STUDY OF HISTOLOGY OF THYMUS GLAND - VARIOUS FOETAL AGE GROUPS

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APRIL - 2016

CERTIFICATE

This is to certify that dissertation titled **"STUDY OF HISTOLOGY OF THYMUS GLAND - VARIOUS FOETAL AGE GROUPS"** is a bonafide work done by **Dr.J.GAYATHRI** under my guidance and supervision in the Department of Anatomy, Thanjavur Medical College, Thanjavur during her post graduate course from **2013 to 2016.**

(Dr.M.SINGARAVELU, M.D.,)
THE DEAN
Thanjavur Medical College
Thanjavur - 4.

DR.T.SIVAKAMI,(M.S)
Professor and Head
Department of Anatomy
Thanjavur Medical College,
Thanjavur -4.

DECLARATION

I, **Dr.J.GAYATHRI** hereby solemnly declare that the dissertation title **"STUDY OF HISTOLOGY OF THYMUS GLAND - VARIOUS FOETAL AGE GROUPS"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under supervision and guidance of my professor and head **Dr.T.Sivakami.M.S.,** This dissertation is submitted to Tamil Nadu Dr.M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch-XXIII) in Anatomy.

Place : Thanjavur.

Date : **Dr.J.GAYATHRI**

GUIDE CERTIFICATE

GUIDE

PROF.DR.T.SIVAKAMI, M.S.,

THE PROFESSOR AND HEAD

Department of anatomy,

Thanjavur medical college & Hospital,

Thanjavur.

Remark of the guide:

The work done by DR.J.GAYATHRI on **"STUDY OF HISTOLOGY OF THYMUS GLAND - VARIOUS FOETAL AGE GROUPS"** is under my supervision and I assure that this candidate will abide by the rules of the Ethical Committee.

GUIDE:Prof.DR.T.Sivakami, M.S.,

THE PROFESSOR AND HOD,

Department of Anatomy,

Thanjavur medical college & Hospital,

Thanjavur.

Thanjavur Medical College

THANJAVUR, TAMILNADU, INDIA-613 001

(Affiliated to the T.N.Dr.MGR Medical University, Chennai)

INSTITUTIONAL ETHICAL COMMITTEE

CERTIFICATE

Approval No. : 002

This is to certify that The Research Proposal / Project titled

STUDY OF HISTOLOGY OF THYMUS GLAND - VARIOUS FOETAL AGE GROUPS

submitted by Dr. J. GAYATHRI **of**

Dept. of ANATOMY **Thanjavur Medical College, Thanjavur**

was approved by the Ethical Committee.

THANJAVUR MEDICAL COLLEGE * THANJAVUR *

Thanjavur

Dated : 06.12.2013

Secretary

Ethical Committee

TMC, Thanjavur.

INTRODUCTION

The name Thymus is from the Latin derivation of the Greek word Thymos meaning "warty excrescence" due to its resemblance to the flowers of the thyme plant .The earliest known reference to the Thymus is attributed to Rufus of Ephesus circa of 100 AD, a Greek anatomist renowned for his investigations of the heart and eye .Rufus attributed the discovery of the Thymus to the Egyptians. It was Galen, who first described the morphology of the gland.

The Thymus is a lymphatic organ that exhibits certain unique structural features.The supporting reticular stroma arises from endodermal epithelium and produces a cellular reticulum. The cells ,designated as epithelioreticular cells, serve as stroma.

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INTRODUCTION

The name Thymus is from the Latin derivation of the Greek word Thymos meaning ”warty excrescence” due to its resemblance to the flowers of the thyme plant .The earliest known reference to the Thymus is attributed to Rufus of Ephesus circa of 100 AD, a Greek anatomist renowned for his investigations of the heart and eye .Rufus attributed the discovery of the Thymus to the Egyptians. It was Galen, who first described the morphology of the gland.

The Thymus is a lymphatic organ that exhibits certain unique structural features. The supporting reticular stroma arises from endodermal epithelium and produces a cellular reticulum. The cells ,designated as epithelioreticular cells, serve as stroma.

Lymphocytes come to lie in the interstices of the cellular reticulum,and these two cellular elements ,the lymphocytes and the epithelioreticular cells, comprise the bulk of the organ. A Blood-thymus barrier is formed by sheathing of perivascular connective tissue of the thymus by the epithelioreticular cells. In addition, there are no afferent lymphatic vessels to the thymus. Thus it cannot react to circulating antigens[1].

Groups of medullary epithelial cells become characteristically arranged in the form of concentric whorls called thymic Hassall's corpuscles.

The thymic components along with the micro environment of thymus gland are responsible for terminal T-cell differentiation and the development and maintenance of cellular immunity. So there is a specific and characteristic histological alteration of thymus gland in the Acquired Immune Deficiency Syndrome(AIDS).

The concept of the thymus as an endocrine gland is now generally accepted and several of its biologically active substances have already been isolated .Among them ,three circulating peptides,thymosin@1,thymopoietin and thymulin have been chemically characterized and obtained in synthetic form. These thymic hormones were shown to play a major role in several intra- and extra-thymic steps of T cell differentiation.[2,3]

Awareness of the anatomical features and a precise knowledge of the histogenesis and histodifferentiation of the various components of the normal thymus is essential in analyzing the different pathologies like thymic neoplasia, myasthenia gravis and certain other autoimmune disorders.

AIM AND OBJECTIVES

1. To study and record the histogenesis and histodifferentiation of the components of human fetal thymus in various gestational ages by haematoxylin and eosin staining.
2. To highlight the various elements in the micro architecture of fetal thymus using special stains.
3. To demonstrate the localization and ultra structure of S-100 immunoreactive cells in the human fetal thymus.
4. To clinically apply the knowledge of histogenesis to diagnose certain autoimmune disorders like myasthenia gravis.

REVIEW OF LITERATURE

HISTORIC REVIEW OF THYMUS

The name Thymus comes from the Latin derivation of the Greek word thymos, meaning ”warty excrescence” due to its resemblance to the flowers of the thyme plant. The earliest known reference to the thymus is attributed to Rufus of Ephesus circa of 100 AD, a Greek anatomist renowned for his investigations of the heart and eye .Rufus attributed the discovery of the thymus to the Egyptians.

Galen was the first to note that the size of the organ changed over the duration of a person’s life. After reaching its greatest weight in proportion to body weight before birth, the thymus continues to grow, reaching its maximum absolute weight at puberty.[1]

Cooper (1833) noted that there was wide variability in thymic size and morphology and reconfirmed Galen’s observation with fetal and infant growth [5]. Hassall AH and Vanarsdale H (1846) used improvements in compound microscope lens quality to study the thymus more thoroughly[6.] They also described differences between the thymus and other lymphoid tissues. It was in 1851, Hassall first described the solid concentric corpuscles in the human thymus. Hassall’s famous corpuscles have been named after him. According to him these bodies were composed of mother

cells, which enclose newly formed daughter cells which are nucleated[7].The nature and origin of the Hassall's corpuscles has raised many doubts and debates.

Watney(1881) described the structure of the thymus in the dog ,and referred to Hassall's corpuscles as cysts lined by ciliated epithelium and felt these cysts increased in size as the animal increased in age[8]. The myoid cells were first noted in 1888 by Mayer,who saw them in frog thymus.They were described as long, spindle shaped cells showing distinct striations and closely resembling rudimentary skeletal muscle fibres.

Subsequently ,Bell (1906) describing the thymus in the pig, believed that the primary function of the thymus lay in the colloid secretion found within the cysts. This colloid formation is similar to one occurring in the neighbouring thyroid gland was one stage in the subsequent formation of the Hassall's corpuscles . In fact, he referred to the non-cystic concentric corpuscles as the 'abortive expressions' of the primary function of the colloid formation[9].According to Pappenheimer(1910) the Hassall's corpuscles represent the sole cell rests of the original epithelial anlage[10].

Hammer(1921) described in detail the morphology and probable functions of the thymus. He had dealt on thymus as early as 1905 and 1910 but his classical work on thymus was in 1921. He regarded the striated cells in the thymus of frogs, chicks, dogs and cattle as hypertrophic reticular cells

.Because their cross-striated fibrils are similar to the fibrils of skeletal muscle, He named them '**Myoid Zellen'**

Referring to the Hassall's corpuscles, he says,"These bodies are started as small fractions of 10 microns to 25 microns in diameter, often in the neighbour hood of some small vessel. One or two reticular cells enlarge in size and assume a spherical shape .More and more cells are added to the periphery and they concentrically enclose like scales of an onion. Compound corpuscles are formed by the union of two or more corpuscles. Though the diameter of a corpuscle during fetal life varies between 25 and 50 microns during postnatal period the maximum goes upto500microns.These bodies called Hassall's corpuscles form the morphological expression of antitoxin activity[11].

Jaffe (1926) considered the corpuscles as ''Spent reticulum cells''[12]. Jordan (1927) described the origin of the corpuscles to stenosed vein.[13]Kingsbury (1928) contradicted Jordan's theory by tracing the origin of the corpuscles to ''expressions of growth transformation in an epithelium as modified and determined through loss of surface relations, and under conditions of marked reticulation.''[14]

Dearth (1928) proposed a theory similar to that of Hammer.[15] Norris (1938) ascribed the origin of the corpuscle to ectodermal remnants of the cervical sinus[16]. In 1931, Wiseman conducted a series of experiments to

note the differential response of lymphoid tissues like tonsil, spleen, lymph node and the thymus to foreign proteins. With repeated injection of egg albumin, the lymphoid tissues, except the thymus undergo marked hypertrophy.

The thymus differs in the following respects from other lymphoid tissues. 1.Germinal centers, which form such a prominent feature in lymph node, normally do not occur in thymus.2 . Whenever a substance with a low molecular weight like trypan blue, is injected parenterally, it does not penetrate into the thymus as readily as it does into the other lymphoid tissues (Kostowieki 1963 & Clark 1963)[17] 3.Whenever as antigen is injected parenterally and the antibody estimated at a scheduled period, it is found that in the spleen and lymph nodes, the antibody titre is increased, whereas there is no increase of antibody titre in the thymus. But if the said antigen is injected directly into the thymus, in a live animal, the following `changes are seen: i) lymphoid follicles with germinal centers appear. ii) The antibody titre is also increased as in other lymphoid organs. These findings go to prove that a haemato - thymic barrier does exist.

Simth (1949) tried to prove that the Hassall's corpuscle is a product of degeneration of epithelial cells, by drawing comparison between the staining characters of the Hassall's corpuscles and the thick skin of the

guinea pig.[18] He also reported the presence of lipid laden foamy cells in the cortex of the mouse thymus.

Metcalf (1956) proposed that the large reticular epithelial cells of the medulla which later constitute the Hassall's corpuscles, had a secretory function.[19] These cells were positive for the PAS reaction and he called the secretion as the LSF (Lymphocytosis stimulating factor) which is thought to be regulator of the rate of lymphocyte production, within the thymus.

Arnesan (1958) described a secretory apparatus in the thymus of the mice. In marked involution of the thymus, alveolar spaces are formed. These spaces lined with cuboidal or columnar cells ,with or without cilia ,contain a colloid material giving a positive PAS reaction."[20]

A large number of research workers have studied the thymus of the mouse under the electron microscope. Koka (1960) did ultra microscopic studies on the thymus, especially on its epithelial cells.[21] Miller (1961) by doing neo-natal thymectomy in the mice showed that although the thymus did not itself form antibodies, it played a crucial role in the development of immune system.[22] Almost simultaneously, Good, Archer and Pierce (1961) had done neonatal thymectomy on rabbits and published similar results.

