

Original article

Immunohistochemical association with histological features of Hassall's corpuscles in human fetal thymus

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Abstract:

Background - Hassall's corpuscles (HC) are balls of flattened medullary epithelial cells which are characteristic features of the thymus. Thymus is truly an 'organ of mystery' which has kept scholars in dark still continues to elucidate researchers till date. Aim- To study the microscopic features of human fetal thymus along with the differentiation and maturation process of the Hassall's Corpuscles (HC) in human fetal thymus by using the immunohistochemical markers. **Materials & Methods**- 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. Another set of microscopic slides were subjected to immunohistochemical studies by staining with primary antibodies (Cytokeratin, S100 and Vimentin) to study the presence of epithelial, histiocytic and mesenchymal components in fetal thymus and to know the nature of differentiation of the Hassall's corpuscles, epithelial reticular cells and dendritic cells in the fetal thymus. **Observations & Results:** Microscopic examination of serial section of thymus revealed few patterns of shapes of HC's. Based on the morphological features, the corpuscles were classified as Solid Hassall Corpuscles (SHC), Cystic I (CHCI) and Cystic II (CHC II) and Degenerating (DHC). Epithelial cells of the HC were positive for cytokeratin which was less intensely positive in Solid Hassall Corpuscles (SHC) and intensely positive in primary and secondary cystic Hassall Corpuscles (CHC I and CHC II). The solid corpuscles (SHC) with homogenous mass were seen to be intensely positive whereas the central core in cystic varieties (CHC I & CHC II) seems to be less positive with S 100 (marker for dendritic cells, histiocytes and keratinocytes). Vimentin is intermediate cytoplasmic proteins expressed in the cells of mesenchymal differentiation found negative in the complete sections studied. **Conclusion:** The various types of HC encountered with different structural morphology could represent a stage of maturation in the developmental process. Hence HCs' were arranged in a sequential order of development stages within the age group of the present study. The presence of Solid Hassall's Corpuscle at the periphery of the medulla and Degenerating Hassall's Corpuscle (DHC) at the central core of the medulla confirms the direction of maturation is from the periphery to the center of the medulla. Hence there is gradual increase in the expression of cytokeratin from solid to cystic Hassall's corpuscles.

Keywords: Hassall's corpuscles; Immunohistochemistry; Solid Hassall Corpuscle; Degenerating Hassall Corpuscle; Thymus microscopy

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Introduction:

Each lobe of thymus is surrounded by loose connective tissue capsule from which septae penetrate to junction of the cortex and medulla, to partially separate them into irregular lobules. Each thymic lobule consists of

an outer cortex with densely packed cells mainly of T – lymphocyte lineage, the thymocytes and an inner medulla with fewer lymphoid cells. The medulla of one lobule is continuous with medulla of the other through a central medullary zone, when sections are

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observed serially.

Thymus is permeated by a network of interconnected thymic epitheliocytes; between which lodge lymphoid and other cells. Epitheliocytes appears in various size and shapes in the diverse 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 Hassall's corpuscles).

Hassall (1849) first described the onion pattern of thymic corpuscles¹. Hassall Corpuscle occurred due to the narrowing of sclerosed blood vessels². Epithelial cells forming the corpuscles are cells which have lost this function of forming a living surface as do epithelial cells elsewhere in the body. Hence, these epithelial cells, as seen in the thymus, undergo hyalinization³.

Studies pointed out probable secretory phenomenon by epithelial cells of thymic corpuscle⁴. Epithelial cells of the medulla are glandular cells by being truly epithelial and possess many of the cytological features of the secretory cells⁵. With advancing age, they produce cysts and cystic foci of degeneration, namely thymic corpuscles. Three types of cytoplasmic inclusions in the medullary epithelial cells as secretory products were described. Furthermore, three stages in the formation of thymic corpuscles were identified: 1. Stage of secretion with follicles, 2. Stage of absorption with disrupted follicle, 3. Stage of hyalinization⁶.

Histochemically rich content of basic non – histone protein, PAS positive substance (glycogen and acid mucopolysaccharides) is detected within HC⁷. HC's usually undergo hypertrophy before inclusion in the outer cell layer of the corpuscles and are structurally organized from medullary reticular epithelial cells. The secretory nature of HC was determined by PAS staining^{8,9}.

HC are composed of concentrically arranged epithelial cells, the more peripheral epithelial cells of the corpuscles often demonstrate cytoplasmic processes connected by desmosomes to processes of medullary epithelial cells which are not a part of the corpuscle. Lymphocytes and macrophages are occasionally observed in HC. The center of corpuscle is either solid or cystic. When cystic there is usually cell debris within the cyst lumen, although an occasional cyst may be empty. Degenerative nuclear and cytoplasmic changes are common in the central cells of the corpuscles¹⁰.

Areas of prevailingly solid (SHC) and prevailingly cystic (CHC) HC's in prenatal thymuses were discovered. Mean areas of the HC's increased with the fetal age, with the greatest difference determined between 16th – 19th week and 20th – 23rd week fetal age groups¹¹. Another study revealed that HC's increased in number and size during 17th – 24th week¹².

