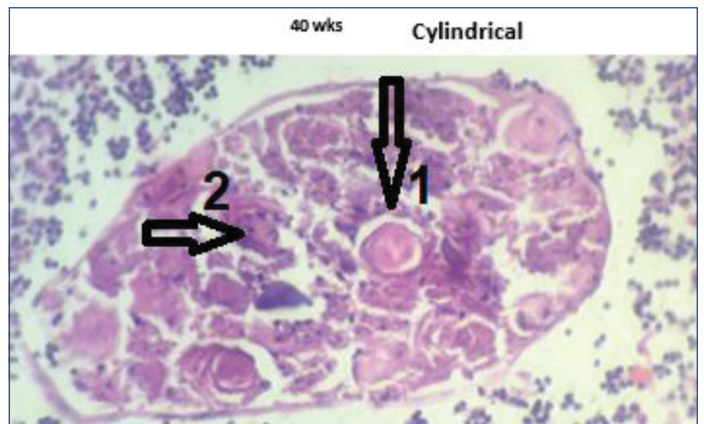
In [Table/Fig-10], at 40 weeks, one of the thymi showed a cylindrical type of Hassall's corpuscle, which enclosed many cystic structures (arrow 1) and degenerating Hassall's corpuscles (arrow 2).



[Table/Fig-8]: Microscopic view of medulla of the thymus stained with haematoxylin and eosin showing of gestational age as observed under 10x objective with 100 times magnification.



[Table/Fig-9]: Microscopic view of medulla of the thymus stained with haematoxylin and eosin showing of gestational age as observed under 100x objective with 1000 times magnification.



[Table/Fig-10]: Microscopic view of the medulla of the thymus stained with haematoxylin and eosin showing of gestational age as observed under 100x objective with 1000 times magnification.

DISCUSSION

The vasculature of the thymus, lymphoid cells, and arrangement of the cortex and medulla have been studied using light microscopy and electron microscopy since as early as 1974 by Harr JL [6]. The interaction between the neural crest (capsule and connective tissue septa development) and endodermal components, specifically the 3rd pharyngeal pouch of the thymic primordia, plays a crucial role in the subsequent differentiation of thymic structure and function [7].

The findings of the present study, indicating a gradual increase in thymic weight (0.1 to 19 gm) with gestational age, are similar to the results of authors Lakshmi KV et al., 2012 (0.1-10 g) and Gouse HS et al., (0.12-14 g) [1,8]. The dimensions of the thymus also showed similar results. In the present study, the length ranged from 0.1-5 cm and breadth from 0.1-4.5 cm, which is consistent with the findings of Sophia MM et al., (length: 0.2-4 cm, breadth: 0.1-2.1 cm) and Lakshmi KV et al., (length: 0.2 cm-5.2 cm, breadth: 0.1 cm-4 cm at birth) [1,7].

As seen in [Table/Fig-11], the present study was conducted on 50 thymi of aborted fetuses. Important microscopic features such as cortico-medullary differentiation, the appearance of Hassall's corpuscles, and epithelial cells were observed at 17-19 weeks, which coincided with Group 1 and Group 2. These findings were consistent with other studies conducted by various authors [1,6,9-13]. The present study focused on both morphology and histogenesis, making it unique and potentially useful in forensic investigations, such as age determination of grossly mutilated fetuses. Correlating both features can enhance the accuracy of the findings.

Future recommendations include increasing the sample size, conducting histometric analysis to validate the findings, and using special stains to demonstrate all the observed features. Immunohistochemistry using markers such as Foxn1 and CD205 (DEC205), which are specific for thymus organogenesis, can also be considered [14].

Author, year, place of study	Stain used	Number of fetuses	Corticomedullary differentiation	Epithelial cells	Hassall's corpuscles	Any other details
Haar JL [6] 1974	0.1% toluidine blue, 0.1% methylene blue		9-14 weeks		15 weeks	
Lakshmi KV et al., [1] 2012, Nellimarla	H&E	50	16 weeks 18 wk more distinct	34 weeks complete differentiation into reticular cells	18 weeks	
Gouse HS et al., [8] 2019, Annamalai	H&E, Masson's trichome and PAS	20	17 weeks		Fusiform solid primary and secondary cystic degenerating	
Khan B et al., [9] 2013, Aurangabad	H&E PAS	53	-	-	12 weeks Max 18-24 weeks	PAS-positive
Krishnamurthy JV et al., [10] 2015, Tirupati	H&E	30	14-16 weeks		14-16 weeks Starry sky hyalinisation and keratinisation at term	
Babu DS et al., [11] 2016, Vishakapatnam	H&E	100	18 weeks		Histometric analysis done	
Bharath CV et al., [12] 2016, Kumool	H&E Masson's trichome	38	12-14 weeks			
Prabhavati G [13] 2021, Chennai	H&E	281	14 weeks		14 weeks	
Present study	H&E	50	17 weeks	Seen at 19 weeks	17 weeks and increased in number	

[Table/Fig-11]: Comparison of various studies on histogenesis of thymus by different authors [1,6,9-13].

Limitation(s)

A limitation of the present study is that special stains were not used. Histometric analysis could be helpful in prenatal imaging as reference values.

CONCLUSION(S)

In the present study, the morphology of the thymus was correlated with the histological changes occurring at different gestational ages. Events in histogenesis, such as the appearance of a thick capsule, lobulation, cortico-medullary differentiation, epithelial reticular cells, and Hassall's corpuscles, mainly occurred from 17 weeks onwards. These findings have potential applications in forensic investigations for determining the gestational age of mutilated fetuses. The varying features of the thymus at different gestational ages can also be considered during thymectomy procedures and prenatal imaging studies.

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