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Histological and Histometrical Study on Human Fetal Thymus

Helen Suban Mohammed Gouse[1]*, Suban Mohammed Gouse[2], JP Gunasegaran[3] and Muniappan V[4]

[1] Department of Anatomy, Ibn Sina Medical College of Medical Studies, Jeddah, Saudi Arabia

[2] Pathologist, QA Consultant and Strategist, Higher Education and Healthcare, Digival Solutions Private Limited, India

[3] Department of Anatomy, Pondicherry Institute of Medical Sciences, Tamil Nadu, India

[4] Department of Anatomy, Rajah Muthiah Medical College, Annamalai University, Tamil Nadu, India

**Corresponding e-mail: dr_helenghouse@yahoo.co.in*

ABSTRACT

Background: *The thymus is one of the primary lymphoid organs other than being the bone marrow. It is responsible for the provision of thymus-processed lymphocytes (T lymphocytes) to the whole body.* ***Aim of the study:*** *To study the microscopic architecture of the cortex and medulla, structure and type of epithelial cells, nature of connective tissue stroma, vasculature of thymus organ, the morphology of Hassall Corpuscle (HC). To study the histometric analysis like estimation of volume and diameter of HC at various gestational age.* ***Method:*** *Total 20 aborted and still born fetuses ranging from 17-39 weeks of gestation were used for the study. After embalming, meticulous dissection, thymus gland was weighed and fixed with formalin. The various histological and histometric parameters were observed.* ***Results:*** *The cortex and medulla were well demarcated by the 17th week. The weight and volume of the thymus were proportionately increased as the fetal age advanced. Early phase had thick mucoid interlobular septae whereas the later phase had thin, reticular interlobular septae. Four different types of HC (SHC, CHC I, CHC II and DHC) in fetus were seen. The presence of solid HC at the periphery of the medulla and degenerating HC at the central core of the medulla tends to postulate the direction of maturation which is from the periphery to the center of the medulla.* ***Conclusion:*** *The findings of the present study is in conformity with studies related to the volume and size of the thymus with respect to gestational ages and histological features related to parenchymal and mesenchymal tissue composition and its various components. However, the present study noted different types of Hassall's Corpuscles which are reported in the adult thymus and the findings lead to further discussion on the maturation and differentiation of Hassall's Corpuscles in human fetal thymus.*

Keywords: Hassall corpuscles, Human fetal thymus, Thymus histology, Thymus histometric

INTRODUCTION

"The first man knew her not perfectly and in like manner the last hath not traced her out" Ecclesiasticus.

The quotation given is apt as far as the thymus gland is concerned. A famous author stated "The literature on the thymus is voluminous. The tissue has been weighed, ground up, injected, sectioned, irradiated, infected and worried but still, its central mystery eludes us." Within the past few years, so much of light has been thrown on the different aspects of this organ, but the proper assessment of its function is still an enigma.

The thymus is derived from a number of sources, including epithelial derivatives of the pharyngeal wall, mesenchyme, haemolymphoid cells, and vascular tissue. These form distinctive components within the mature thymus, interacting functionally to create its unique immunological properties.

The cells of the thymic microenvironment are T-lymphocytic lineage, sessile epithelial cells, myoid cells,

macrophages, interdigitating cells, hemopoietic cells (eosinophil, neutrophils, B-cells, plasma cells, and erythroid cells) and fibroblasts. Majority of the blood vessels travel in connective tissue septa (outside the parenchyma of the gland) to the corticomedullary junction and enter the cortex and medulla to drain back into the septal blood vessels. Lymphatics forms at the corticomedullary junction, and leave the organ through blood vessels and efferent lymphatics present in the connective tissue septa.

Unlike other lymphoid organs, where the supportive framework is chiefly reticular tissue, the thymus is permeated by a network of interconnected thymic epitheliocytes; between lodge lymphoid and other cells of the organ. Epitheliocytes vary in size and shape in the different positions within the thymus. Cortical epitheliocytes are branches whereas those in the medulla form solid cords as well as the characteristic whorls of partially keratinized epithelium (thymic or HC).

HC are balls of flattened medullary epithelial cells which are characteristic features of the thymus. Their function is not clear, although in the past it has been suggested that they are the graveyards for thymic cells or regions where immunoglobulins are concentrated [1]. The functions of Hassall's corpuscles remain a mystery. Proposed functions include:

- The site of thymocytes death [2,3]
- Production or storage of antigen
- Antibodies [4]
- The site of thymic hormone production [5-7]
- Remnants of the thymic primordium without any significant function
- Lymphocyte rich HC's is involved in the negative selection of thymocytes [8]
- Differentiation of thymocytes at medulla [9]
- Removal of apoptotic thymocytes and maturation of developing thymocytes [10]

PATIENTS AND METHODS

Collection of Fetuses

Total 20 aborted and stillborn fetuses varying from 17-39 gestational weeks were obtained from the Department of Obstetrics and Gynecology, Rajah Muthiah Medical College, Annamalai University. Consent form for autopsy and embalming of the fetus was obtained from the parents and from the hospital authority. The gestational age was confirmed with the corresponding Crown-Rump Length (CRL) as well as with the LMP [11]. Every aborted and stillborn fetus was subjected to detailed physical examination and any fetus with external anomalies was excluded. They were segregated into 4 groups based on CRL and gestational age as illustrated in Tables 1 and 2. Two groups were considered together (Group I and II) called as early phase and Group III and IV as a late phase for comparative purposes.

Table 1 Gestational age based on CRL

Gestational age (weeks)	Crown-Rump Length (mm)	Number of Fetuses
15-18	61-100 mm	1
19-22	101-150 mm	3
23-26	151-200 mm	2
27-30	201-260 mm	5
31-34	261-320 mm	5
35-39	321-390 mm	4

Table 2 Grouping of fetuses

Phase	Groups	Gestational Age (Weeks)	Number of Fetuses
Early	I	17-24	5
	II	25-30	6
Late	III	31-35	5
	IV	36-40	4

Dissection of Thymus

After embalming, the thymus was dissected out by making a window in the thoracic region. The window was made using 2 vertical incisions on either side of the sterno-costal junction and joined by a transverse incision. This flap was reflected upwards to expose thymus lying in the superior and anterior mediastinum (Figure 1). The thymus was weighed with a digital balance and the total volume of the organ was measured using the water displacement method. A minimum of 4 pieces was collected from each thymus from different regions to represent the entire organ and preserved in formalin. Tissues were processed for light microscopy. One set of glass slides were routinely stained with Hematoxylin and Eosin (HandE) while another set of slides were stained with special stains like Masson's Trichrome and Periodic Acid Schiff (PAS).

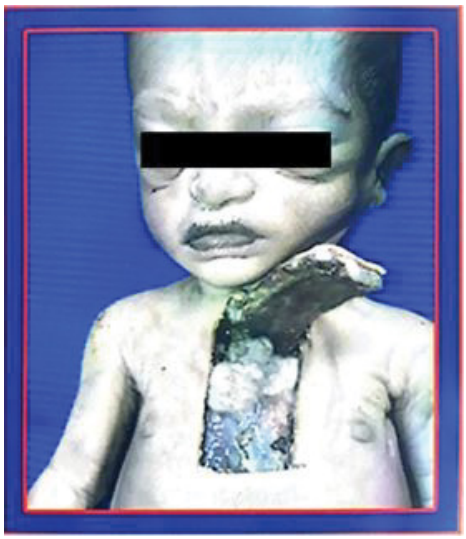

Figure 1 Dissection of the thymus

The stained slides were viewed under a light microscope to study the following parameters:

Histological parameters:

- Lobulation and differentiation of cortex and medulla
- Structure and type of epithelial reticular cells
- Morphology of Hassall's corpuscle
- Nature of connective tissue stroma and vasculature of the organ

Histometric parameters:

(The principles emphasized by authors were strictly employed [12]. The following stereological formula has been employed for histometry)

- Estimation of volume: Point count method was used with the help of eye piece, line grid-reticule (Figure 2). The volumes of major tissue components of the thymus; cortex, medulla and connective tissue were computed.