There is similarity in the staining reactions of the epithelial cells of thymic corpuscles and cells of skin¹³. The study of HC tends to support this view as epidermis (ectodermal origin) is positive with monoclonal antibodies MR14 and MR19 which also react with cells around HC. Immunoreactivity of outer cell layer of Hassall bodies with antibodies is similar to epidermal granulososa cell layer¹⁴.

Thorough immunohistochemical analysis on the relationship of the keratinization process in skin and thymus by studying 67 and 55 KD keratins showed certain keratin antisera are markers for HC's and epithelial reticular cells¹⁵. Studies found some 30, 36, 34 and 70 KD proteins associated with keratohyaline granules in both skin and HC's of the rat^{16,17}. Four different epithelial cell types according to their varied reaction to specific antibodies were identified¹⁸.

Study confirmed the deep similarity between keratinization process of the skin and thymic corpuscles¹⁹. Filaggrin, a marker for advances stage of keratinization was distinguished in HC's by histochemical and Immunohistochemical technique. Moreover, different patterns HC's at different developmental stages in postnatal human thymus and mammals were found. More intensive and homogeneously stained corpuscles were found in their initial formation of development stages than secondary or cystic corpuscles.

During fetal development, the keratin composition of thymic epithelium changes from staining predominantly with low to high molecular weight keratin²⁰. Also epithelial proliferation is an important component of thymic hyperplasia along with B cell proliferation.

HC derived from endocrine medullary thymic epithelium are antigenically distinct regions of endocrine thymic epithelium²¹. HC represent end stages of maturation of thymic medullary epithelium²². Lympho-epithelial content within elongated and ovoid spherical cysts, structurally and antigenically reflect similar to cortical parenchyma²³.

Anti cytokeratin antibodies with single cytokeratin can be regarded as a useful tool to study the heterogeneity

of thymomas²⁴. Study reported positively staining cells for Cytokeratin at 13 weeks of gestation²⁵. The immunohistochemical study in postnatal normal human thymus showed existence of four different forms of HC namely juvenile, immature, mature and senescent^{26,27}. On immunohistochemical staining, the following reactions were negative: cytokeratin 7 & 8, vimentin, desmin, CD 3, CD 68, CD 34, and neuron specific enolase. Two cases showed isolated positive chromogranin cells and one case showed positive intracorpuseular CD 20 cells. In the epithelial cells of the HC's polyclonal cytokeratin was positive. In high molecular weight cytokeratin it is strongly expressed in mature corpuscles. Within the corpuscles, distributed among the epithelial cells, having dendritic morphology positive S 100 cells demonstrated in all specimens. Positive S 100 cells in HC in juvenile and immature forms were detected in the present study.

In a recent study of cytokeratin patterns on human fetal thymus showed cytokeratin (CK) 5, & 8 positive from 13 weeks and cytokeratin 14 was seen after 16 weeks. CK8 and CK14 positivity was seen around the periphery, while CK5 positivity was observed both at periphery and central part of HC²⁸.

The functions of Hassall's corpuscles remain still an enigma²⁶. Proposed functions include; 1) site of thymocytes death^{10,23,29,30,31}, 2) production or storage of antigen, 3) antibodies^{29,32}, 4) site of thymic hormone production^{33,34,35}, 5) remnants of the thymic primordium without any significant function^{36,37}, 6) lymphocyte rich HC's is involved in negative selection of thymocytes²⁶, 7) differentiation of thymocytes at medulla³⁸, 8) Removal of apoptic thymocytes and maturation of developing thymocytes³⁹.

However, controversies still exist in relations to their structural heterogeneity, maturation and their line of differentiation. In the present study, the microscopic features of human fetal thymus are correlated with differentiation and maturation process of the Hassall's Corpuscles (HC) in human fetal thymus by using the Immunohistochemical markers such as cytokeratin, S100 and Vimentin.

Materials and Methods:

Collection of Fetuses: Twenty aborted and stillborn fetuses varying from 17 – 39 gestational weeks were obtained from the department of Obstetrics and Gynecology, Rajah Muthiah Medical College, Tamil Nadu, India. Information gathering the mother especially pertaining to parity, date of last menstrual

period (LMP), consanguinity, duration of pregnancy, exposure to radiation, drugs intake, previous miscarriages and mode of terminations was obtained from the case records and entered in present study proforma. 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 LMP⁴⁰. Every aborted and stillborn fetus was subjected to detailed physical examination and any fetus with external anomalies was excluded. They were segregated into four groups based on CRL and gestational age as illustrated in the Table I. Two groups were considered together (Group I and II) called as early phase and Group III and IV as late phase for comparative purposes.

Table I – 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

Embalming: All fetuses were embalmed by means of multiple local and cavity injections of embalming fluid using 100 cc syringe & 8 – 9 gauge needle. The quantum of fluid depending on the fetal body weight was injected (250 ml/kg body weight)⁴¹. The fetuses were well labeled & preserved in jars filled with the embalming fluid. Thymus was dissected and tissue processed for histological studies⁹.