Marjan (1962) reported on the Hassall's corpuscles in the gunieapig. Many views have been expressed regarding the functions of the thymus in general and of the Hassall's corpuscle in particular. Hammer believed in an antitoxic activity for the Hassall's corpuscles.

Tanaka (1962) observed the mesenchymal and epithelial reticulum in the thymus of mice.[23] Marshall & White (1962) first postulated the theory of a barrier similar to blood-brain barrier.[24] Burnet & Mackay (1962) have suggested that a breakdown of the haemato - thymic barrier is responsible for the onset of any auto-immune disease. When this barrier breaks down the organisms own protein enter the thymus, for whose cells they then provide an antigenic stimulus, which gives rise to the auto-immune disease. This results in the formation of lymphoid nodules with germinal centers within the thymus.[25]

Clark (1963) proved the presence of a secretion rich in mucopolysaccharides within the Hassall's corpuscles. Analysing the cellular constituents of the thymus, it has been observed that there are lymphoid and non lymphoid types of cells. . He reported on the electron microscopic appearance of the thymus in the mice.[26] The epithelial reticular cells within the thymus had received enormous attention by several workers.

Weiss (1963) postulated the presence of the epithelial reticular cell as the peripheral element of the vessel wall in the thymus. These cells extend processes\ which enclose a portion of the circumference of the vessel, forming a boundary to the extra-cellular tissue and thus becoming the most peripheral vascular elements. Two or three reticular cells enclose the whole perimeter of the vessel. The reticular cells may form a complete or incomplete covering for haemato thymic barrier.[27]Electron microscope studies by Clark and Weiss have more or less confirmed the presence of such a barrier.

Hoshino (1963) almost simultaneously published his observations on the epithelial reticular cells of the mouse thymus.[28] Cells containing tonofilaments and attached to each other by desmosomes, lining along the inner surface of the capsule, and along the blood vessels have been observed by Clark, Hoshino and Weiss, under the electron microscope.

Cells (1963) was the earliest author to describe the ultra structure of the epithelial cell of the thymus. He described the barrier as interposed between lymphoid cells and connective tissue and as consisting of a continuous layer of epithelial cells, closely joined by desmosomes and resting on a basement membrane. There was a perivascular space around the venules, but a very narrow space around arterioles and capillaries.[29]

Kohnan and Weiss had conducted ultra structural studies on the Hassall's corpuscles in the guinea pig and mouse and had observed similarities between the two. Izard (1964) had reported on the ultra structure of the intracytoplasmic bodies in the thymus of guinea pig.[30]

Saint Marie and Leblond (1964) first described the existence of a perivascular space between these reticular epithelial cells and the vessels which these cells surround.[31] Schoeider adopting method of fractionation and thymocytolysis in the thymus of guinea pigs had studied the isolated fractions of the Hassall's corpuscles and thymic stroma, and found that both these elements increased during the involution period. Kohnen and Weiss have reported on the highly variable electron microscopic appearance of the reticular epithelial cells in the guinea pigs. They have described cell junctions marked by complex interdigitating processes, a major portion of the cell surface being involved in desmosomal formation.

Lundin and Schelin (1965) elaborated on the ultra-structure of the rat thymus.[32] Kamaya and Watnabe (1965) had presented his observations on the human thymus and found them similar to those of any mammalian thymus.[33] Izard (1965) a, b, c had published three classical papers on the electron microscopic appearance of the thymus in guinea pig. He also reported on the ultra structure of the thymic reticulum in the guinea pig, discussing the cytological aspects of the problem of thymic secretion.[34,35,36]

Clermont and Pereira (1965) reported on the distribution of the epithelial reticular cells in the rat thymus with TPA(Tannic acid, Phosphomolybdic acid and Amido black) technique of Leblond (1965).The topography of the epithelial reticular cells have been studied by the presence of the cell web within these cells being specifically stained by the TPA technique.[37]

Ito and Hoshino (1966) had discussed the electron microscopic observations on the vascular pattern of the thymus in the mouse.[38] Izard (1966) had dealt in detail on the desmosomal reticular cells in the thymus of guinea pigs and described the reticular cells as being inter-connected by typical desmosomes with tonofilaments extending from the desmosomes.[39]

Metcalf (1966) believed that the epithelial aggregates in the whorled patterns gave rise to the Hassall's corpuscles ,but these bodies appear in different morphological forms in different species .The significance of these different forms may be related to different functional status of the cells concerned. These epithelial cells which are connected by desmosomal bridges are not phagocytic and appear to be secreting a PAS(periodic acid Schiff) positive material. Metcalf said, that though at first sight, the microscopic picture of thymus appeared simple, with detailed study, it is found to be having a highly complex structure. He had quoted the presence

of cells specific for the thymus like the reticular epithelial cells. According to him, the lymphocytes and macrophages are the non-specific cells in the thymus.[40]

Mackay thought that the Hassall's corpuscles are complex tubular structures with feature suggestive of derivation from either epithelial cells or thick walled venules. Auerbach formulated the possibility of dual functions for the thymus and suggested that it produced two factors (1) a diffusible factor and (ii) a migratory factor. Blau (1967) found the localization of antigen antibody in the Hassall's corpuscles suggesting an immunological function[41].

Haelst (1967) dealt on the ultrastructural study of the normal and pathological thymus of the rat.[42] Bockman and Winborn (1967) had studied the ultra structure of the thymus in two species of snakes.[43] Blau (1973) found that a substance like trypan blue did enter the thymus and was found both in the macrophages and in the Hassall's corpuscles.[44] Norris believes that there is a partial haemato thymic barrier in all adult animals, but this barrier is much less effective in the new born, and he has proved this fact by auto-radiographic studies.

Mendel (1968) described the ultra structure of the Hassall's corpuscles.[45] Goldstein et al studying the ultra structure of the human thymus believed that the basic structure of the mammalian thymus ,human or not consisted of an epithelial 'sponge' or 'lattice', the interstices of which were filled with lymphocytes and into which vessels had invaginated. According to all these workers, the structure of the mammalian thymus is similar in all species [3,4]

Ito has studied the relationship of blood vessels to parenchyma of thymus and found that a continuous layer of epithelial cells surround the thymic capillaries and separate the capillaries from the parenchyma where lymphocytosis is taking place, and thus suggesting a blood-thymus barrier. When cortical capillaries are traced in low power, the perivascular spaces are found to be continuous directly with the thymic parenchyma. In the medulla, the vein is also surrounded by an incomplete layer of epithelial cells. According to Ito, the blood thymus barrier is more a selective functional entity than a structural one.

Goldstein and Mackay have done three dimensional reconstructions of the Hassall's corpuscle from serial sections of human thymus. The Hassall's corpuscle increase in size with the central cells undergoing degeneration as evidenced by pyknotic nuclei. With further growth, a central cavity containing cellular debris, polymorphs and lymphocytes are

found. This is the cystic type of Hassall's corpuscles. In a healthy human thymus, the epithelial form of Hassall's corpuscles predominates with approximately only three out of every ten having a cystic appearance.[3,4]

Kathiresan (1969) has described three stages in the formation of Hassall's corpuscles, namely, 1) stage of secretion, ii) stage of absorption and iii) stage of degeneration.[46] Blau had discussed the relationship of Hassall's corpuscles to the reticuloendothelial system.

Norris noted that the thymus is the first organ to contain lymphocytes in an embryo, and that shortly after birth, it exports cells briskly into the peripheral lymphoid system. According to Norris, the thymus produce a humoral factor, which helps the bone marrow stem cells to differentiate into immunocompetent lymphocytes, and these twin roles are indispensable over the first few weeks of life.

Goldstein and Mackay reported on thymic substance affecting neuro-muscular function and called this substance as the 'thymin'. According to them 'thymin' inhibits transmission at the neuromuscular synapse. In myasthenia graves, it is excessive thymin which is considered to be responsible for the lesion. Using immune fluorescent technique they also described special cells called 'myoid cells' seen close to Hassall's corpuscles. Myoid cells with antigenic properties of striated muscle has

been demonstrated by these authors.[3,4] These myoid cells are the same as the epithelial cells of thymic medulla described by Vander Gold et al and the 'Myoid Zellen cells' quoted by Hammer.

Goldstein described these epithelial cells of the barrier under the electron microscope.According to his study, the thymus consists of a cytoreticulum of inter connected epithelial cells with numerous lymphocytes in the interstices of the cytoreticulum. The epithelial cytoreticulum arises from the third branchial cleft and the lymphoid tissues from the mesenchyme.[3]

The work done by Kathiresan on Human Foetal thymus (1970) shows the epithelial reticular cells forming part of the perivascular sheath when stained with the TPA (Tannic acid, Phosphomolybdic Acid and Amido black) technique. The Electron microscopic picture, show the perivascular epithelial cells with the basement membrane and the desmosomal junctions. He also reported on the presence of mast cells in the thymus of the echidna (an egg laying mammal) available in Australia.[47] Hoshino (1970) had mentioned about the presence of cells containing birbeck granules in the human thymus.

Pereira and Clermont (1971) have described the topographical distribution of the epithelial reticular cells in the thymus of young adult rats. Such a distribution was revealed by the study of sections of thymus stained with TPA technique, which is known to stain intracytoplasmic fibril known as the cell-web (Puchtler and Leblond 1958). This descriptive work by Pereira and Clermont on the spatial arrangement of the cell web containing epithelial reticular cells led to a better classification of thymus into two compartments: (1) Epithelial compartment, (2)Connective tissue compartment.

The medulla itself consists of an outer medulla with an abundance of T.P.A. stained reticular cells and an inner medulla which is faintly stained and devoid of such cell. These epithelial cells of the inner medulla form a discontinuous layer along the perivascular spaces enclosing the venules. A basement membrane is seen underneath the epithelial sheet. At the boundary between the two zones of the medulla, a large number of what is called the 'Stellate epithelial reticular cells' were seen. The Hassall's corpuscles have their origin from the epithelial reticular cells, as proved by T.P.A. technique. The deep cells near the centre of the Hassall's corpuscles contain faint T.P.A. positive, cytoplasmic processes rich in tonofibrils. The flattened epithelial reticular cells of outer medulla are morphologically identical to those of the cortex and form a lining along the perivascular

spaces. This delicate layer of epithelial cells is not always continuous and as such the so called haemato thymic barrier formed by the epithelial reticular cells is not a complete barrier.

Haemato thymic barrier was investigated by Rappey et al (1971).They reported on the fine structure, distribution and function of the rat thymic reticular cells in the perinatal life.[48] Schwarz (1971) observed the epitheloid cells in thymus of the cat.[49] Pereira and Clermont (1971) had observed the Hassall's corpuscles to be mainly formed by reticular epithelial cells and that newly formed corpuscles were oval structures consisting of hyalinised epithelial reticular cells massed together in irregular fashion. According to them, older corpuscles contained a homogenous colloid substance or remnants of degenerating epithelial cells and lymphocytes.