Vi=Pi/PT

Vi:Volume of that particular histological structure in question per cubic unit

Pi:Number of points against that particular histological structure

PT:Total number of points in the line grid taken into consideration

- The diameter of Hassall's Corpuscle: The diameter of Hassall's corpuscle was measured using an ocular micrometer.

D=L+B/2

D: Diameter of the corpuscle

L: Maximum width of corpuscle in length

B: Maximum breadth of corpuscle at a right angle to 'L'

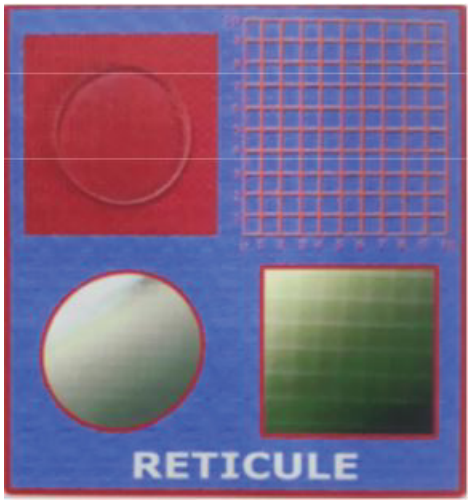

Figure 2 Reticule

Statistical Analysis

Histometric data were routinely entered in the Microsoft Excel and standard software was used for statistical analysis of various tissue components studied. The statistical tests applied were the Kruskal-Wallis test. Non-parametric correlations were applied to identify the existence of a significant correlation between the parameters studies.

RESULTS

General Features of the Thymus

Thymus appeared soft, bilobed and pink in color, located in the superior and anterior mediastinum. The maximum weight and volume of thymus reached during the gestational period were 14 gms and 16 cc respectively. The weight and volume of the thymus were proportionately increased as the fetal age advanced (Tables 3 and 4).

Table 3 Analysis of weight and volume in fetal thymus

Group	Gestational Age (weeks)	Weight (Grams)	Volume (mm^3)
		Mean ± SD	Mean ± SD
I	17-24	0.742 ± 0.591	1001 ± 704.310
II	25-30	1.602 ± 0.853	2000 ± 894.420
III	31-35	4.844 ± 0.945	4300 ± 670.820
IV	36-40	11.070 ± 3.501	11250 ± 4272.002
Kruskal-Wallis Test		16.277	16.413
p-value		0.001	0.001

Table 4 The weight of thymus and fetus was correlated during intrauterine life

S. No	Weight of the fetus (in grams)	Weight of the thymus (in grams)
1	200	0.30
2	250	0.12
3	450	0.90
4	600	1.63
5	650	0.76
6	500	1.92

7	900	0.86
8	550	2.88
9	300	1.12
10	700	2.10
11	1000	0.69
12	1750	4.42
13	1700	3.52
14	1600	5.62
15	1200	4.80
16	900	5.86
17	2500	6.85
18	2900	9.53
19	2400	14.00
20	2600	13.90

Histological and Histometric Observations

Lobulation, differentiation and volume estimation of cortex and medulla: Lobulation and differentiation of cortex and medulla were well demarcated by 17th gestational week, the lowest age group in the present study (Figure 3).

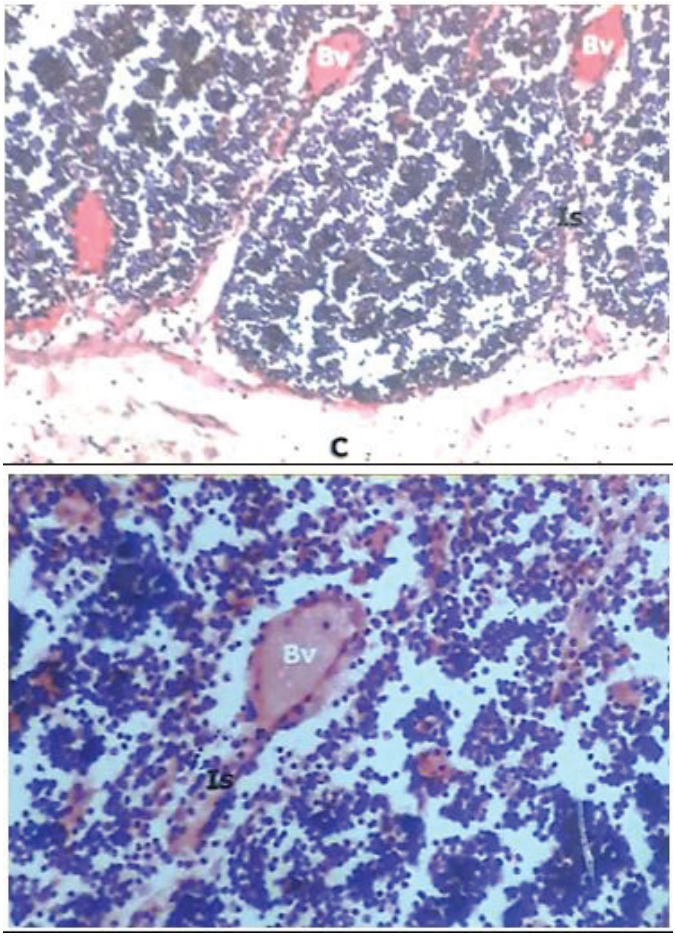

Figure 3 A: 17-weeks Hematoxylin and Eosin (x100). Lobules separated by interlobular connective tissue septa (Is) carrying blood vessels (Bv), C-Capsule. B: 17-weeks Hematoxylin and Eosin (x200). Lobules separated by interlobular connective tissue septa (Is) carrying blood vessels (Bv)

Presence of a pale sub capsular zone in the periphery of cortex separated from the darkly stained deep cortex was noticed. This zone was chiefly made up of epithelial reticular cells (Figure 4).

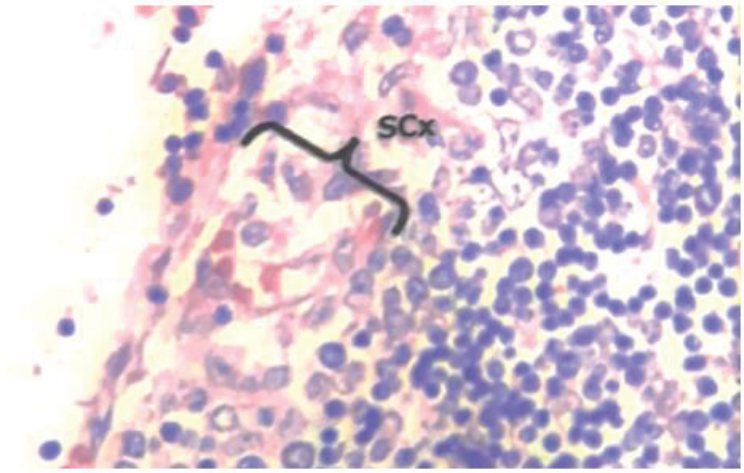

Figure 4 39-weeks Hematoxylin and Eosin (x400). Lightly stained sub-capsular cortex (SCx) showing many epithelial reticular cells

Histometric analysis of cortex and medulla revealed that there was no significant variation in their ratio on the various age groups (Table 5).

Table 5 Ratio of cortex and medulla

Group	Gestational Age (Weeks)	Cortex (mm^3)	Medulla (mm^3)	Cortex/Medulla Ratio
		Mean ± SD	Mean ± SD	Mean ± SD
I	17-24	0.4686 ± 0.592	0.3918 ± 0.1857	3.6619 ± 1.7666
II	25-30	0.4948 ± 0.862	0.4035 ± 0.1111	4.0040 ± 1.2422
III	31-35	0.4770 ± 0.1001	0.3636 ± 0.0560	4.7858 ± 3.496
IV	36-40	0.4797 ± 0.0175	0.3830 ± 0.0455	3.6797 ± 0.718
Kruskal-Wallis Test		0.269	4.367	0.522
p-value		0.966	0.224	0.914

Nature of connective tissue and vasculature of the organ: In the early phase (Group I and II), the capsule and interlobular septa were thick and made of a mucoid or mesenchymal type of connective tissue. The septa were seen penetrating into the cortex to further divided into smaller lobules. This connective tissue was composed of few fibroblasts, immature or fine collagen fibrils and a large amount of ground substance (Figure 5).