Tissue Processing for Immunohistochemical Studies:

Tissues were processed in the same way as for paraffin embedding. Thin sections 4 – 5 μ m thickness were cut with a Leica rotary microtome and subjected to immunohistochemical technique (super sensitive polymer HRP methods). Paraffin infiltration and block making procedure done then sections were flattened and mounted on Poly – L – Lysine coated slides and incubated at 37 degrees Celsius for one day and further kept for 58 degrees Celsius overnight.

Immunohistochemistry is the technique used to detect and localize antigens by means of labelled antibodies through Ag – Ab interactions that are visualized by enzyme – substrate – chromogen reaction. In this technique, the positive area of specific antigen appears brown in color and nuclei appear blue. The line of differentiation (epithelial, histiocytic and mesenchymal) is identified by the antibody binding against the expressed cytoplasmic intermediate

filamentous proteins such as Cytokeratin, S100 and Vimentin in the particular cells. In these techniques an enzyme labelled antibody is used to link a cellular antigen specifically to a chromogen that can be more readily visualized under light microscope.

The microscopic slides were stained with primary antibodies (Cytokeratin, S100 and Vimentin) to study the presence of epithelial, histiocytic and mesenchymal components in fetal thymus and to know the nature of differentiation of the Hassall's corpuscles, epithelial reticular cells and dendritic cells in the fetal thymus.

Ethical clearance: This study was approved by Rajah Muthiah Medical College Tamil Nadu, India.

Results & Observations:

Histological and Histometric observation of Hassall's Corpuscles: Based on the morphological features, the corpuscles were classified as Solid Hassall Corpuscles (SHC), Cystic I (CSC I) and Cystic II(CSC II) and Degenerating (DHC)⁹.

Hassall's corpuscles were well distinguished with Masson's Trichrome staining where the composition of the central mass of HC is keratin (red stained) and outer capsule is made of epithelial cells (Figure 1a & 1b).

There were various types of HC with different structural morphology which could represent a stage of maturation in the developmental process. Hence, an arrangement in a sequential order of development stages is made within the present study age group (Figure 2).

Immunohistochemical observations of Hassall's Corpuscles: Cytokeratin is an intermediate cytoplasmic protein expressed in the epithelial cells and it represents a good marker for epithelial differentiation. Antibody against cytokeratin was applied to demonstrate cytokeratin in Hassall's corpuscles. Epithelial cells of the HC were positive for cytokeratin (Figure 3a) which was less intensely positive in Solid Hassall Corpuscles (SHC) and intensely positive in primary and secondary cystic Hassall Corpuscles (CHC I and CHC II) (Figure 3b and 3c).

S100 is an acidic, dimeric calcium binding proteins expressed mainly in dendritic cells, histocytes and keratinocytes. In the present study, it was positive in many cells like Hassall corpuscles, epithelial reticular cells and cells with dendritic morphology (Figure 4a). In all age groups, all types of Hassall corpuscles were seen positive. The solid corpuscles (SHC) with homogenous mass were seen to be intensely positive whereas the central core in cystic varieties (CHC I & CHC II) seems to be less positive (Figure 4b and 4c). The lowest gestational age group fetus of our series (17 weeks) was seen to be positive with S 100.

Vimentin is intermediate cytoplasmic proteins expressed in the cells of mesenchymal differentiation and found negative in the complete sections studied (Figure 5a and 5b).

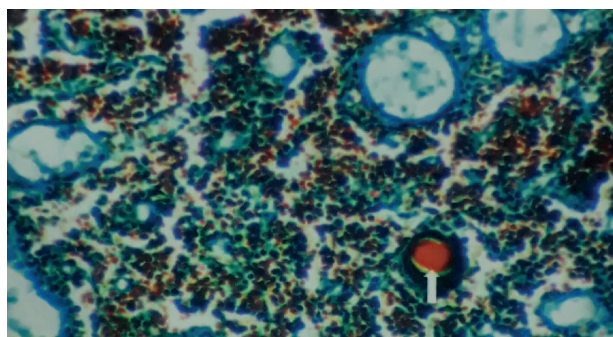


Figure 1a – Arrow indicate Hassall's Corpuscles. (Masson Trichrome x200)

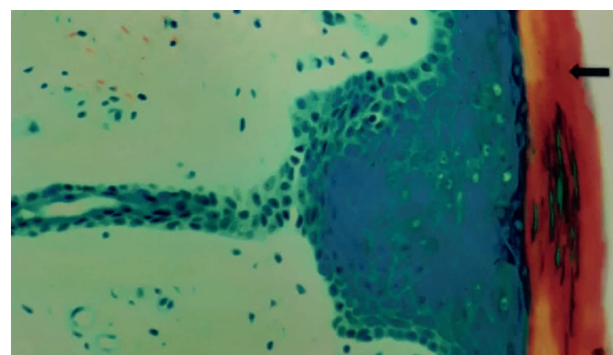


Figure 1b – Skin – Stratified squamous epithelium showing keratinization, Arrow indicates the keratin. (Masson Trichrome x200)