Hayward (1972) had observed the myoid cells in the human foetal thymus.[50] Croxatto (1972) described the appearance of epithelial cords in adult thymic remnants in man.[51] Blau (1973) by auto radiographic studies had provided evidence that the DNA from the thymocytes accumulated and later disintegrated within the Hassall's corpuscle and thus making the Hassall's corpuscle a grave-yard for the thymocytes.[44]

Ushiki.T et al(1984) in their study dealt with the localization and ultra structure of S-100 immunoreactive cells in the human thymus.[52]

Lobach et al(1985)has suggested the appearance of mature T cell antigens, T3 and p80 on thymocytes by 12 week of gestation. It implies that the T cell antigen repertoire may be established in the thymus during the first trimester. Thus, a critical period of T cell maturation appears to occur between 7 and 12 week of human fetal gestation.[53]

Liberti et al(1994)classified the Hassall's corpuscles into solid and cystic types,depending on the presence or absence of empty space inside it.[54]

Ravinder K.Suniara et al(2000) have found that mesenchyme derived fibroblasts are still required for early T cell development in the presence of mature epithelial cells and hence mesenchyme might have a direct role in lymphopoiesis.[55]

According to Helen et al (2006) ,the epithelial reticular cells were the predominant cells in the medulla during early gestational period in the human foetal thymus. Hassall's corpuscles first appeared during the 17th week and increased in size subsequently.[56]

Raica et al (2006) classified the Hassall's corpuscles into 4 types ;they are juvenile, premature, mature, senescent or advanced.[57]

R.K.Ajita et al (2006) in their study of structure of the thymus in human foetus 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 increase in number and size during 17th and 24th week. Macrophage cells could be observed at 12th week[58].

K.Karl et al(2012) has stated that fetuses with trisomy 18 or 21, but not trisomy 13, have a small thymus, suggesting accelerated thymic involution *in* utero . IUGR(intrauterine growth restriction) may contribute to the reduced thymic size in trisomy 18 fetuses. Trisomy 21 fetuses seem to have additional factors leading to a small thymus which could be a possible confirmation of the reduced immune response observed in fetuses and neonates with Down syndrome.[59]

Eviston DP et al (2012) were first to study and suggested that fetal thymus growth is reduced before the clinical onset of preeclampsia and precedes any described fetal anomalies or maternal immunological changes associated with preeclampsia. They proposed that the fetal adaptive immune system is either passively affected by maternal processes preceding clinical preeclampsia or is actively involved in initiating preeclampsia in later pregnancy.[60]

Interestingly, thymic mesenchyme is derived from neural crest cells, and extirpation of the region of the neural crest involved results in impaired thymic development and craniofacial abnormalities similar to the group of clinical defects found in the DiGeorge syndrome.

Vijayalakshmi et al (2013) in her study on Histomorphogenesis of Thymus in human fetuses found lobulation of the thymus was observed at 16th week ,cortex and medulla were differentiated at 16th week .Hassall's corpuscles were found at 18 weeks of gestation.[61]

Bashir khan et al(2013) in Histogenesis of endodermal component of human fetal thymus concluded that the epithelial cells are observed at 10th week. Hassall's corpuscles appeared to be PAS positive .They are first visible at 12th week .Maximum growth is observed between 18th and 24th weeks, thereafter they increase in size and number with increase in gestational age.[62]

Prabavathy(2014) in Histogenesis of human fetal thymus in different gestational age groups stated ,lobules had started forming during the 9th week and the formation of lobules become clearly evident after 12weeks,where as differentiation of cortex and medulla became well distinguished from 14th week onwards. Presence of Hassall's corpuscles was observed from 14th week onwards and was present in all sections from 15th week onwards.[63]

Bashir khan et al(2014) in Histogenesis of mesodermal components of human fetal thymus concluded that during development of human fetal thymus, invasion of blood vessels , lymphocytic and other haemopoietic cells is followed by lobular organization.The differentiation of human thymus starts at 9th week and all significant structural changes of thymus such as lobulation and corticomedullary differentiation occur within 17th week of gestation and thereafter thymus shows microscopic growth and maturity in the form of increase in size of lobule and blood vessels.[64]

Aksh Dubey et al (2014) in their study of Estimation of gestational age from histogenesis of the thymus in human fetuses concluded that the lymphocytes first appeared in the thymus at the 9th week,trabeculae developed from the 9th week onwards,lobulation started to develop at the 9thweek 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 occur till 38th week.[65]

Shunichi Suzuki et al (2014) detected the medulla formation more clearly, they performed an immunohistochemical analysis with cytokeratin 5 (CK5), which is a marker protein for the thymic medulla.[66]

Sezin Erturk Aksakal et al(2014) found that both the transverse diameter and area measurement of the thymus are more significant than sedimentation and CRP(C reactive Protein) values in predicting histological CA(chorioamnionitis). Fetal thymus measurements can be used in early diagnosis of infections among high risk patients.[67]

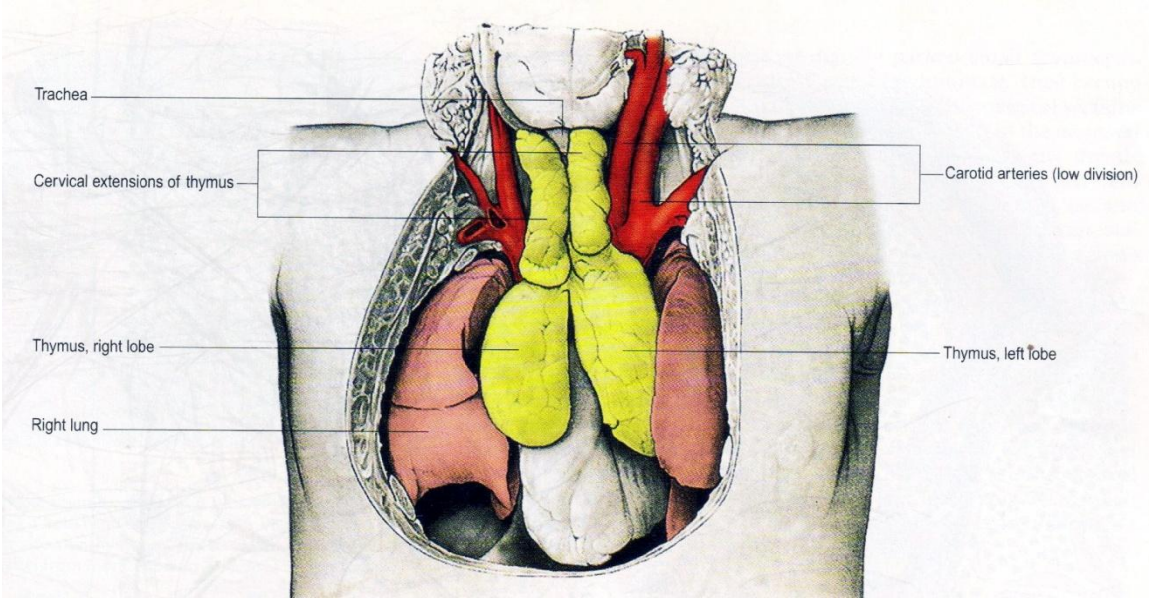
Krishnamurthy et al (2015) in their study found that there is a delay of 5 weeks in corticomedullary differentiation in South Indian fetuses when compared to those of West Bengal region of India.There is a delay of 3 weeks in the time of At 36 weeks ‘starry- sky ’appearance an indication of emperipolesis was observed at the corticomedullary junction.[68]

GENERAL FEATURES

The Thymus is one of the two primary lymphoid organs;the other is the bone marrow.It is responsible for the provision of the thymus processed lymphocytes(T-lymphocytes)to the entire body and provides a unique microenvironment in which T-cell precursors (thymocytes) undergo development, differentiation and clonal expansion .During this process ,the exquisite specificity of T cell responses is acquired, as is their immune tolerance to the body’s own components.These steps involve intimate interactions between thymocytes and other cells(mainly epithelial cells and antigen presenting cells) and chemical factors in the thymic environment. The Thymus is also part of the neuroendocrine axis of the body,and it both

influences and is being influenced by the products of this axis.Its activity , therefore varies throughout life under the influence of different physiological states ,disease conditions and chemical insults, such as drugs and pollutants.

Fig.1 - Gross Anatomy of Fetal Thymus



POSITION AND RELATIONS

The greater part of thymus lies in the superior and anterior inferior mediastinum and the lower border of the thymus reaches the level of the fourth costal cartilages. Superiorly extensions into the neck are common ,reflecting the (bilateral) embryonic origins of the thymus from the third pharyngeal pouch. It sometimes reaches the inferior poles of the thyroid gland or even higher.Its shape is largely moulded by the adjacent structures. Inferiorly ,the lower end of right lobe is commonly between the right side

of the ascending aorta and the right lung,anterior to the superior cava.Anterior to the gland in the neck are sternohyoid and sternothyroid and fascia;in the thorax the gland is covered anteriorly by the manubrium ,the internal thoracic vessels,the upper three costal cartilages,and laterally by the pleura.

Posteriorly,it is in contact with the vessels of the superior mediastinum especially the left brachiocephalic vein,which may be partly embedded in the gland and with the upper part of the thoracic trachea and the upper part of the anterior surface of the heart.

Ectopic thymic tissue is sometimes found. Small accessory nodules may occur in the neck .They represent portions that have become detached during their early descent .The thymus may be found even more superiorly as thin strands along this path,reaching the thyroid cartilage or above.Connective tissue marking the line of descent during early development may occasionally run between the thymus and the parathyroids.

VASCULAR SUPPLY

Arterial Supply-The thymus is supplied mainly from branches of the internal thoracic and inferior thyroid arteries,which also supply the surrounding mediastinal connective tissue.A branch from the superior

thyroid artery is sometimes present.There is no main hilum,but arterial branches pass either directly through the capsule or more often into the depths of the interlobar septa before entering the thymus at the junction of the cortex and medulla.

Venous Drainage-Thymic veins drain to the left brachiocephalic ,internal thoracic and inferior thyroid veins.One or more veins often emerge medially from each lobe of the thymus to form a common trunk opening into left brachiocephalic vein.

LYMPHATIC DRAINAGE

The thymus has no afferent lymphatics.Efferent lymphatics arise from the medulla and corticomedullary junction and drain through the extravascular spaces in company with the arteries and veins entering and leaving the thymus.Thymic lymphatic vessels end in the brachiocephalic,tracheobronchial and parasternal nodes.It is not known whether there is perithymic lymphatic drainage.

INNERVATION

The thymus is innervated by the sympathetic chain via the cervico - thoracic (stellate) ganglion or ansa subclavia and by the vagus .Branches from the phrenic nerve and the descending cervical nerve mainly innervate the capsule of the thymus.

RADIOLOGICAL FEATURES

The appearance of the thymus varies considerably with age. It is largest in the early part of life up to the age of 15 years,although it persists actively into old age. It is a soft,bilobed ,and its two parts lie close together side by side,joined in the midline by connective tissue that merges with the capsule of each lobe.The thymus is visible on CT and MRI axial sections just anterior to the aorta and inferior to the brachiocephalic vein.The CT density in younger individuals is homogenous and similar to or greater than that of muscle.With MRI on T_2- weighted images,the signal intensity is similar to or greater than that of fat.[69]

MICRO ARCHITECTURE

The thymus is derived from number of sources including epithelial derivatives of the pharyngeal pouches, mesenchyme ,haemolymphoid cells and vascular tissue.

The thymus is a primary lymphoid organ that is the site of maturation of T lymphocytes.The capsule of the thymus composed of dense,irregular collagenous connective tissue, sends septa into the lobes,subdividing them into incomplete lobules. Each lobule is composed of a cortex and a medulla,although the medullae of adjacent lobules are confluent with each other. In section ,the thymus can be seen to consist of

an outer cortex of densely packed cells mainly of the T-lymphocyte lineage,the thymocytes and an inner medulla ,with fewer lymphoid cells.

The thymic cortex serves as a lifelong source of T-lymphocytes,but it is most active in fetal and early postnatal life. The thymus produces T-lymphocyte continuously and its rate of lymphocyte production remains unaffected by antigen levels or the number of lymphocytes in the peripheral blood .Hence the thymus produces T- cells autonomously.

The supporting, tissue framework in the thymus consists of two components. Reticular fibers (type III collagen) are noted in the trabeculae,the septa and vessel adventitia but are absent from cortical lobules and in central medulla.The other supporting network in the lymphoid regions of the thymus is the epitheliocytes.