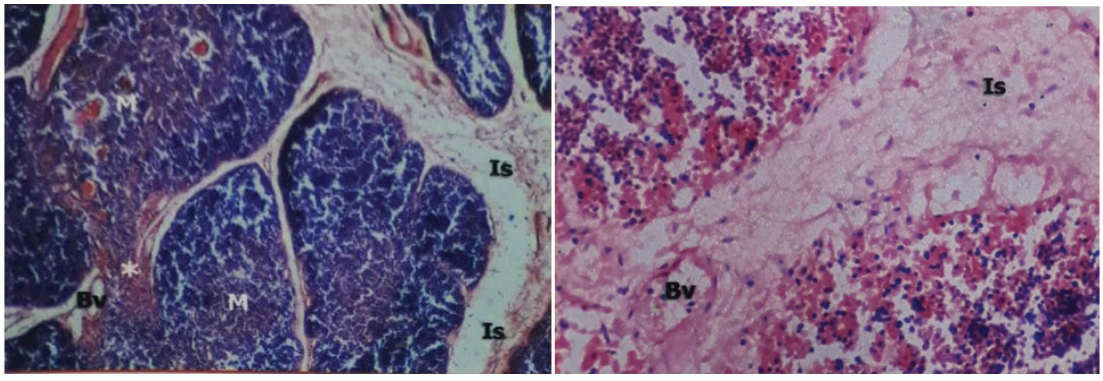

Figure 5 A: 28-weeks, Hematoxylin and Eosin (x40). Note the interlobular septum (Is) is interrupted by a bar of medullary tissue (*) connecting the medulla of adjacent lobules. Bv-Blood vessels. B: 28 weeks, Hematoxylin and Eosin (x200). Note the thick interlobular septa (Is) made of mucoid connective tissue

In the later phase (Group III and IV), the capsule and interlobular septa were thin and made of loose areolar connective tissue. Here the composition was mainly fibroblasts and well differentiated collagen fibers embedded in less amount of ground substance (Figure 6).

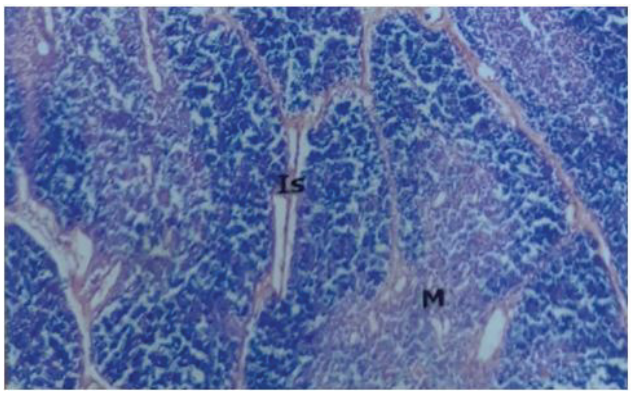

Figure 6 39-weeks, Hematoxylin and Eosin (x40). Note the thin interlobular septa (Is) made of well differentiated collagen fibers

Infiltration of adipose tissue has been noticed by 39th gestational week in the capsule and also in the interlobular septa (Figure 7).

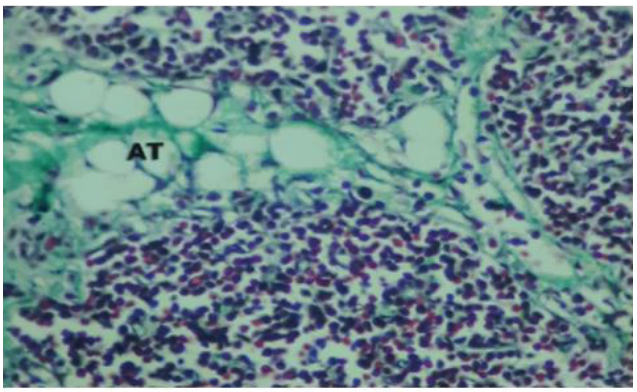

Figure 7 39-weeks, Masson's Trichrome (x100). Infiltration of adipose tissue (AT) in the interlobular septum

Thymus appeared to be more vascular in the later phase than in the earlier phase. Both interlobular and intralobular blood vessels were plenty during the later gestational weeks (Figure 8).

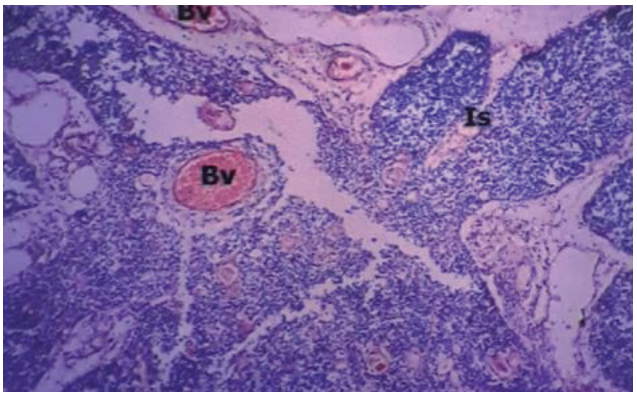

Figure 8 32-weeks, Hematoxylin and Eosin (x40). Inter and intra lobular blood vessels are seen (Bv). Is-Interlobular septum

At higher magnification, many small blood vessels were seen at the cortico-medullary junction. These blood vessels were mostly posted capillary venules and often closely related to HC's (Figure 9).

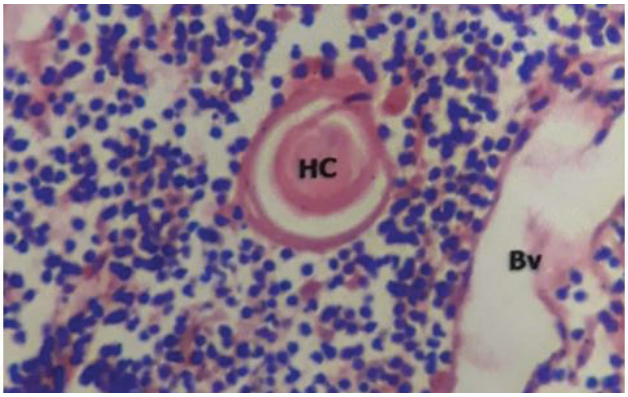

Figure 9 36-weeks, Hematoxylin and Eosin (x200). Hassall's Corpuscles (HC) and blood vessels (Bv) at cortico-medullary junction

The histometric analysis of parenchyma (cortex and medulla) and connective tissue indicates that there was no significant variation in their ratio (Table 6).

Table 6 Ratio of parenchyma and connective tissue

Group	Gestational Age (Weeks)	Connective Tissue (mm^3)	Parenchyma (Cortex+Medulla)/ Connective Tissue
		Mean ± SD	Mean ± SD
I	17-24	0.1528± 0.0707	6.6036 ± 2.816
II	25-30	0.1313 ± 0.0365	7.4296 ± 2.734
III	31-35	0.148 ± 0.0879	8.4040 ± 6.187
IV	36-40	0.135 ± 0.0274	6.6631 ± 1.553
Kruskall Wallis Test		0.197	0.177
p-value		0.976	0.981

Structure of Epithelial Reticular cells

Many epithelial reticular cells have been noted in thymuses of early and late phases in the subcapsular cortex (superficial cortex). They were numerous around the blood vessels. Epithelial reticular cells were also located in the deep cortex. The presence of epithelial reticular cells in the medulla was noticed where some were related to HC. The morphology of the epithelial reticular cells in the cortex and medulla appeared to be different.