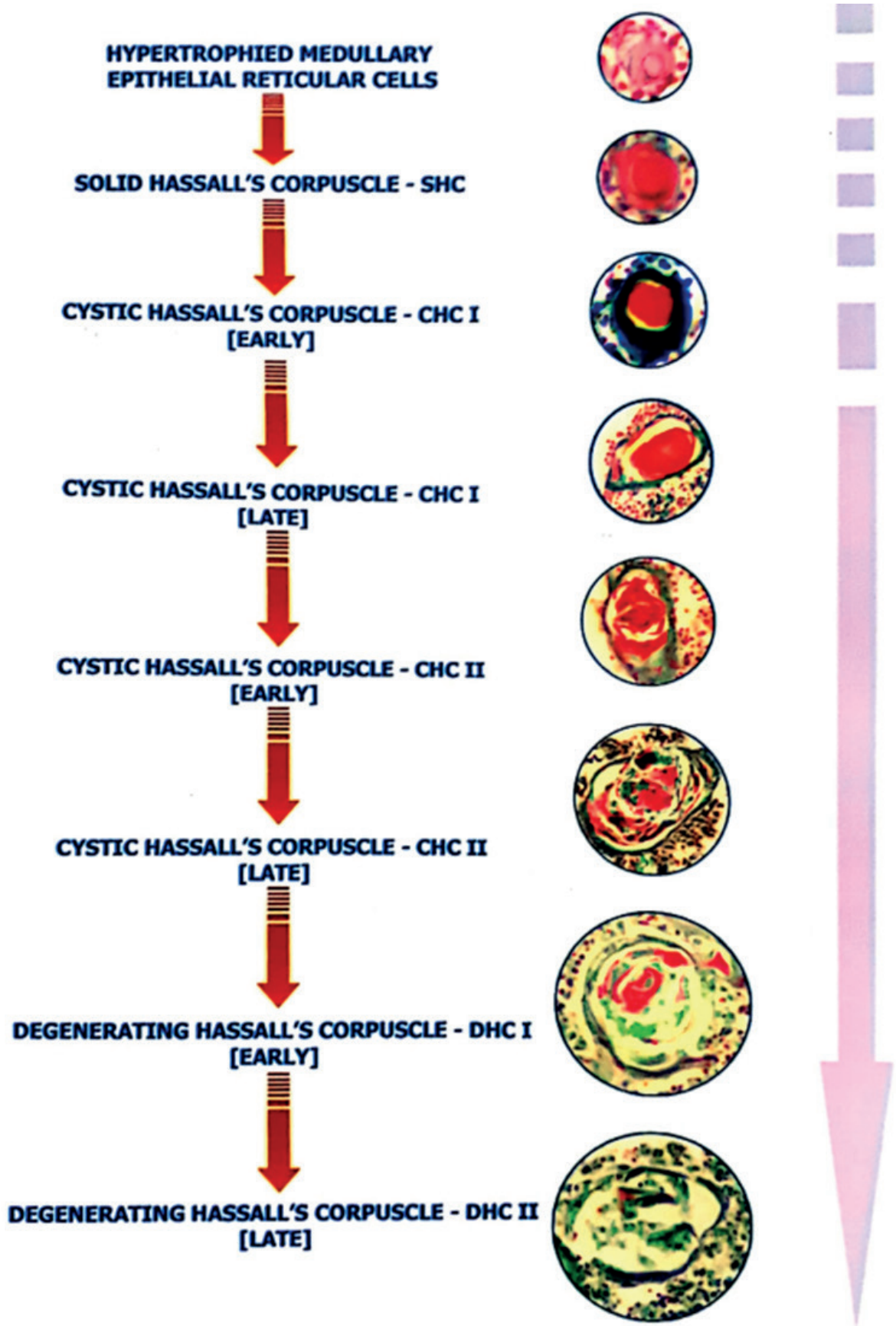
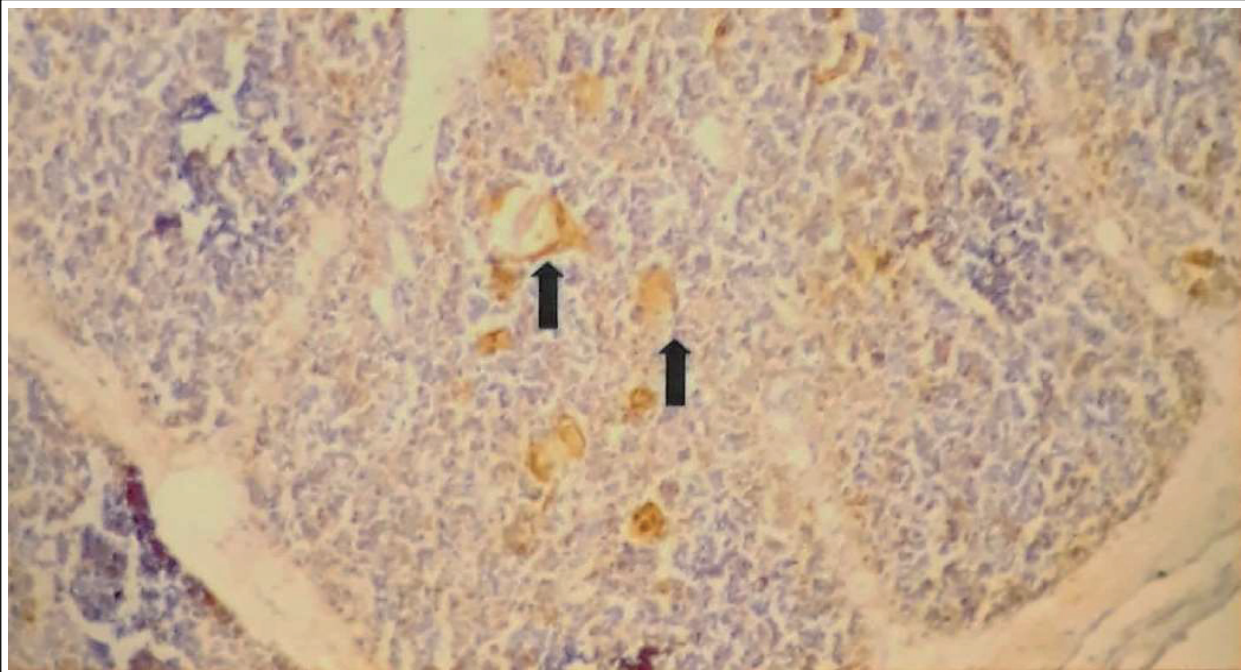
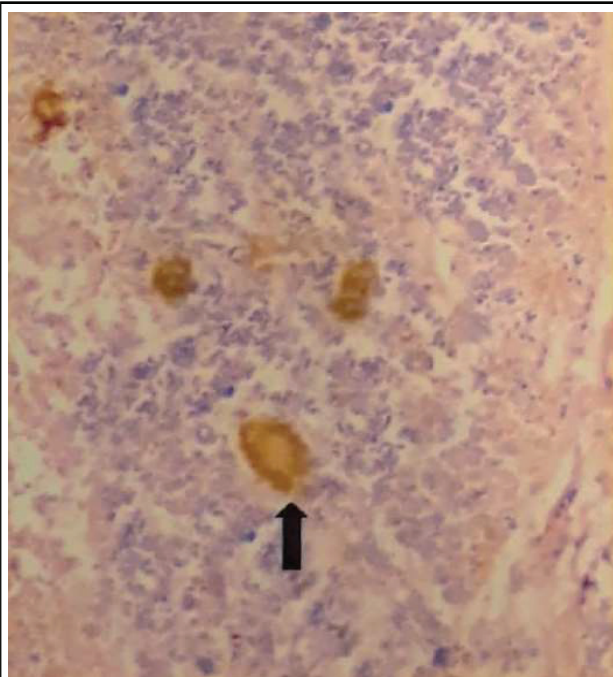