The main cellular constituents of the thymus are lymphocyte (thymocytes),with characteristic small, round,dark staining nuclei and epithelioreticular supporting cells with large pale-staining nuclei.The thymus also contains macrophages,however ,they are difficult to distinguish from the epithelioreticular cells.

By the proliferation of lymphocytes in the cortex ,immature T lymphocytes are produced in large numbers and accumulate in this region. Although most of these lymphocytes die in the cortex by apoptosis and are

removed by macrophages, small number migrate to the medulla and enter the blood stream through the walls of the venules. These cells migrate to nonthymic lymphoid structures and accumulate in specific sites as T-lymphocytes.

In addition to the lymphocytes, the cortex houses macrophages and epithelial reticular cells. The human epithelial reticular cells are derived from endoderm of the third pharyngeal pouch. The three types of epithelial reticular cells present in the thymic cortex are;

Type I cells- separate the cortex from the connective tissue capsule and trabeculae and surround vascular elements in the cortex.These cells form occluding junctions with each other, completely isolating the thymic cortex from the remainder of the body.The nuclei of type I cells are polymorphous and have a well-defined nucleolus.

Type II cells-located in the midcortex.These cells have long,wide ,sheath- like processes that form desmosomal junctions with each other.Their processes form a cytoreticulum that subdivides the thymic cortex into small,lymphocyte-filled compartments.The nuclei are large ,pale structures with little heterochromatin.The cytoplasm is also pale and is richly endowed with tonofilaments.

Type III cells -located in the deep cortex and at the cortico medullary junction.The cytoplasm and the nuclei of these cells are denser than those of type I and typeII epithelial reticular cells. The RER(rough endoplasmic reticulum) of type III cells display dilated cisternae, which is indicative of protein synthesis.

These three types of epithelial reticular cells completely isolate the thymic cortex and thus prevent developing T cells from contacting foreign antigens. Developing T lymphocytes whose TCRs recognize self-protein or whose CD4 or CD8 molecules cannot recognize the MHCI or MHC II molecules ,undergo apoptosis before they can leave the cortex. It is interesting that 98% of developing T cells die in the cortex and are phagocytosed by resident macrophages, which are referred to as tingible body macrophages. The surviving cells enter the medulla of the thymus as naïve T lymphocytes and from there they are distributed to secondary lymphoid organs via vascular system.

The thymic medulla stains much lighter than the cortex because its lymphocyte population is not nearly as profuse and because it houses a large number of endothelially derived epithelial reticular cells. The medulla contains Hassall's corpuscles,which are characteristic of this region.These structures are concentrically arranged,flattened epithelial reticular cells that become filled with keratin filaments, degenerate and sometimes

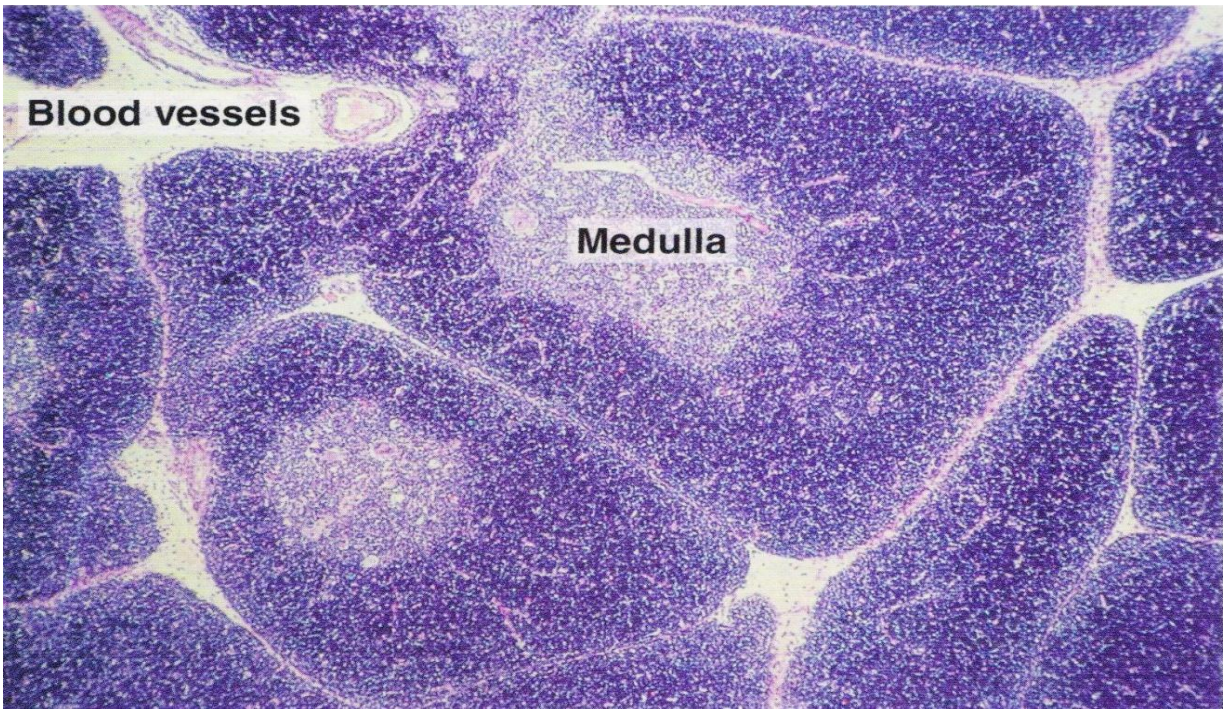
calcify.Their function is unknown.The medulla has the same cell population as the cortex,with a large number of epithelial reticular cell. The three types of epithelial reticular cells in medulla are;

Type IV cells - found in close association with type III cells of the cortex and assist in the formation of the corticomedullary junction.The nuclei of these cells have coarse chromatin network ,and their cytoplasm is dark staining and richly endowed with tonofilaments.

Type V cells -form the cytoreticulum of the medulla.The nuclei of these cells are polymorphous,with well-defined perinuclear chromatin network and a conspicuous nucleolus.

Type VI cells - compose the most characteristic feature of the thymic medulla.These large ,pale staining cells coalesce around each other,forming whorl shaped thymic corpuscles (Hassall's corpuscles),whose number increase with a person's age.TypeVI cells may become highly cornified and even calcified.Unlike types IV and V ,type VI epithelial reticular cells may be ectodermal in origin.The function of thymic corpuscles is not known although they may be the the site of T lymphocyte cell death in medulla. The corticomedullary junction is a clear zone in the foetal thymus. The cells seen here are Myoid cells also called Myoid-Zellen cells ,monocytes and Hassall's corpuscle.[70]

Fig.2- Histology of Fetal Thymus (Haematoxylin & Eosin Staining)



Microvasculature of the thymus:

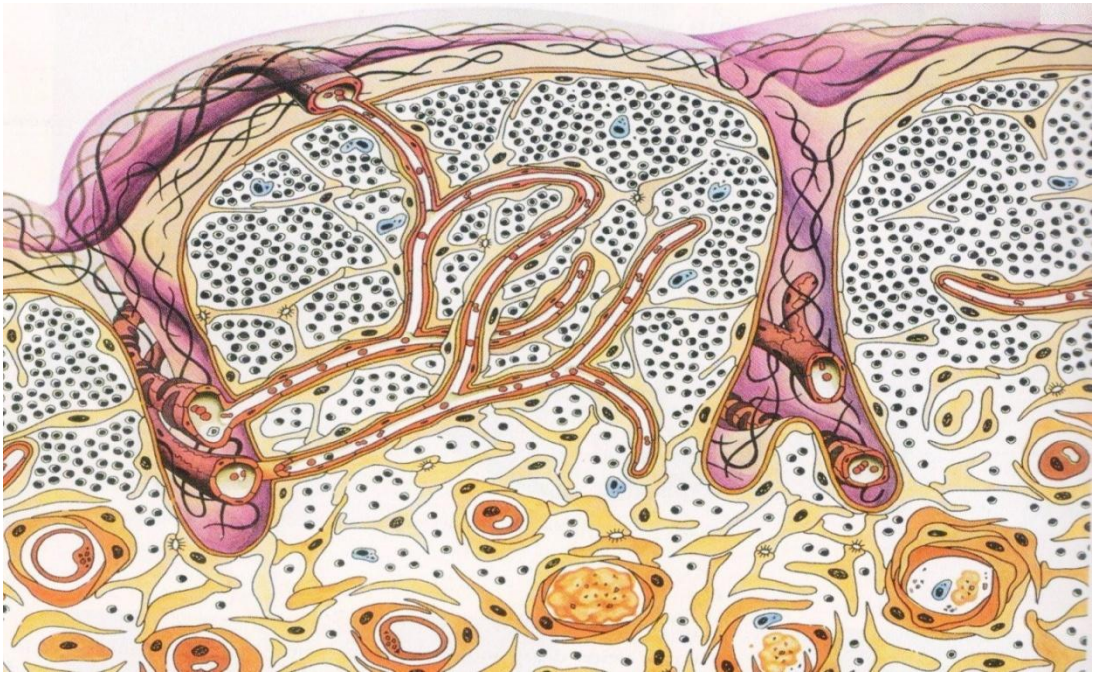
The arteries are from the internal thoracic and inferior thyroid. The thymus receives numerous small arteries,which enter the capsule and are distributed throughout the organ via the trabeculae between adjacent lobules.The blood supply of the thymus first gains entry into the medulla and forms a capillary bed at the junction of the cortex and medulla. Branches of these capillaries enter the cortex and immediately become surrounded by a sheath of type I epithelial reticular cells that are held to one another by fasciae occludentes.These epithelial reticular cells form the blood thymus barrier in thymic cortex,which ensures that macromolecules carried in the blood stream cannot enter the cortex and interfere with the

immunologic development of T cells. The endothelial cells of the cortical capillaries and the type I epithelial reticular cells possess their own basal lamina, which adds support to the barrier. The space between the epithelial sheath and the endothelium is patrolled by macromolecules that manage to escape from the capillaries **.**

From these arterioles a series of radial capillaries pass into the cortex and some into the medulla. Thymus capillaries have a nonfenestrated endothelium and a very thick basal lamina.These capillaries are particulary impermeable to proteins ,preventing most circulating antigens from reaching the thymus cortex where T lymphocytes are being formed. The returning post capillary vessels of corticomedullary junction present a thickened endothelium, across which passage of lymphocytes occur frequently. The endothelial venules have a cuboidal endothelium, specialized for the exit of lymphocytes.These endothelial cells express on their surface Lymphocyte Binding Molecules known as “addressings” which allow lymphocytes to bind to endothelium. This is the first step of migration of the lymphocyte into the tissues from the blood vessel to bind to endothelium. Venous return occurs via capsular venous plexus. The circulation from the arteriole to the capillary continues its transcortical centrifugal path as radial venules and small veins drain into capsular veins. These micro vascular routes may be significant in the cell dynamics of the thymus, especially in relation to the

partial blood thymus barrier. It is the epithelial reticular cell and the perivascular space forming part of the barrier. The cortex of the thymus drains into the venous network of the medulla. Medullary veins penetrate the connective tissue septa and leave the thymus through its capsule.There is no blood thymus barrier in the medulla.[71]

Fig.3 - Microvasculature of Thymus Gland



Development of Thymus

The third pharyngeal pouch expands and develops a solid,dorsal bulbar part and a hollow,elongated ventral part.Its connection with the pharynx is reduced to a narrow duct that soon degenerates.By the sixth week the epithelium of each dorsal bulbar part begins to differentiate into

an inferior parathyroid gland.The epithelium of the elongated ventral parts of the third pair of pouches proliferates,obliterating their cavities.