The subcapsular epithelial reticular cells had large lightly stained euchromatic nuclei with conspicuous nucleoli. They showed slender amphophilic cytoplasmic processes with the indistinct cell membrane (Figures 10 and 11).

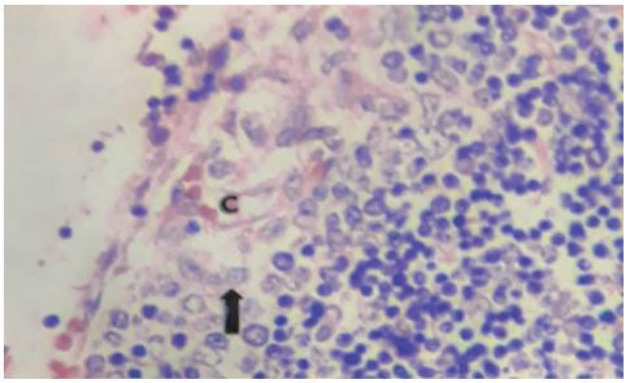

Figure 10 39-weeks, Hematoxylin and Eosin (x400). Epithelial reticular cells (arrow) in the subcapsular cortex. C-Capillary

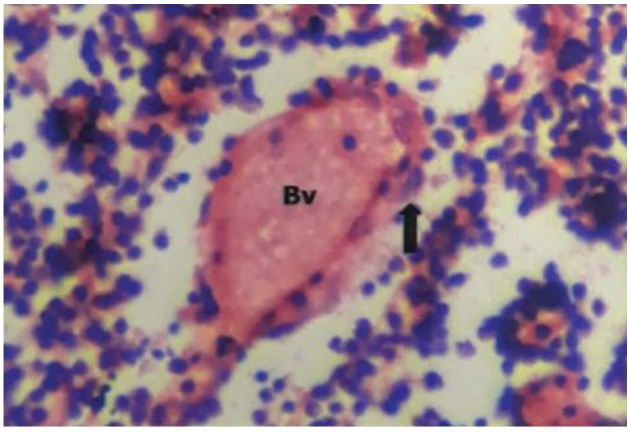

Figure 11 17-weeks, Hematoxylin and Eosin (x400). Epithelial reticular cells (arrow) around blood vessels (Bv)

Epithelial reticular cells present in the cortex were few, paler cells widely spread apart with thin cytoplasmic processes compared to the subcapsular cells. They also showed euchromatic nuclei with conspicuous nucleoli (Figure 12).

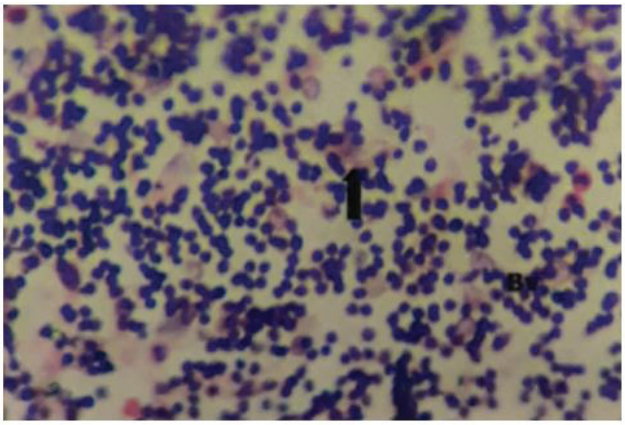

Figure 12 32-weeks, Hematoxylin and Eosin (x400). Epithelial reticular cells (arrow) in deep cortex

On the contrary, the medullary epithelial reticular cells had darkly stained heterchromatic nuclei with inconspicuous nucleoli. They showed acidophilic cytoplasmic processes and distinct cell membrane (Figures 13-15). The presence of a group of hypertrophied medullary epithelial reticular cells was seen in the process of forming HC.

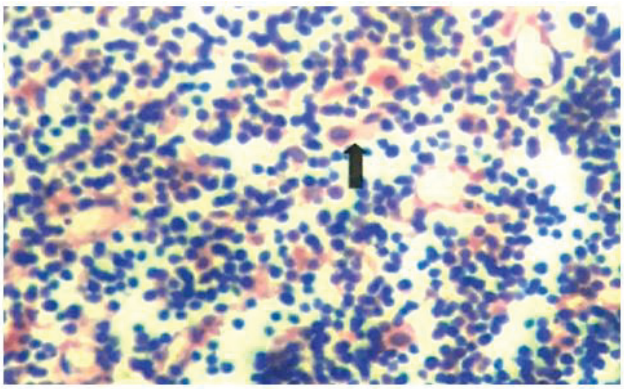

Figure 13 39-weeks, Hematoxylin and Eosin (x400). Epithelial reticular cells (arrow) in the medulla

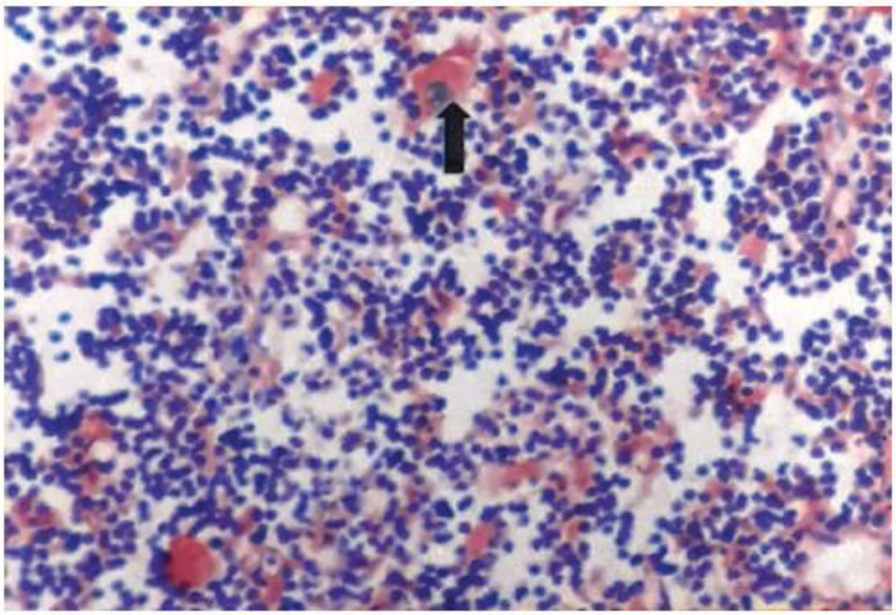

Figure 14 39-weeks, Hematoxylin and Eosin (x400). Hypertrophied epithelial reticular cells (arrow) tend to form HC's

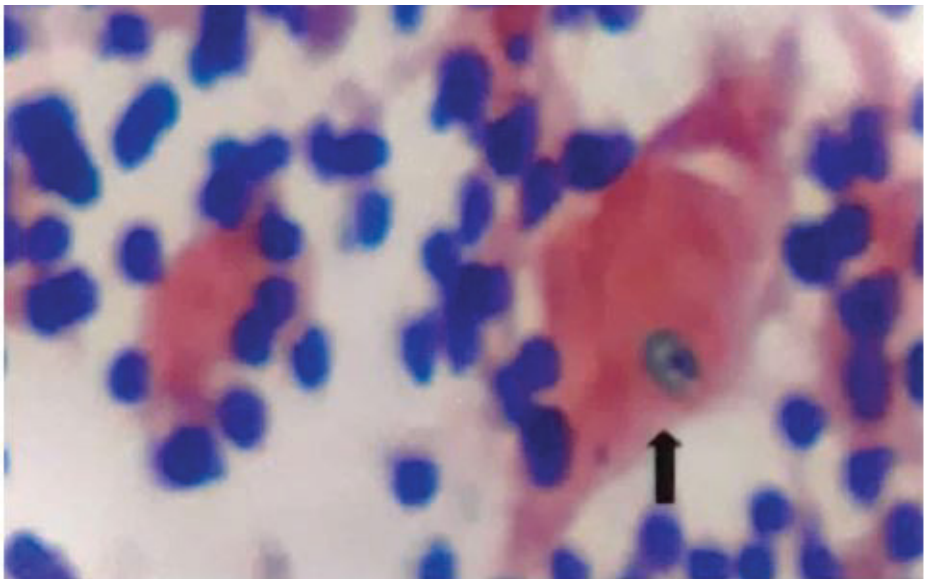

Figure 15 32-weeks, Hematoxylin and Eosin (x1000). Hypertrophied medullary epithelial reticular cells (arrow) shown in higher magnification