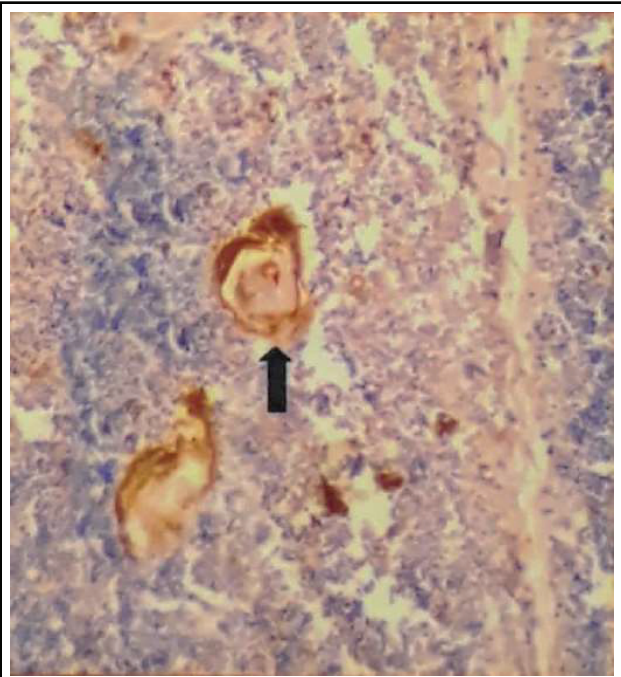
Figure 2: Comparison of Hassall's Corpuscles with keratinizing stratified squamous epithelium (skin)



**Figure 3a – Cytokeratin positive (brown) in all types of Hassall's Corpuscles (arrow).
(Immunohistochemistry: Cytokeratin x100)**



**Figure 3b – Cytokeratin, less intensive positive (brown) in Solid HC (arrow)
(Immunohistochemistry: Cytokeratin x400)**