These bilateral primordium of the thymus come together in the median plane to form thymus, which descends into the superior mediastinum. The bilobed form of this lymphatic organ remains throughout life, discretely encapsulated; each lobe has its own blood supply.

The primordia of the thymus and parathyroid glands lose their connections with the pharynx and migrate into the neck.Later the parathyroid glands separate from the thymus and lie on the dorsal surface of the thyroid gland.[72]

Histogenesis of Thymus

This primary lymphoid organ develops from epithelial cells derived from endoderm of the third pair of pharyngeal pouches and from mesenchyme into which epithelial tubes grow.The tubes soon become solid cords that proliferate and give rise to side branches.Each side branch becomes the core of a lobule of the thymus.Some cells of the epithelial cords become arranged around a central point, forming small groups of cells-the thymic corpuscles(Hassall's corpuscles).Other cells of the epithelial cords spread apart but retain connections with each other to form an epithelial reticulum.The mesenchyme between the epithelial cords forms thin incomplete septa between lobules.Lymphocytes soon appear and fill the

interstices between the epithelial cells. The lymphocytes are derived from hematopoietic stem cells**.**[73]

The thymic primordium is surrounded by a thin layer of mesenchyme that is essential for its development.This mesenchyme as well as certain epithelial cells in the thymus and a peculiar muscle cell in the medulla of the organ is derived from neural crest cells.[74]

Cellular components: There are different types of cells seen in the thymus. These are;

1. Lymphocyte
2. Epithelial reticular cells
3. Macrophages
4. Mast Cells.
5. Myoid Cells
6. Plasma Cells
7. Hassall`s Corpuscle
8. Adipose Cells
9. Eosinophils

1. Lymphocytes: The lymphocytes are small and are called as T-lymphocytes and are responsible for cell-mediated immunity. They are packed in the reticular mesh of the cortex. Stem cells in the bone marrow travel to thymus. Here they lie on the superficial part of the cortex and undergo repeated divisions to form small lymphocytes. The medulla also has lymphocytes, but less densely packed. As a result, the cytoreticulum is

more clearly seen in the medulla. As the lymphocytes divide, they pass deeper into the cortex and then into the medulla. After getting trained as immune competent cells, these T cells pass into the blood vessels. These comprise 20-50% of white cells in circulation. Some are small (6-9 µm) and others large measuring 9-15µm. Small cells have a round nucleus filling 90% of cell volume and only a thin rim of basophilic cytoplasm. [75]

Lymphocytes are patrolling cells of the body, seen in circulation of blood, lymph and extracellular fluid. It is in the lymphoid organs like the spleen, lymph node, tonsil and lymphoid tissues of the gastrointestinaltract.The primary lymphoid organs are the bone marrow and the thymus. All the others are called secondary lymphoid organs. The primary organs are responsible for lymphopoiesis, but the secondary organs are responsible for the activation of a potentially reactive lymphocyte in meeting an antigen. If an antigen binds to a lymphocyte surface receptor, the lymphocyte will be activated and a specific response to that antigen is triggered.

The immune response must be tightly controlled, so as to be very active, when there is a potentially severe infection. On the other hand, the immune response should not be there at all, against harmless component

parts of everyday life in foreign food protein and even against normal components of the body itself. This is called auto-immunity.

There are a large number of pathogens but still the effusiveness of immune system is the ability of the lymphocytes to produce a huge range of antigen receptors -surface immunoglobulin (SIg) or 'B' cells and T cell receptors (TCR) for T cells. The ability of the antibody to bind the antigen is determined by the physico-chemical properties of the antibody the closer the fit of binding site to antigen, the stronger the bond formed and the more likelihood of the lymphocyte being stimulated.

Immature T cells migrate from bone marrow to the thymus to undergo maturation or schooling by the epithelial reticular cells of the thymus. The process of maturation includes proliferation and rearrangement of TCR genes and the acquisition of surface receptors and necessary molecules of the mature T cells. At this stage, T cells with the ability to react with self-antigens are removed by APOPTOSIS (programmed cell destruction and phagocytosis) creating a state of self-tolerance. Mature T cells (which do not react with self antigens) then reach the secondary lymphoid tissues and from there as a continuous process re-circulate via the blood stream in the quest for antigen.

Functional subsets in T cells are:

1. T helper cells (TH cells) - these secrete mediators called INTERLEUKINS help β cells, cytotoxic T cells and macrophages

2. Cytotoxic (TC) T cells - able to kill virus infected and some cancer cells. Require interaction with TH cells.

3.Suppressor T cells (TS cells) - may suppress immune responsiveness to self antigens, switch off response, when antigen is removed.

4. Memory T cells develop from activated T cells provide a RAPID REACTING FORCE for a subsequent encounter with the same antigen.

2. Epithelial Reticular Cells: The origin of Epithelial Reticular Cells from epithelium of third pharyngeal pouch is confirmed by (a) the presence of basement membrance and (b) cell connections through tonofibrils and desmosomes.

The identity of these cells has been established by their consistent ultra-structural features being 1. presence of tonofilaments and desmosomes 2. basal laminar associated with cell membrane.

The cells have long cytoplasmic process getting connected with adjacent cells. These connections could be seen in Electron microscope and also by light microscope by the special staining called TPA technique.These cells form a three dimensional frame work of thymic parenchyma.These reticular cells are distinguished from the reticular cells of mesodermal origin in the

spleen and lymph nodes. Their epithelial origin is proved by the keratinizing feature in the Hassall's corpuscle and by the presence of tonofilaments and desmosomes. Hence these cells are also called 'EPITHELIOCYTES'. Some are named as THYMIC NURSE CELLS because they play a role in maturation of the T-lymphocytes, making them responsible for cell mediated immunity.

The cells are seen in the following areas:1. outside the capsule 2. Just deep to the capsule in sub-capsular zone 3. within the trabeculae forming the septae. 4. They form a sheath, covering blood vessels within the gland, and probably play a role in the formation of the partial blood-thymic barrier. 5. The cells are seen in the corticomedullary zone. Lastly, they form the lattice like structure both in the cortex and medulla.

The lymphocytes lie in this network of epithelial reticular cells.Since there is crowding of the lymphocytes in the cortex,the reticular cells are not clearly visible in the cortex. But in the medulla, there a few lymphocytes and hence epithelial cells are clearly seen and they form the Hassall's corpuscles.

As forming part of the blood thymic barrier, these cells prevent the antigens present in the blood from reaching the T-lymphocytes. The epithelial cell also promote proliferation of T cells and T cell differentiation. Several workers have described ultra structural differences

between the cortical and medullary epithelial reticular cells. It is not clear whether the cells of the same origin and same type are seen differently according to their functions in different situations.

In the electron microscope studies according to their electron density, two main types are described, the pale and dark epithelial reticular cells (DER) created by the increased density of cytoplasmic ground substance.The pale epithelial cell shows the heterochromatin along the inner nuclear membrane as a thin rim and rarely clumped. Nuclei are distinct and there is space distribution of ribosomes. Some pale cells form the Hassall's corpuscles.The dark cells are associated with collagen fibres. The long cytoplasmic processes extend from the cell body to encompass the bundle of collagen fibres. The collagen fibres are definitely extracellular in position.[76]

Both pale and dark epithelial reticular cells have in common the rough endoplasmic reticulum, moderately developed golgi bodies, membrane bound vesicles, electron dense granules and lysosome like bodies. A few of these cells showed vacuoles and small cystic inclusions in their cytoplasm.The vacuoles may contain degenerating material-may be lymphocytes reacting with self antigens and hence getting destroyed.This give the 'Coffee seed appearance'[77]

Fig.4 - Electron Microscopic view of Epithelial Reticular Cells

At least eight hormones have been isolated since 1966. But the details of the synthesis of production and its transportation have not been made clear. The proliferation of T-lymphocytes and their conversion into cells capable of reacting to antigens are events dependent upon the hormones produced by the epithelial reticular cells. The hormone affects lymphopoises in the peripheral lymphoid organs. If thymus is removed during neo-natal period, the peripheral lymphoid organs do not develop in the normal way. Recent studies have identified some of these hormones having origin from the epitheliocytes.

a. THYMULIN - enhances function of T-cells.

b. THYMOPOIETIN stimulates production of cytotoxic T-cells.

c. THYMOSIN - alpha 1- stimulates lymphocyte and antibody production.
d. THYMOSIN BETA -4
e. Thymic humoral factor controls the multiplication of helper and suppressor T-cells.

Apart from actions on the lymphocytes, hormones formed in the thymus probably influence the adeno hypophysis and the ovaries.In turn, the activities of thymus is influenced by the hormones of adenohypophysis , adrenal cortex and gonads.

3. Macrophages: Macrophages are cells belonging to the mononuclear phagocytic system. They are large cells and are seen in the subcapsular zone,cortico medullary junction and in the medulla.The central mass of Hassall's corpuscles may contain degenerating macrophages.[78]

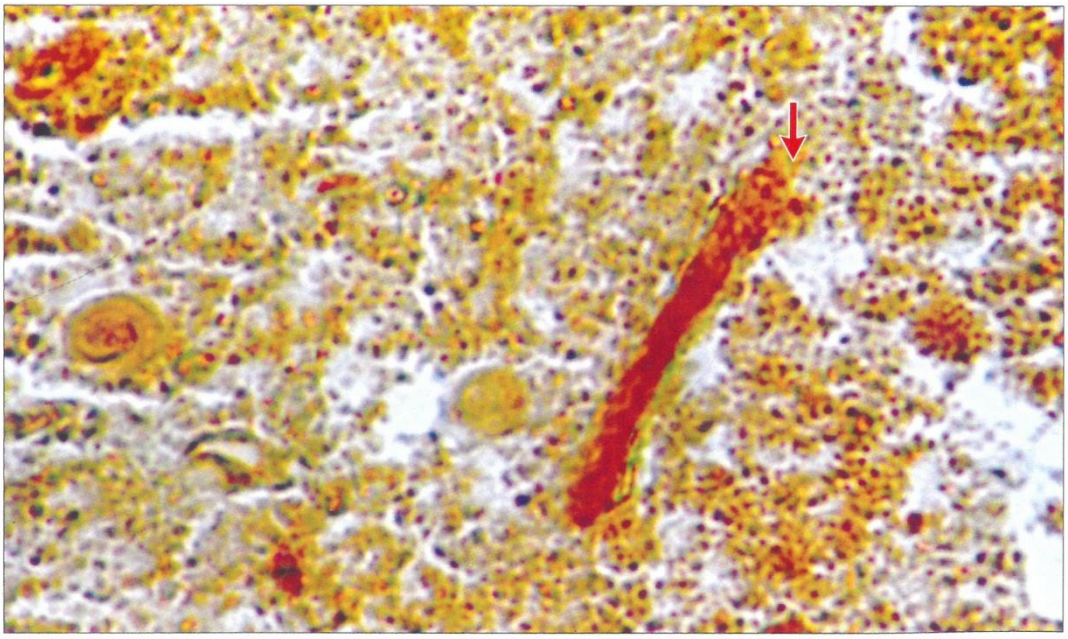
4. Mast Cells: Mast cells are seen in the sub capsular zone and also in the septae. The cells appear big and dark and contain granules. The granules are responsible for the three 'H' substances Heparin, Histamine and Five Hydroxyl tryptamine. During involution of the thymus due to stress, the mast cells are more. The granules packed in the cytoplasm when stained

with toludine blue,bind to the dye and present red or dark red colour. Mast cell is hence called metachromatic cell.