In addition to the above cells, two other cell population were noticed they were:

- Epithelial reticular cells with ingested or attached lymphocytes forming rosettes seen in the thymic cortex and cortico-medullary junction near blood vessels. These complexes were called as 'Thymic nurse like cells' (TNC) (Figure 16)

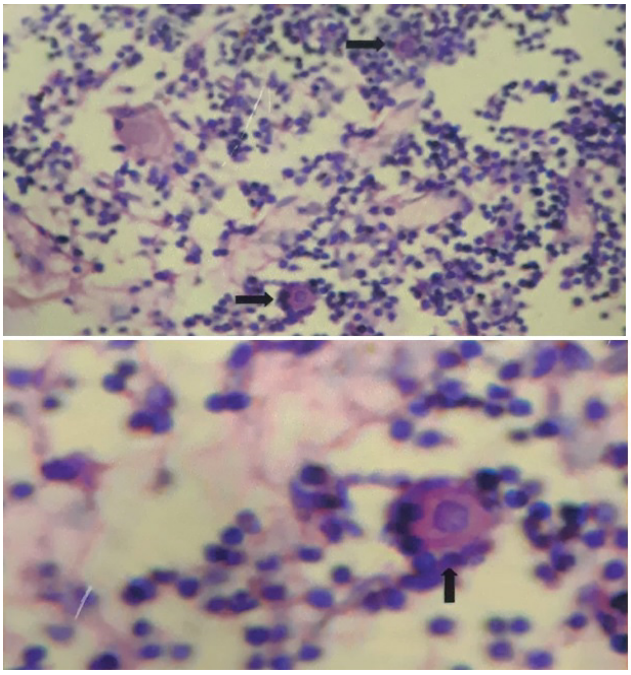

Figure 16 A: Arrow indicates thymic nurse like cells. (Hematoxylin and Eosin x400). B: Arrow indicates Thymic Nurse Like Cells in higher magnification. (Hematoxylin and Eosin x400)

- Few blasts like cells-with scanty cytoplasm and large nuclei with fine chromatin were seen in the subcapsular zone (Figure 17)

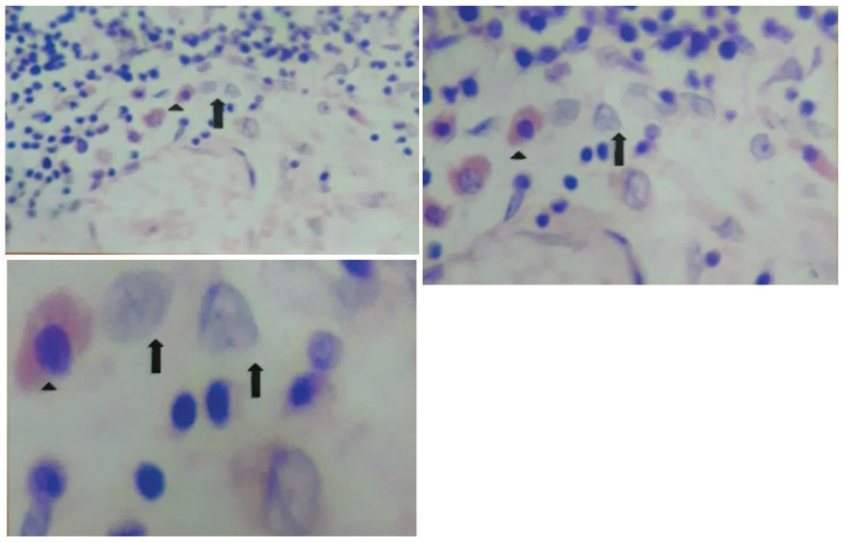

Figure 17 A: Hematoxylin and Eosin (x100), arrow: blast like cells, arrow head: thymic epithelial cell. B: Hematoxylin and Eosin (x100), arrow: blast like cells, arrow head: thymic epithelial cell. C: Hematoxylin and Eosin (x100), arrow: blast like cells, arrow head: thymic epithelial cell

Morphology of Hassall's Corpuscles

The presence of HC's is in the 17th week thymus which is the lowest age of foetus in this series was noticed. Many types of HC's with varying morphology were seen in thymuses within the age group of the present study.

Microscopic examination of a serial section of thymus revealed few patterns of shapes of HC's. While most of them were spherical and oval nevertheless fusiform and cylindrical forms were rarely encountered (Figures 18).