**Figure 3c – Cytokeratin, intensively positive (brown) in Cystic HC (arrow)
(Immunohistochemistry: Cytokeratin x400)**

(Figure 3a,3b,3c) Immunohistochemical Analysis of Cytokeratin

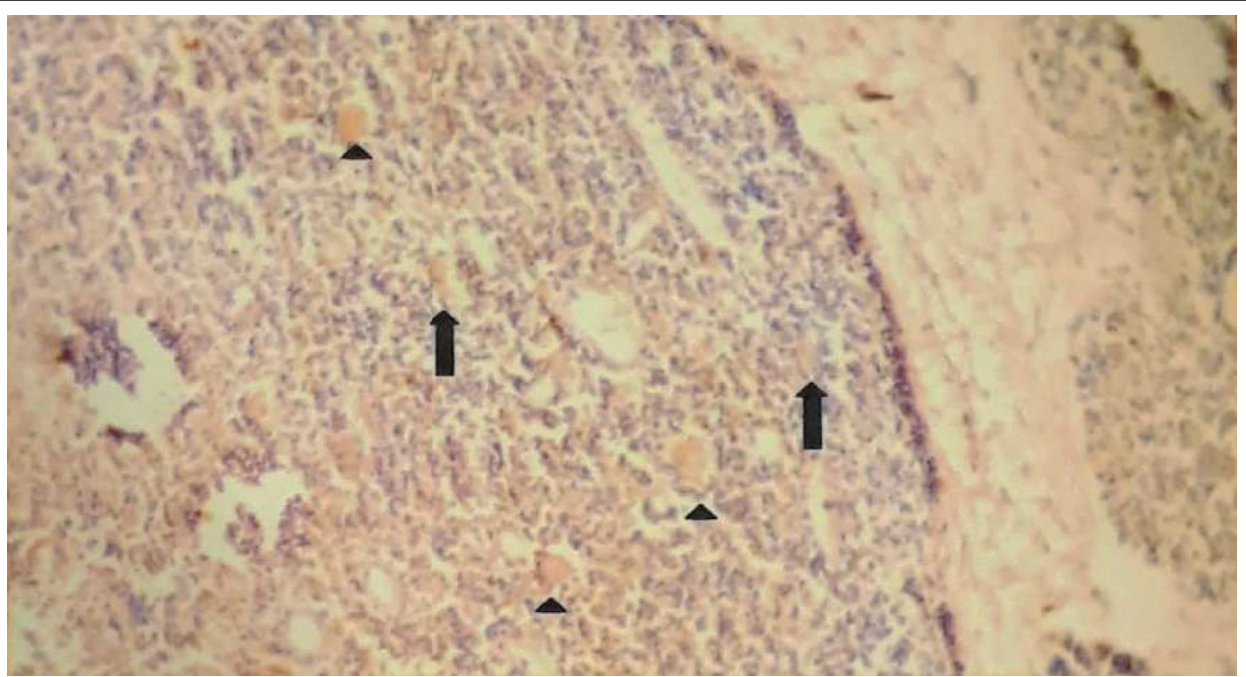


Figure 4a – S100 positive (brown) in all types of Hassall's Corpuscles (arrow head), epithelial reticular cells and cells with dendritic morphology (arrow). (Immunohistochemistry: S100 x100)

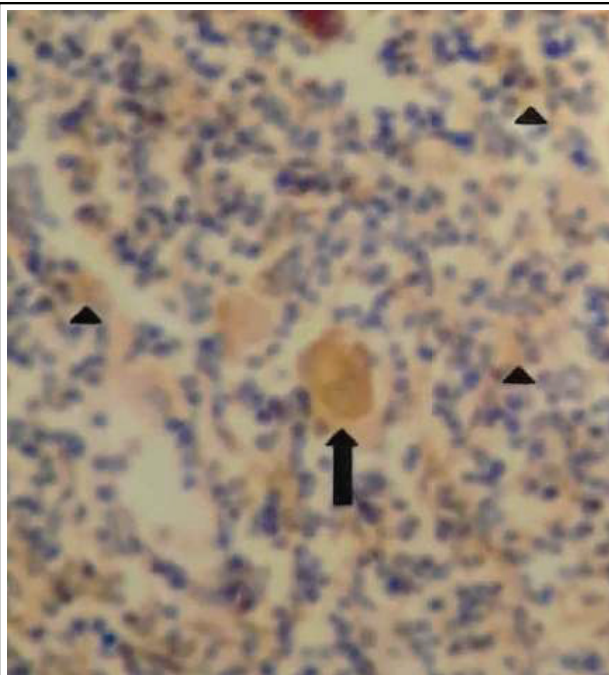


Figure 4b – S100, intensive positive (brown) in Solid HC (arrow), cells with dendritic morphology (arrow head). (Immunohistochemistry: S100 x400)