5. Myoid Cell : Myoid cell is a large cell in close relation with the Hassall's corpuscle. Hammer called it as 'Myoid Zellan Cell'.The cell has a broad head and a fusiform tail. These cells are seen at the corticomedullary junction.The cells have the antigenic properties of striated muscle. The cells increase with ageing of thymus. They appear to originate in the perithymic mesenchyme and become secondarily included within the glandular parenchyma.These elements are also found in thymic tumors and lesions associated with myasthenia in human patients.They undergo involutionary changes and may be phagocytosed by reticular cells.It is suggested that this process might be related to the development of the antimuscle antibodies which appear in the sera of myaesthenic patients.[79]

The number of myoid cells in the thymus varies from species to species and even in members of the same class.As a rule,younger animals contain more myoid elements per unit mass of thymic parenchyma than older individuals.They are especially abundant in the thymuses of young repitiles and amphibians.Two categories –the elongated cell and the rounded variant—are commonly seen.

Fig.5 - **Light Microscopic view of Myoid Cell**



Elongated myoid elements usually appear as uninucleated ,fusiform bodies but they can be multinucleated and branched. Generally they appear as aggregates of independent cells or as single myocytes,partially surrounded by reticular cells.Their fibrillar nature and similarity to striated muscle fibres are clearly seen in thin plastic sections.Elongated forms are usually located in the medullary region of the thymus,but are sometimes found along the corticomedullary junction.

Occasionally they are noted in the cortex and in loose connective tissue stroma surrounding the gland.The extramedullary location is encountered more often in the early fetal thymus;it is not seen in adult thymus.

Elongated striated fibrils resembling fibrils of somatic muscle are seen by electron microscopy .Measurements indicate that the thick and thin filaments are about 110$A^0$and 50A^0in diameter and about1.5to2microns long respectively. The sarcoplasm,which is enclosed in a cell membrane ,measuring 70-80 A^0 in diameter and is abundant in the larger myoid cells,contains some glycogen and some free ribosomes . Desmosomes are occasionally observed,connecting adjacent myoid cells.Mitochondria appear to be scattered,at random,peripheral to the cytoplasmic fibrils,occasionally occurring with the sarcoplasmic cisternae between individual fibres.Many are observed around the nucleus.The oval-shaped nuclei situated at the periphery of the cell are enclosed by a smooth usually homogeneous membrane.

Rounded myoid cells display considerable variation in size and shape with some being pear shaped.The majority of fibrils are oriented along long axis but at the point where the cell appear bulbous the fibrils are curved and concentrically arranged about the nucleus[80]

No myoid cells were observed in the normal human infantile,adolescent or adult thymus whereas these elements can be identified in human thymic tumors at all ages. Myoid cells are common in the early stages of human fetal thymic development but they are rarely seen after the seventh month of gestation. A number of myoid cells were

observed in serial sections of the thymus of a 12-week old human fetus but only 4 were seen in sections of the thymus from 7 month old still born fetus.[50]

Myoid cells are present in the thymus and in thymomas from both youthful and adult patients suffering from myasthenia gravis[79]. The source of acetylcholine receptors in the thymus is considered to be myoid cells,which are in very close contact with antigen presenting interdigitating cells.Thymectomy improves myasthenia gravis in some patients regardless of whether thymoma is present.

6. Plasma Cells: Plasma cells are B lymphocytes which undergo changes to become antibody forming cells, called plasma cells. Cells are big, have cart wheel appearance of nucleus, seen in the medulla. These cells are derived from PRECURSOR cells in the bone marrow .They undergo maturation there.When stimulated B cells mature into plasma cells which synthesise large amounts of antibodies. These immune globulins are classified as; IgG, IgA,IgM,IgE and IgD Immunoglobulin is the antigen receptor for the B-lymphocytes.When it binds, the B cell is activated, generally with the help of TH cell responding to the same antigen.Once activated, the B cell undergoes mitotic division to produce a clone of cells able to synthesis immunoglobulin of the same antigen specificity. Most of the B cells of such a clone mature into plasma cell .

7. Adipose cell: Infiltration of fat cells in seen even in foetal life. The infiltration starts around the blood vessels in the septae. As age advances the fat replaces large areas of the cortex. In some cases the fat invades the medulla also.

8.Eosinophils: Eosinophils appear only in late foetal life. Rounded bilobed nucleus is seen. In some cases eosinophils are seen within cysts of the Hassall's corpuscles.

9. Hassall's corpuscles: Hassall's corpuscles are characteristic components of the medulla of mammalian thymus. These Hassall's corpuscles had variable sizes from very small to very large. The smallest size class was represented by corpuscles in early stages of organization, composed of one or two hypertrophic Epithelial reticular cells(juvenile stage).Next was represented by small groups of hypertrophic cells showing early processes of keratinisation ,but without a flattened aspect or a tendency to concentric disposition (pre mature stage).

In mature stage, the Epithelial reticular cells appeared flattened and disposed concentrically around keratin and a mix of degenerated lymphocytes and macrophages ,with or without empty space. In advance stage (mainly observed in older fetuses) some Hassall's corpuscle showed varying degrees of deposition of materials at their center or periphery, where as Hassall's corpuscle with a distorted shape seemed to try and fuse with other nearby one.[57]

The keratinization process of Epithelial reticularcells was triggered at different moments from one corpuscle to another, with no obvious correlation between the size of the Hassall's corpuscle and the development of this process. Most Hassall's corpuscles showed a well-organised peripheral zone, consisting of concentric Epithelial reticularcells,with the central area occupied with material derived from both keratinization and degeneration of Epithelial reticularcells,in different proportion from one formation to another.Large corpuscles had same general organization and structure as the medium size ones ,only differences were in the dimensions and degree of degeneration of the components in the central area.

Hassall's corpuscles are bodies made up of concentric cells derived from the epithelial reticular cells. There are only two main types of the corpuscles

a. the concentric type of corpuscle giving the onion peel appearance

b. the cystic pattern where the cysts are filled with degenerated material or dead cells.[54]

Each corpuscle starts as a unicellular or bicellular body made up of epithelial reticular cells.The size of the corpuscle is highly variable.The variations of structure in the Hassall's corpuscle suggest a cyclic process.

1. Early alteration of the epithelial reticular cell
2. Migration and peripheral application of other cortical epithelial cells.
3. Formation of central cavity in the cell aggregate.
4. Process of cell intrusion into the central cavity.
5. Digestion of contents of cavity as evidenced by loss of intruded cell outlines and loss of staining ability.
6. Rupture of cyst and after digestion, eosinophil enters the opened cyst.

HAEMATO - THYMIC BARRIER

The Haemato - thymic barrier is a concept arising from the observation that materials injected intravascularly do not penetrate to the extra vascular spaces of the thymic cortex, where the lymphocytes are proliferating. This suggests that the walls of the blood vessel may act as efficient barrier to the passage of antigens in to the thymic tissue (especially to cortex) which could, thus be an "IMMUNOLOGICALLY SEQUESTERED SITE" allowing the untroubled differentiation of lymphocytes - the T cells. Whenever epithelial cells borderd upon connective tissue, a basement membrane separating the two was seen. The barrier interposed between the lymphoid cells and connective tissue consisted of a layer of epithelial cells, joined by desmosomes and resting on a basement membrane. The barrier extended completely around the

periphery of each lobule and surrounded each of the penetrating blood vessels.

The components of the haemato-thymic barrier are 1.The capillary endothelium 2.The basement membrane 3.The perivascular space 4.The epithelial reticular cell resting on basement membrane.

The main component being the reticular epithelial cell, a detailed study of this cell had been done both under the light microscope and electron microscope. The light microscope work done with the TPA techique, a non specific staining method to bring out the cytoplasmic fibrillar material found in epithelial reticular cells. The intra-cytoplasmic protein fibrils referred to as 'cell web' form the cytoskeleton giving rigidity and resistence to the cytoplasm.These fibrils correspond to the bundles of tonofilaments first described by Clark in the epithelial reticular cells of mouse thymus under electron microscope. The epithelial reticular cells are either stellate or flattened. The stellate epithelial reticular cells show a well stained nuclear envelope due to accumulation of cell web filaments close to the nucleus.The morphological characteristics of the tonofibril-containing epithelial reticular cells suggest the fact the supporting frame work of the thymic cortex is formed by both the stellate and flattened epithelial reticular cell.

The reticular epithelial cell had a large polygonal nucleus, mitochondria and golgi apparatus . Large inclusion bodies and organelles were observed and vacuoles bounded by smooth membrane were seen. These vacuoles were optically empty granules or related linear structures varying in size . A charcteristic feature of these crystals was the very dense peripheral component surrounding a large inner moderately dense material.

The barrier between the lymphoid cells and the capillary consists of a layer of epithelial cells resting on a basement membrane. The continuous layers of epithelial cells are joined by desmosomes. The two important features which enable the epithelial reticular cell to act as the barrier are (1) the desmosomal junctions connecting the adjoining epithelial reticular cells and (2) the basement membrane in which they lie. The other features of the barrier as seen in ultra thin sections are (1) the extension of the epithelial reticular cells in between the lymphocytes (2) the extra-capillary space otherwise known as the perivascular space.[47]

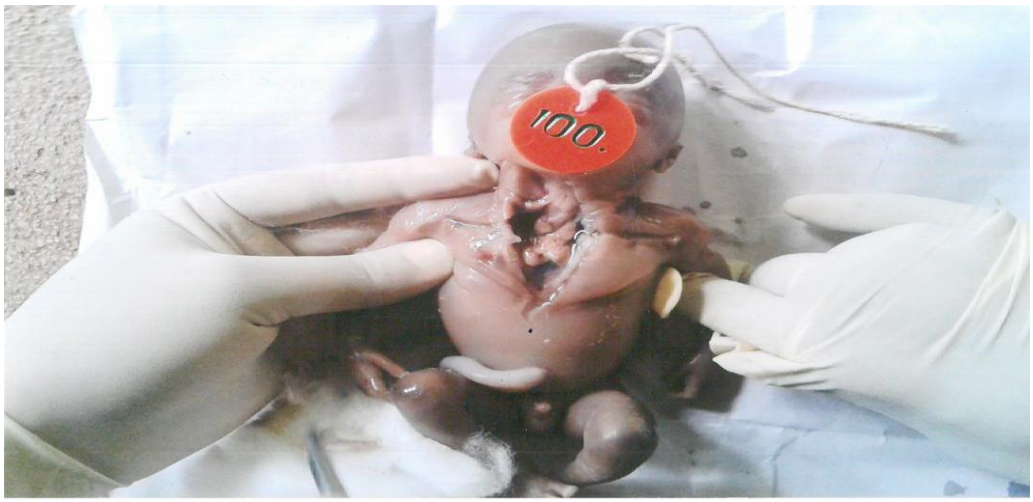
MATERIALS AND METHODS

20 human fetuses of different groups ranging from 10 to31 gestational weeks were procured from the Department of Obstetrics and Gynaecology ,Raja Mirasudar Hospital ,Thanjavur Medical College. These fetuses were the products of terminated pregnancies under the Medical Termination of Pregnancy Act of India, 1971 and still births. Anomalous fetuses and twins were excluded from the study.

Collection of Data

Fetuses were obtained within 4-5 hours of birth to avoid postmortem changes and immediately fixed in 10% formalin .Gestational age of the fetus was calculated from first day of last menstrual age(LMP).Fertilization age was obtained by subtracting two weeks from gestational age. Fertilization age was also determined from Crown Rump Length of fetus and using table in the Moore and Persaud.[72]

Fig.6 - Dissection of Fetus



The fetuses were dissected and the sternoclavicular joints were disarticulated and costal cartilages were cut. Thus the entire thoracic cavity was open and lower part of neck was also dissected for complete exposure of thymus in its natural location. The tissue sample was fixed in, processed to prepare paraffin embedded blocks and 4-5 micron thick sections were cut.