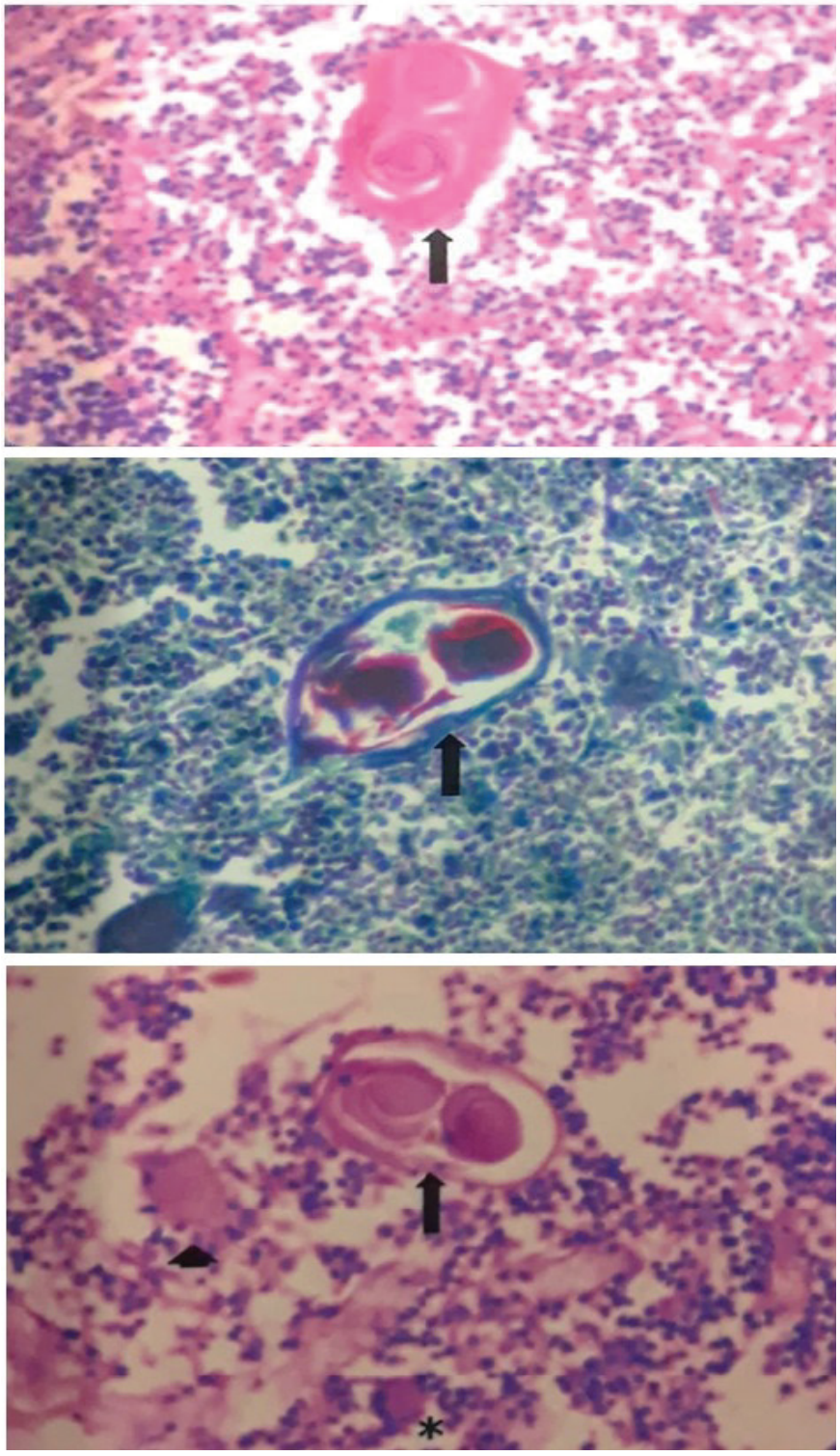

Figure 18 A: Hematoxylin and Eosin (x400). Fusiform type of HC seen containing two corpuscles enclosed in a single capsule (arrow). B: Masson's Trichrome (x400). Fusiform type of HC seen containing two corpuscles enclosed in a single capsule (arrow). C: Periodic Acid Schiff (x400). Fusiform type of HC seen containing two corpuscles enclosed in a single capsule (arrow). Also seen SHC (arrowhead) and TNC (*)

The fusiform ones often contain two corpuscles enclosed in a single capsule. Occasionally a bunch of HC of similar type (CHC II) which were interconnected with one another was also seen (Figure 19).

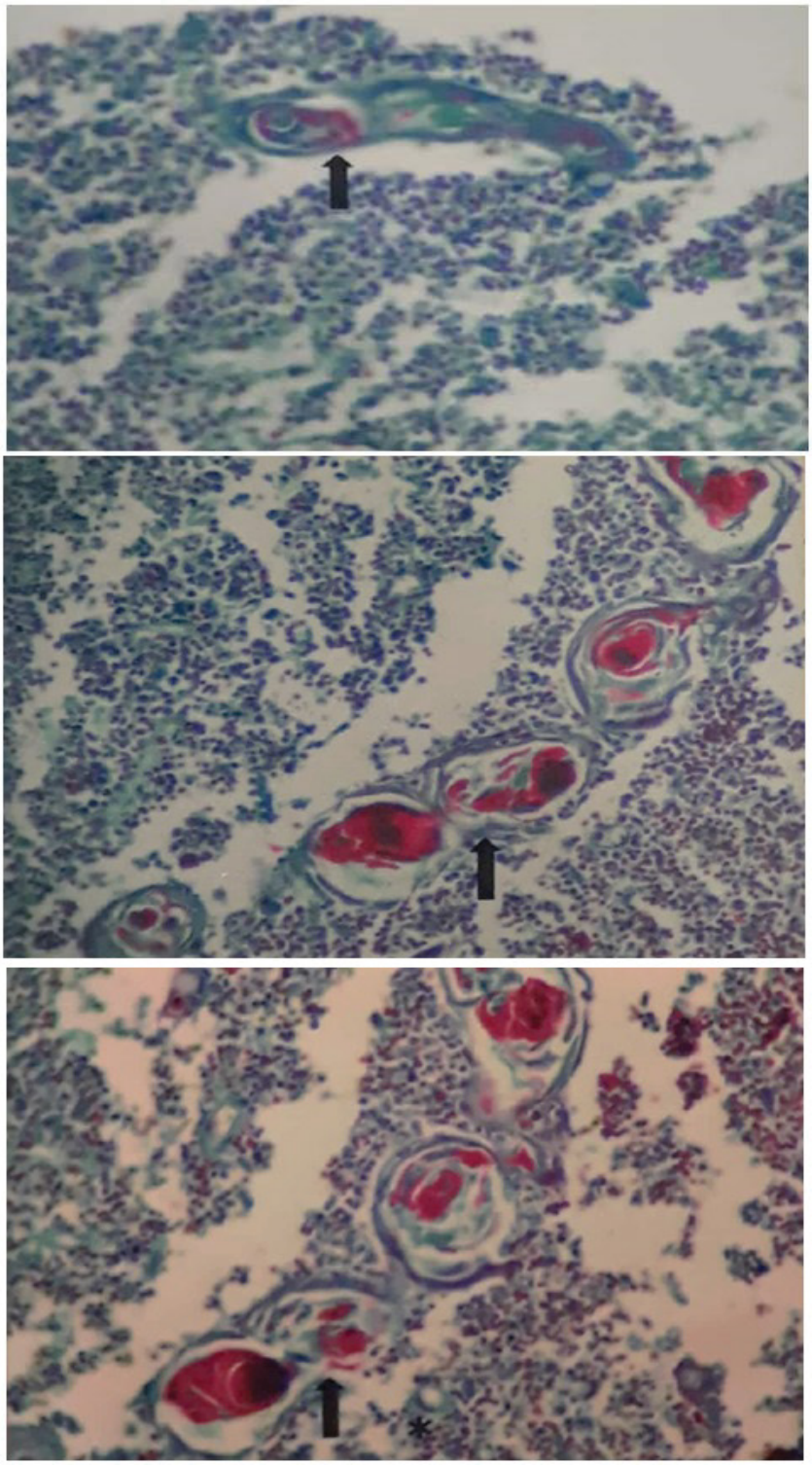

Figure 19 A: Masson's Trichrome (x400). Tangentially cut HC seen as tubular or cylindrical shaped structures (arrow). B: Masson's Trichrome (x400). Bunch of HC of similar type (CHC II) seen (arrow). C: Masson's Trichrome (x400). On serial section, the same bunch of HC mentioned above, are interconnected together (arrow)

Some corpuscles were made of a group of hypertrophied medullary epithelial reticular cells in the center bordered by few flattened epithelial cells (Figure 20).

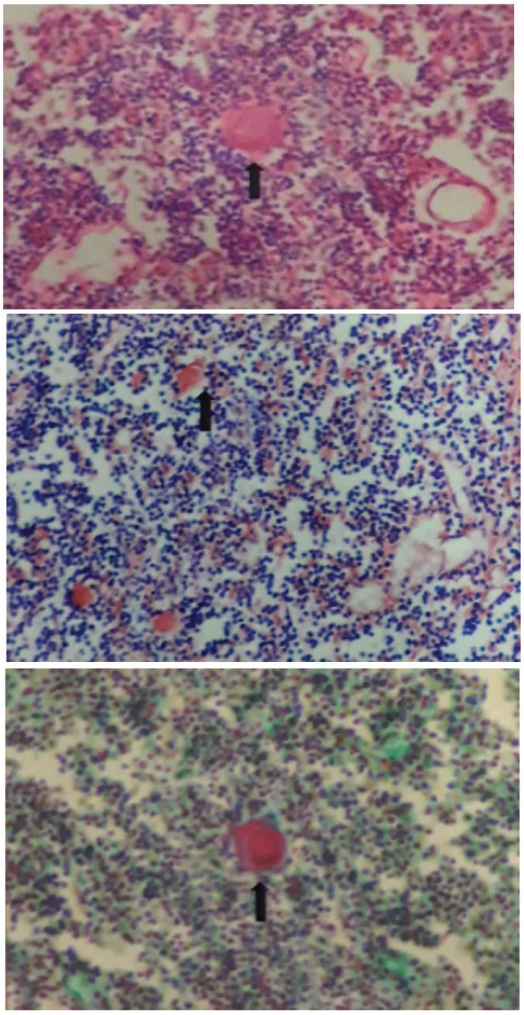

Figure 20 A: Hematoxylin and Eosin (x400). Solid corpuscles (SHC) seen close to the corticomedullary junction (arrow). B: Hematoxylin and Eosin (x400). SHC made of a hypertrophied epithelial reticular cell is seen (arrow). C: Hematoxylin and Eosin (x400). SHC made of a central hypertrophied epithelial reticular cell surrounded by a ring of flattened epithelial cells (arrow)

These corpuscles were frequently seen in thymuses of the early gestational period which were called as Solid Hassall Corpuscle (SHC) and were located at the periphery of the medulla within the age group of the present study. Their size ranged from 25-35 µm with a mean of 27.156 µm (Table 7).