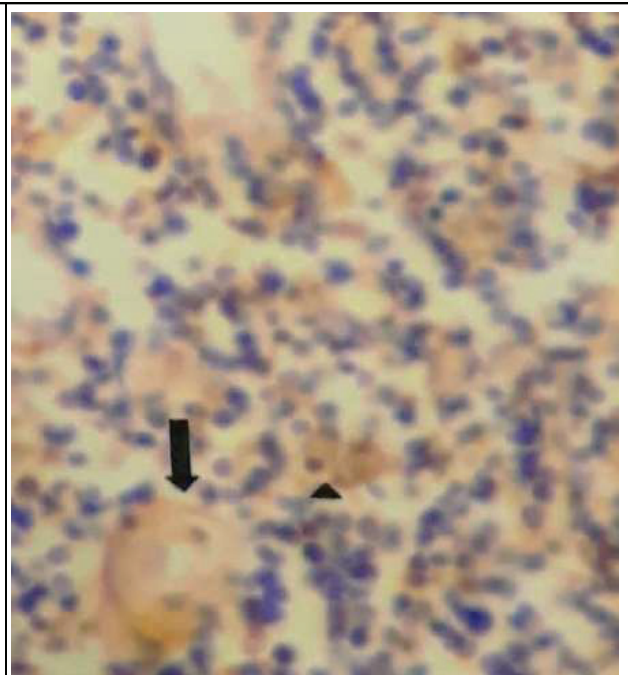


Figure 4c – S100, less intensively positive (brown) in Cystic HC (arrow)& cells with dendritic morphology, intense positivity (arrow head). (Immunohistochemistry: S100 x400)

(Figure 4a,4b,4c) Immunohistochemical Analysis of S – 100

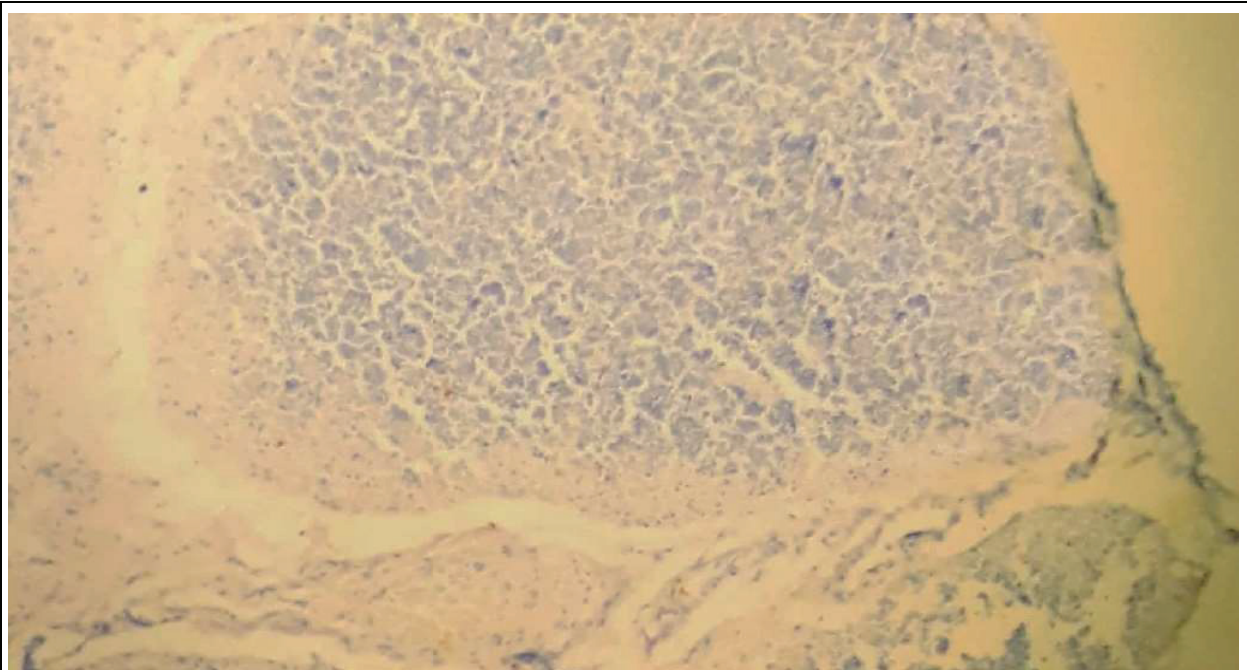


Figure 5a – Vimentin negative (no brown) in the entire tissue section. Nuclei – Blue. (Immunohistochemistry: Vimentin x100)