The slides were stained with Haematoxylin and Eosin, Mason's Trichrome, Von-Gieson's, and Gomori's Reticulin stains and Periodic acid Schiff were studied under light microscope.

Method of tissue processing

The formalin fixed thymus tissue was then processed using Automated Tissue Processor (Leica TP1020).

Fig.7 - Automated Tissue Processor

Procedure

1. Dehydration-with Isopropyl alcohol in ascending grade.

   40% - single change 10 minutes; 50% -single change 30 minutes;

   70% - single change 2 hours; 90% -single change 6 hours;

   Absolute alcohol – two changes 2 hours each.

2. Clearing –done with Xylene (Sulphur free).

   First change -1 hour 30 minutes; second change -2hours.

3. Impregnation- done with Paraffin Wax (Melting point 55^{o}- 60^{0} C)

   First change and Second change- 2 hours each.

Fig.8 - Rotary Microtome and Tissue Floatation Bath

The impregnated tissue was then embedded in molten Paraffin Wax (Melting point 55^{o}- 60^{0} C) using Leuckhart's L molds and the block was

cooled, trimmed and, labeled. The blocks are then cut into thin section of 3-4 microns in thickness using Rotary Microtome (MT-1090A) and floated in Tissue Floatation Bath (Dalal) at 48^{o}C (below the melting point of wax) and mounted on the glass slide coated with Meyer's egg albumin.The mounted slides were deparaffinized, dipped in Xylene and treated with descending grades of isopropyl alcohol and brought to water.

STAINING PROCEDURE

HEMATOXYLIN AND EOSIN STAIN

After the slide was brought to water ,they were stained with Hematoxylin and Eosin stains. Slides were kept in Hematoxylin (Erhlic's) trough for 20 minutes and washed in water., then dipped in 1% acid alcohol for differentiation and immediately washed in water Slides were kept for blueing in running tap water for 10 minutes.Next the slides were dipped in Eosin for 5 seconds,washed with water then air dried, and mounted using DPX mountant.The slides were then studied under 4X,10X.40X magnification,using binocular light microscope (Magnus) and observation noted and analyzed.

DIFFERENTIAL STAINING

Procedure for the differential staining of connective tissue fibres and muscle are important part of histological technique and their use is often helpful in the diagnosis of pathological changes in the tissues. Because of

this, many methods have been described for the demonstration of the components , some of them selectively staining different types of fibres by the use of several dyes, in combination or in sequence. Metallic impregnation methods however, are necessary for the complete demonstration of reticulin fibres.

Four special stains namely Van Gieson's ,Masson's Trichrome , Reticulin (Gomori's method),Periodic acid Schiff (PAS) had been used to bring out the arrangement of connective tissue elements composed of collagen, muscle tissue,reticulin fibres and Hassall's corpuscles respectively.[81]

1.**VAN GIESON'S (1889) STAIN**

Van Gieson's mixture of picric acid and acid fuchsin is the simplest method for the differential staining of collagen. Its main disadvantages are its inability to stain young fibrils the red that is imparted to mature collagen and the tendency for the red colour to fade, whatever mounting medium is used.

Fixation: formalin is used.

Sections: Paraffin embedded sections.

Preparation of stain:

Saturated aqueous picric acid-100ml mixed with 1 % acid fuchsin in distilled water 5-10ml

Staining technique:

1. Take sections to water.
2. Stain nuclei either with Weigert's iron haematoxylin for 5 minutes, wash with running water.
3. Dip in acid alcohol
4. Wash water for 5-10minutes.
5. Stain in Van Gieson's solution (picrofuchin) for 2 minutes.
6. Do not wash in alkaline tap water which extracts the red stain.
7. Dehydrate in absolute alcohol, clear in xylene and mount in a synthetic resin

2. MASSON'S TRICHROME STAIN (MODIFIED FROM MASSON, 1929)

Fixation: Formalin

Sections : Thin paraffin sections.

Preparation:

Cytoplasmic (plasma) stain-1percent ponceau de xylidine (ponceau 2R) in 1 percent acetic acid 2 parts ,1percent acid fuchsin in 1 percent,and

acetic acid 1 part .Differentiator and Mordant-1Percent phosphomolybdic acid in distilled water .Fibre stain -2 percent aniline blue in 2 percent acetic acid.

Staining technique:

1. Take sections to water
2. Stain nuclei with Weigert's iron haematoxylin .
3. Wash well in water.
4. Differentiate nuclear stain with 1% acid alcohol.
5. Wash well in tap water, rinse in distilled water.
6. Stain in the red cytoplasmic stain 5-10 minutes
7. Rinse in distilled water.
8. Differentiate in 1percent phosphomolybdic acid until collagen is decolorized, muscle, red blood cells and fibrin remaining red.
9. Rinse in distilled water.
10. Counterstain in aniline blue for 5 minutes
11. Wash well in 1 percent acetic acid (CH3COOH) for atleast 1 minute.
12. Blot, dehydrate in absolute alcohol, clear in xylene, mount in a synthetic medium.

3. GOMORI'S RETICULIN METHOD

Fixation: formalin used

Sections : Thin paraffin sections.

Preparation of Silver Solution:

To 5ml. of 10.2 percent silver nitrate, add strong ammonia drop by drop until the resulting precipitate is just dissolved. Add 5ml. of 3-1 percent sodium hydroxide and re-dissolve the precipitate with a few more drops of ammonia. Dilute to 0.5ml with distilled water.

Staining technique

1. Take sections to water
2. Oxidize for 1-2 minutes in 0-5 percent potassium permanganate,
3. Wash briefly in water.
4. Bleach in 2 percent oxalic acid.
5. Rinse in distilled water followed by thorough washing in tap water.
6. Sensitize in silver nitrate solution for 3 minutes
7. Wash well with distilled water.
8. Reduce with 10percent neutral formalin for 3 minutes.
9. Wash in tap water followed by distilled water.
10. Tone in 0.2 percent, gold chloride for 10 minutes (the sections turn a purplish colour).
11. Wash briefly with distilled water.
12. Fix in 2 percent potassium metabisulfite for 2 minutes
13. Fix in 2percent, sodium thiosulphate (‘hypo’) for 2 minutes.
14. Wash well in water.
15. Dehydrate, clear and mount in a synthetic resin medium.

4. PERIODIC ACID SCHIFF (PAS)

Reagent preparation:

1. 0.5% periodic acid -0.5gm periodic acid dissolved in 100ml of water. 2.Schiff's Reagent: Basic fuchsin- 1gm,sodium metabisulphate-1 gm dissolved in hydrochloric acid 20ml and water 200ml.Boil the distilled water, then add basic fuchsin and stir ,boil to 50^{o}C,then filter and add 1N hydrochloric ,cool to 25^{o}C,then add sodium metabisulphate. This solution is ready to use when it becomes nearly colourless, which may take upto two days in the dark.

3. Sulphurous acid - Sodium metabisulphate 10%-6ml and 10% of Hydrochloric acid 5ml mixed with 100ml 0f distilled water.

4. 0.2% light green or Mayer's hemalum.

Staining procedure:

1. Place the section to water.
2. Add 0.5% periodic acid(5-10 minutes)
3. Rinse in distilled water
4. Add Schiff agent (15 mins)
5. Rinse three changes of sulphurous acid between two minutes
6. Rinse in distilled or tap water for 5minutes.
7. Then add counter stain for 30 seconds.
8. Wash in running tap water for 5 minutes.
9. Air dry and mount in DPX

Method used to study histogenesis in fetal thymic tissue

Specimens were divided into five groups according to the gestational age (weeks) based on the study of Ajita et al (Table-1),who studied in 70 fetuses as given below.

Table -1

GROUP	AGE(WEEKS)	NO OF FOETUSES
GROUP I	9-11	13
GROUP II	12-14	16
GROUP III	15-17	20
GROUP IV	18-24	12
GROUP V	25-40	9

The slides were studied with the binocular research microscope , using powers 10x,40x,100x and 400x.The appearance and histogenesis of the various cellular components were noted and analyzed as suggested by Ajita et al.(table-2) as given below.

Table - 2

Group	Lobulation	Cortex	Medulla	Cortico medullary junction	Trabeculae	Hassall's Corpuscle
I	started	Started developing	Started developing	Not seen	Seen ,with blood vessels	Not seen
II	continues	Recognizable	Recognizable	Seen	Seen	Not seen
III	Increases further	Recognizable	Recognizable	Seen	Seen	Seen
IV	Number increases	More densely lymphocyte seen	Less dense	Seen	More extensive	Number and size Increase
V	Number increases	Densely packed with lymphocyte	Hassall's corpuscles with maturity	Clearly seen	More extensive	Number and size increases with maturity

Immunohistochemistry (S100) to demonstrate Interdigitating cell

Staining procedure:

Sections were cut and mounted on APES(Amino Propyl triEthoxy Silane) coated slides and treated with 3 changes of acetone (first and third change for 2 minutes,second with 10 ml of APES for 30 seconds).The slides were then dewaxed. Retrival of antigen was done with citrate buffer and then treated successively with commercially available power block S100 antibody, super enhancer, SS label and DAB(Diamino benzidine)

chromogen, after washing with buffer in each stage, Harris hematoxylin was then used as counter stain(1min),washed ,dried and mounted with DPX.

To demonstrate the Interdigitating cells present in the medulla of thymus. Three slides were stained from 12 weeks(sample no.19), 16 (sample no.13) and 31weeks (sample no.2) ,belonging to groups II,III and V respectively.

OBSERVATIONS

20 fetal thymuses of 10 to 31weeks of gestational age were considered and classified into five groups as described by R.K.Ajita et al . All the specimens were analyzed and plotted against age groups.

Table- 3

Group	Age(weeks)	No of fetuses
Group I	9-11	1
Group II	12-14	3
Group III	15-17	3
Group IV	18-24	9
Group V	25-31	4

The appearance of various cellular components and their period of development was noted and plotted against each group as mentioned by Ajita et al in the following table.

Table 4

Group	Lobulation	Cortex	Medulla	Cortico medullary Junction	Trabeculae	Hassall's Corpuscle
I	Not seen	Not recognizable	Not seen	Not seen	Seen	Not seen
II	Started appearing	Not seen	Not seen	Not seen	Seen	Not seen
III	Increases further in number	Seen in some	Seen in some	Seen in some	Seen	Started developing
IV	Number increases	More densely packed lymphocyte seen	Less dense	Seen	More extensive	Number and size Increase
V	Number increases	Densely packed with lymphocytes seen	Hassall's corpuscle with maturity Seen	Clearly seen	More extensive	Number and size increases with maturity

Group I - The Gland was seen to be composed of lymphocytes with a delicate capsule.The lobulation and corticomedullary differentiation were not seen. Trabeculae associated with blood vessels were observed .Spindle shaped epithelial cells were noted .No Hassall's corpuscles were observed.

Group II - Well formed connective tissue capsule surrounds the gland. The lobulation of the gland was still advancing, with developing connective tissue trabeculae between lobules. Cortex and medulla were not recognizable. No Hassall's corpuscles were seen

Group III - The number of lobules increased further. The peripheral part of each lobule is heavily infiltrated with lymphocytes that are the cortex. The central parts of the lobule contain fewer lymphocytes, hence lightly stained. The cortex and medulla were differentiated from 15^{th} week. Hassall's corpuscles were seen in some sections from 15^{th} week.