Table 7 Diameter in different types of Hassall's corpuscles

Gestational Age (Weeks)	Solid (SHC) µm	Cystic I (CHC I) µm	Cystic (CHC II) µm
17	27.00	55.27	70.00
19	35.01	68.00	64.00
20	34.58	70.38	69.00
22	30.00	65.00	96.00
24	28.33	46.88	80.00
26	31.07	55.50	70.00
27	32.50	38.75	65.83
28	27.50	38.75	65.00
28	32.50	40.01	95.00
29	30.83	50.00	101.67
30	27.50	47.50	76.00

31	26.00	46.25	80.00
32	25.00	40.00	69.17
32	26.00	46.25	62.00
33	22.50	42.50	73.00
34	27.50	52.50	75.00
36	22.50	49.36	69.00
37	22.50	33.16	82.00
38	34.70	37.50	58.75
39	27.50	37.50	50.00
Mean	27.156	48.153	70.071

Certain corpuscles had a homogenous hyalinized eosinophilic mass in the center encircles by well defined, compactly packed concentric layers of epithelial cells which formed a capsule like structure. This epithelial capsule was separated from the central mass by a subcapsular space that gave a cyst like an appearance hence named primary cystic Hassall's corpuscle (CHC I) (Figure 21). Their size varied from 35-70 µm with a mean of 48.153 µm thickness (Table 7).

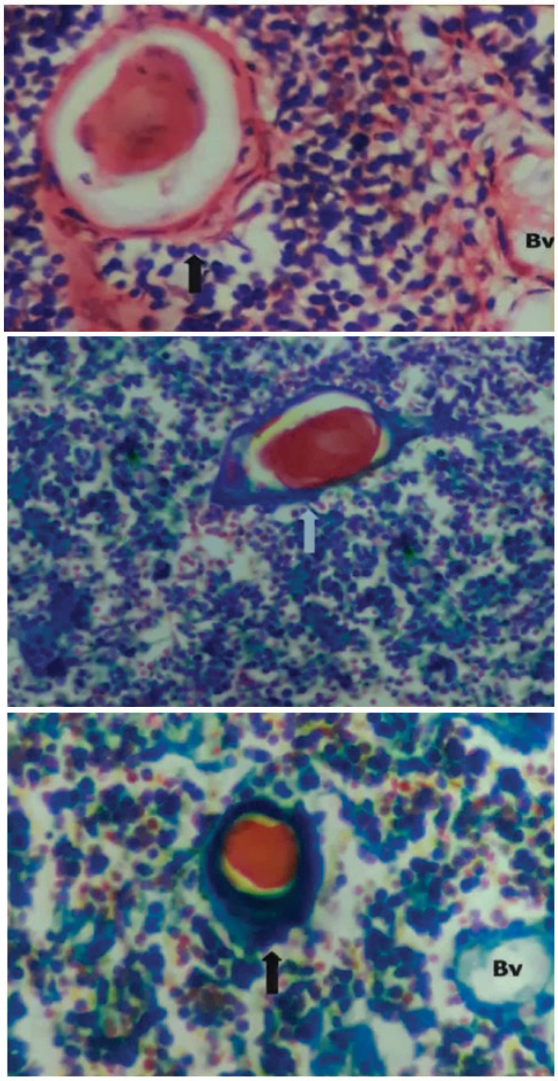

Figure 21 A: Hematoxylin and Eosin (x400). Primary cystic corpuscles (CHC I) made of hyalinized homogeneous acidophilic mass in the center surrounded by space of cyst, encapsulated by flat epithelial cells (arrow). B: Masson's Trichrome (x400) Primary cystic corpuscles (CHC I) shows a red color homogeneous mass in the center surrounded by space, encapsulated by greenish black capsule like structure (arrow). C: Masson's Trichrome (x400) CHC I near the corticomedullary junction (arrow). Bv: Blood vessels

The largest corpuscles were made of a central core of hyalinized mass surrounded by concentric layers of eosinophilic plates giving a whorl like or onion peel appearance with spaces among them, entrapping thymocytes and basophilic matrix (Figure 22).

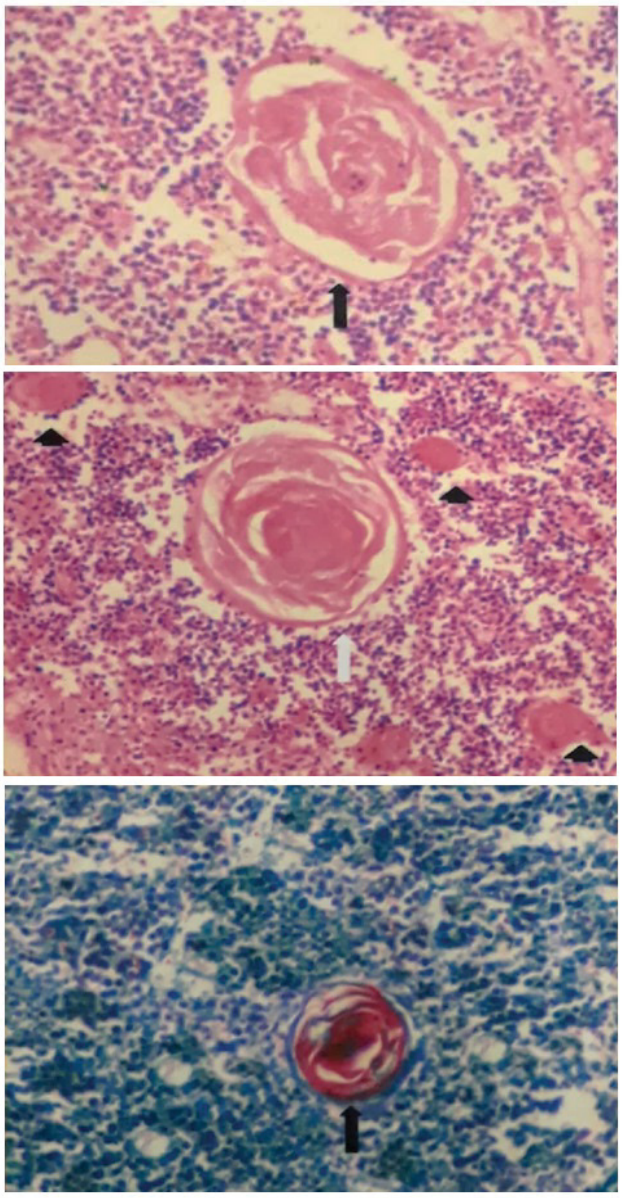

Figure 22 A: Hematoxylin and Eosin (x400) Secondary cystic corpuscles (CHC II) with whorled/onion peel appearance (tangentially cut) (arrow). Hematoxylin and Eosin (x400) Secondary cystic corpuscles (CHC II) with typical whorled/ onion peel appearance (arrow). And SHC is also seen around (arrow head). Masson's Trichrome (x400) CHC II, note the whorled (onion skin) appearance near the corticomedullary junction (arrow)

Externally the whole structure was surrounded by an epithelial capsule as found in CHC I, hence named as secondary cystic corpuscles (CHC II). They were mainly observed in the central core of the medulla. Their size ranged from 50-100 µm with a mean of 74.171 µm thickness (Table 7).

In a few corpuscles, the central core was replaced by pigmented basophilic material and fine collagen like fibers which was encircled by a collapsed epithelial capsule (Figure 23).