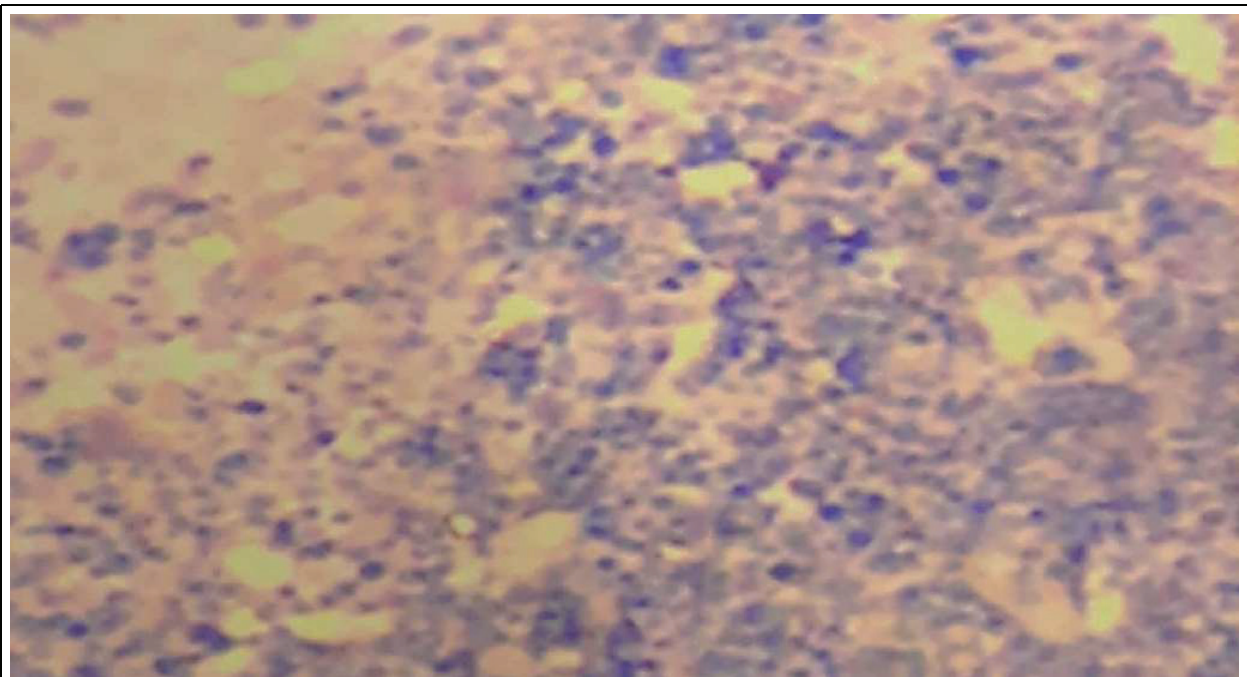


Figure 5b – Vimentin negative (no brown) shown in higher magnification. (Immunohistochemistry: Vimentin x400)

(Figure 5a & 5b) Immunohistochemical Analysis of Vimentin

Discussion:

The keratinization process of HC is similar to the granulosal layer of the epidermis^{14,15,16,17,19,44}. The presence of four different types of HC (SHC, CHC I, CHC II and DHC) earlier reported in adult and later in fetus^{26,45}. The present findings of hypertrophied medullary epithelial reticular cells, SHC, SHC to become cystic, CHC I, CHC II (early – granular appearance, late – whorled appearance), DHC (early and late) suggests the maturational stages of HC's. The presence of Solid Hassall's Corpuscle at the periphery of the medulla and Degenerating Hassall's Corpuscle at the central core of the medulla confirms that the direction of maturation is from the periphery to the center of the medulla.

The immunohistochemical observation on Hassall's corpuscle showed positive reaction to Cytokeratin, S100 and negative reaction to Vimentin which is similar to the findings in postnatal thymus^{26,28}. Cytokeratin expression in the epithelial cells of the Hassall's corpuscles showed less intensity in the Solid HC's (SHC) and high intensity in the primary cystic (CHC I) and secondary cystic (CHC II) Hassall's Corpuscles. It confirms their differentiation towards the epithelial type and maturation goes in order from the solid to cystic. This correlates with the histological findings of the HC⁹.

S 100 expression was seen in the outer layer of the HC's and cells with dendritic morphology. It was observed in all the gestational weeks of the fetuses. The positivity was high intense in the SHC and less intense in the CHC I and CHC II which confirms the presence of dual differentiation (Epithelial – Reticular) but the difference in the intensity of positive reaction suggests that reticular activity is more active in the SHC than compared with CHC I and CHC II. It again suggests that active involvement of Hassall's Corpuscles in modulating the differentiation of thymocytes formation in the early stages of HC's and it tends to decrease in the late stages. Even earlier studies also suggested that modulating function of

HCs' is mediated by the protein S100²⁶.

Vimentin was not expressed in any of the components of the fetal thymus studies is similar to the previous study²⁵. It suggests that there is no mesenchymal differentiation in the thymic tissue components, however to confirm this statement further studies are required with other mesenchymal markers on the fetal thymus.

Summary and Conclusion:

In the present study, different types of Hassall's corpuscles were observed in fetal thymus. The presence of Solid Hassall's Corpuscles (SHC) at the periphery of medulla whereas Secondary Cystic Corpuscles (CHC II) and Degenerating Corpuscles (DHC) in the central core of the medulla confirms that the line of maturation is from periphery to the center of the medulla. Hence there is gradual increase in the expression of cytokeratin from solid to cystic Hassall's corpuscles. The Hassall's corpuscles sequential stages of differentiation and maturation also supported by the immunohistochemical markers provides additional value to the existing human fetal thymus literature and this study enriches the information on the Hassall's Corpuscles structure.

Authors' contribution:

Data gathering and idea owner of this study: Dr. Helen Suban Mohammed Gouse

Study design: Prof. JP Gunasegaran, Dr. Helen Suban Mohammed Gouse

Data gathering: Prof. Muniappan V, Dr. Kamala Esakkimuthu, Dr. Helen Suban Mohammed Gouse

Writing and submitting manuscript: Dr. Helen Suban Mohammed Gouse, Dr. Suban Mohammed Gouse

Editing and approval of final draft: Dr. Helen Suban Mohammed Gouse, Dr. Suban Mohammed Gouse

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