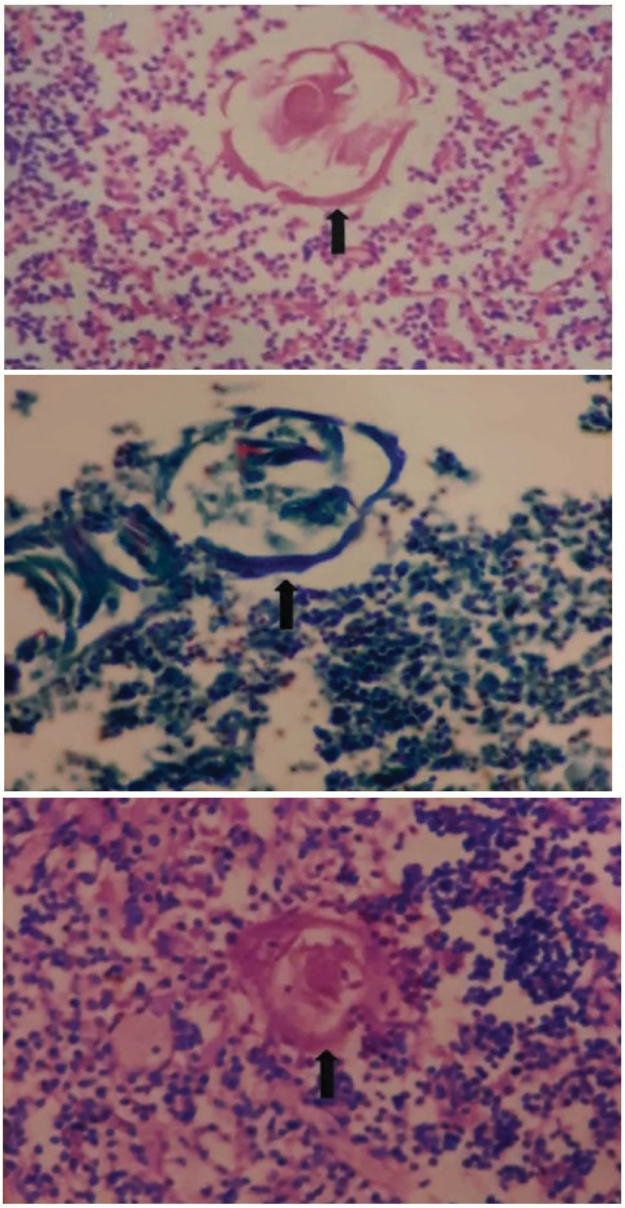

Figure 23 A: Hematoxylin and Eosin (x400). Degenerating corpuscle (DHC) shows amorphous acidophilic degenerating changes in the center and disruption of the capsule. However, an appreciation of DHC is difficult in H and E when compared with Trichrome stain (arrow). B: Masson's Trichrome (x400) Degenerating corpuscle (DHC) seen at the central core o the medulla with basophilic material and fine collagen like fibers encircles by a collapsed epithelial capsule (arrow). C: Periodic Acid Schiff (x400) DHC, seen near the corticomedullary junction (arrow)

These corpuscles show atrophic changes hence termed as degenerating Hassall's Corpuscles (DHC), both early and late stages were noticed. They were identified especially in the central core of the medulla from late gestational age. The diameter of Hassall corpuscle was calculated and readings are given in Table 7.

DISCUSSION

The present finding of the gradual increase in the thymic weight and volume related to gestational age is similar to findings of authors [13,14]. Researchers stated that cortex and medulla differentiation was observed from the 14th week which could not be confirmed as the fetal age in the present study started from 17th week [15-17]. However, the observation on the presence of lobulation, differentiation of the parenchyma in 17th gestational week thymus confirms the earlier findings [11,18-21].

Similarly, the observation on the presence of Hassall's Corpuscles in the 17th week of gestation confirmed the earlier findings [20,22-25]. There is an existence of a pale subcapsular zone at the periphery of the cortex till 39th week suggesting that the differentiation of cortex and medulla is not yet fully accomplished.

The mean volumes of cortex (0.4808 ± 0.7045 mm^3), medulla (0.386 ± 0.0674 mm^3) and their ratio (4.0490 ± 1.9824 mm^3) as well as the mean volumes of connective tissue (0.1413 ± 0.5692 mm^3) and the ratio between parenchyma (cortex and medulla) and connective tissue (7.3134 ± 3.5522 mm^3) in fetal thymus. These findings are dissimilar to the author stated that the cortex was 9 times more than the medulla which was reversed postnatally [26]. This difference of findings could be due to the inclusion of parameters like connective apart from parenchyma (cortex and medulla).

Although the histometric analysis of parenchyma and connective tissue indicates that there was no significant variation in their ratio but their histological observation showed a substantial difference. It is evident that the volume of mucoid connective tissue is more in the earlier phase than that of the late phase. This may be correlated to the functional importance of extracellular matrix, fibroblasts, and mesenchymal tissue in the development of T lymphocytes at early stages [27].

Early researchers have noticed the presence of adipose cells in adult thymus, their presence in a 39th gestational week is a feature hitherto not reported [28]. In the present study, thymus appears to be more vascular with increased number and size of blood vessels when compared to the earlier gestational group (Group I and II). The proximity of Hassall's Corpuscles (HC) with post capillary venules, the most permeable segment of the vasculature, may be a significant feature of the thymic microenvironment with important functional consequences [29].

Analogous to the liver, where hepatocytes closest to portal venules are exposed to the highest concentration of blood borne nutrients from the gut [30], HC adjacent to post capillary venules would likely be exposed to high intrathymic levels of blood borne self-antigens [31].

The different epithelial reticular cells observed with different morphology may have various functional correlations. The morphology of subcapsular and deep cortical epithelial cells with large lightly stained euchromatic nuclei and conspicuous nucleoli tends to suggest that they are immature and metabolically active. These two cortical epithelial cells may be involved in blood thymus barrier and may interact with infected cells. On the other hand, the other two medullary epithelial reticular cells with darkly stained heterochromatic nuclei and inconspicuous nucleoli are mature and may be involved in structural support and formation of HC's [32]. Studies remarked that the cortical epithelial cells seemed to be arranged in a graded morphological series which extended from cells in the subcapsular region right across the cortex into some of the medullary epithelial cells. These morphologically distinct epithelial cells help in creating a microenvironment where T-cell maturation occurs.

The presence of positive periodic acid Schiff reaction exhibited by the cytoplasm of epithelial reticular cells is similar which could be related to its morphology and function [25,33]. The features associated with protein synthesis observed in cellular types 2 and 6 make them likely candidates for humoral factor producing and/or secreting elements.

Presence of the blast like cells seen in the subcapsular zone is synchronous [34]. According to the author, the presence of such cells supports the unifying concept of the origin of the thymic epithelium from step cells and their ability to differentiate into other epithelial cell forms and the possibility of niches around that region.

In the present finding, the epithelial-lymphocyte interaction probably thymic nurse cells is similar to Kendall MD, and Kaymaz, et al., [28,35]. The thymic nurse cells were noticed mainly in the cortico medullary junction whereas earlier authors observed it at the cortex. Their location close to adjacent blood capillaries infers that they are involved in lymphocyte traffic in the thymus [32].

In the present study, 4 different types of HC (SHC, CHC I, CHC II and DHC) in the fetus is seen whereas the author reported 4 types in the adult [9]. The presence of Solid HC at the periphery of the medulla and degenerating HC at the central core of the medulla tends to postulate the direction of maturation from the periphery to the center of the medulla.

CONCLUSION

The findings of the present study are in conformity with studied related to the volume and size of the thymus with respect to gestational ages as well as with previously mentioned histological features. However, the present noted

different types of HC observed which is reported in the adult thymus. The presence of Solid Hassall's Corpuscles (SHC) at the periphery of the medulla and secondary Cystic Hassall's Corpuscles (CHC II) and Degenerating Hassall's Corpuscles (DHC) in the central core of the medulla tends to suggest that the line of maturation is from the periphery to the center of the medulla.

The study has its limitation due to less number of thymus studies and further advanced morphometric studies using images and confocal laser microscopy is needed to do an in-depth analysis of the present histometric findings. However, the present histological and histometric observations of human fetal thymus can be used as a reference note for further studies in the specific population.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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