Group IV Lobules, blood vessels, and connective tissues of its capsule become more extensive. The corticomedullary differentiation becomes distinct by 18^{th} week. Hassall's corpuscles found to increase in size and number.

Group V - The thymic tissue of each lobule is continuous in the more central part of the adjacent lobule . The trabeculae were seen extending up to the cortex, leaving the medulla remain undivided. The parenchyma of the cortex seen to be consisting of dense population of lymphocytes of all sizes, closely and uniformly packed. These cells occupy the spaces in the cytoreticulum and obscure it. The lymphocytes are less in number in medulla and hence cytoreticulum is seen well. There is a sharp demarcation between the cortex and medulla forming a clear corticomedullary junction.

Hassall's corpuscles of varying shapes and sizes , immature solid to mature cystic types seen. The number increases with gestational age. The gland during this stage had an internal architecture similar to that seen in the adults.

Table- 5

Appearance of Lobulation in different gestational age groups

Group	Number of specimens	Number seen	Percentage (%)
Group I	1	0	0
Group II	3	1	33 %
Group III	3	3	100 %
Group IV	9	9	100 %
Group V	4	4	100 %

In the group I (9-11 weeks) lobulation was not seen.In the group II (12-14 weeks) lobulations were seen in 1 out of 3 specimens (33%).In the group III (15-17 weeks), the lobulations were seen in all the specimens (100%).In group IV (18-24 weeks) a total of 9 specimens were included and lobulations were seen in all the specimens(100%). In the group V (25-31 weeks) lobulations were seen in all the specimens (100%).

CHART-1

APPEARANCE OF LOBULATION IN DIFFERENT GESTATIONAL AGE GROUPS

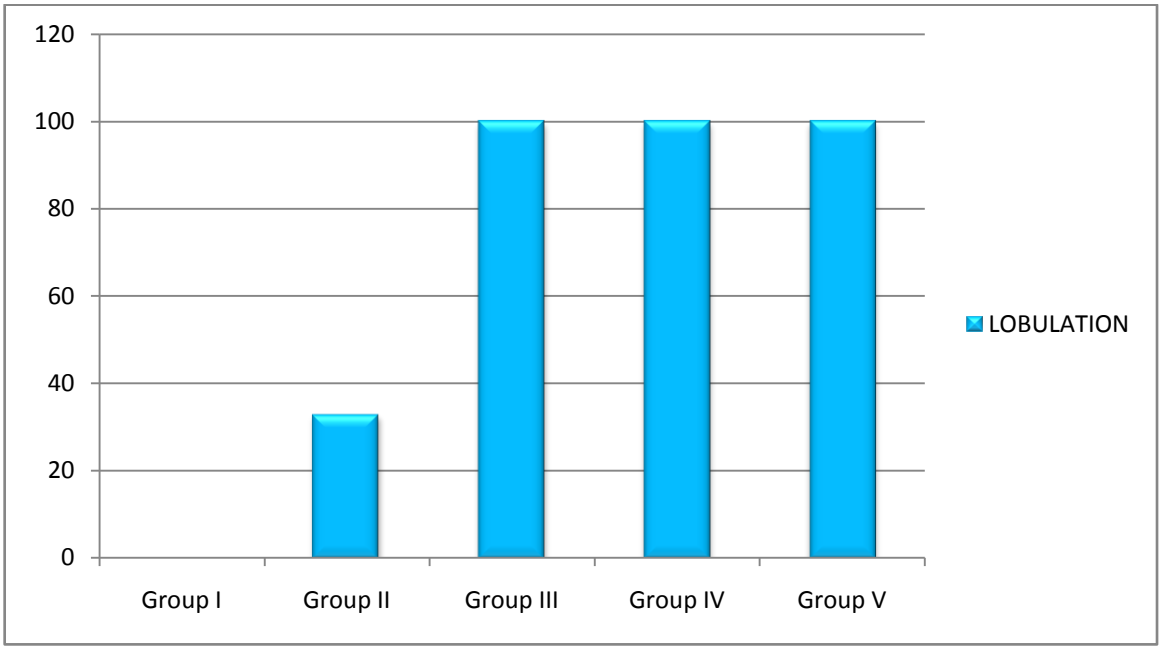


Table-6

Appearance of cortex in different gestational age groups

Group	Number of specimens	Number seen	Percentage
Group I	1	0	0
Group II	3	0	0
Group III	3	1	33%
Group IV	9	9	100%
Group V	4	4	100%

In the group I (9-11 weeks)cortex was not seen.In the group II (12-14 weeks) cortex was not recognizable in any of them.In the group III (15-17 weeks) out of 3 specimens, the cortex was recognizable in only one specimen(33%).In group IV (18-24 weeks) the cortex was recognizable in all the specimens(100%). In the group V (25-31 weeks) the cortex was recognizable in all the specimens(100%).

CHART-2

APPEARANCE OF CORTEX IN DIFFERENT GESTATIONAL AGE GROUPS

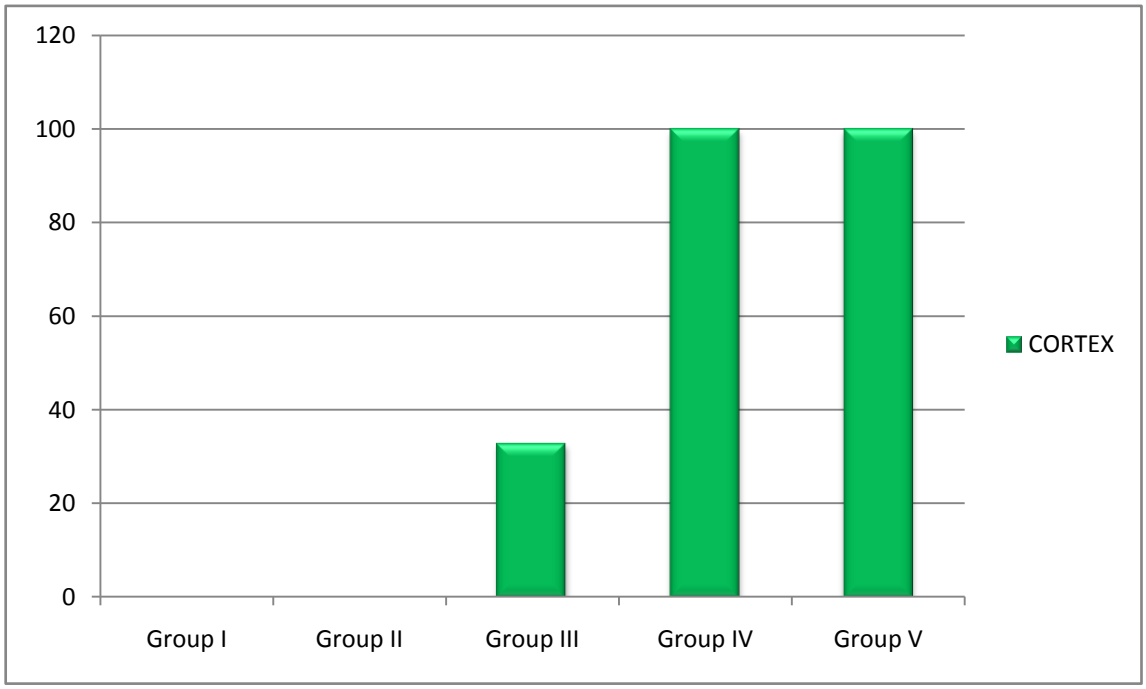


Table-7

Appearance of medulla in different gestational age groups

Group	Number of specimens	Number seen	Percentage
Group I	1	0	0
Group II	3	0	0
Group III	3	1	33%
Group IV	9	9	100%
Group V	4	4	100%

In the group I (9-11 weeks) medulla was not seen. In the group II (12-14 weeks) the medulla was not recognizable. In the group III(15-17 weeks) out of the 3 specimens, the medulla was recognizable in only one specimen (33%).In group IV (18-24weeks),the medulla was recognizable in all the specimens(100%).In the group V (25-31 weeks) it was recognizable in all the specimens(100%).

CHART-3

APPEARANCE OF MEDULLA IN DIFFERENT GESTATIONAL AGE GROUPS

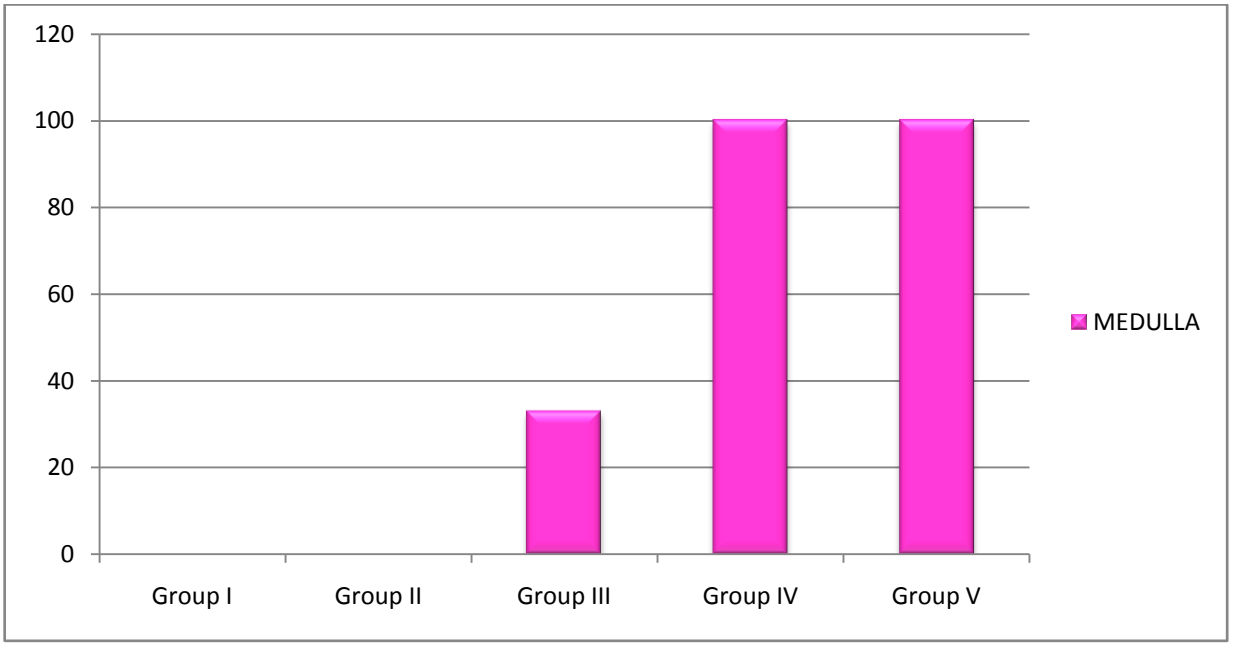


Table-8

Appearance of Corticomedullary junction in different gestational age groups

Group	Number of specimens	Number seen	Percentage
Group I	1	0	0
Group II	3	0	0
Group III	3	1	33%
Group IV	9	6	67%
Group V	4	4	100%

In the group I (9-11 weeks) the corticomedullary junction was not seen. In the group II (12-14 weeks) the corticomedullary junction was not recognizable in any of them.In the group III (15-17 weeks) corticomedullary junction was clearly seen in only one specimen (33%).In group IV (18-24 weeks) a total of 9 specimens were included and the corticomedullary junction was clearly seen in 6 specimens(67%).In the group V (25-31 weeks) the corticomedullary junction was clearly seen in all the specimens(100%).

CHART - 4

APPEARANCE OF CORTICOMEDULLARY JUNCTION IN DIFFERENT GESTATIONAL AGE GROUPS

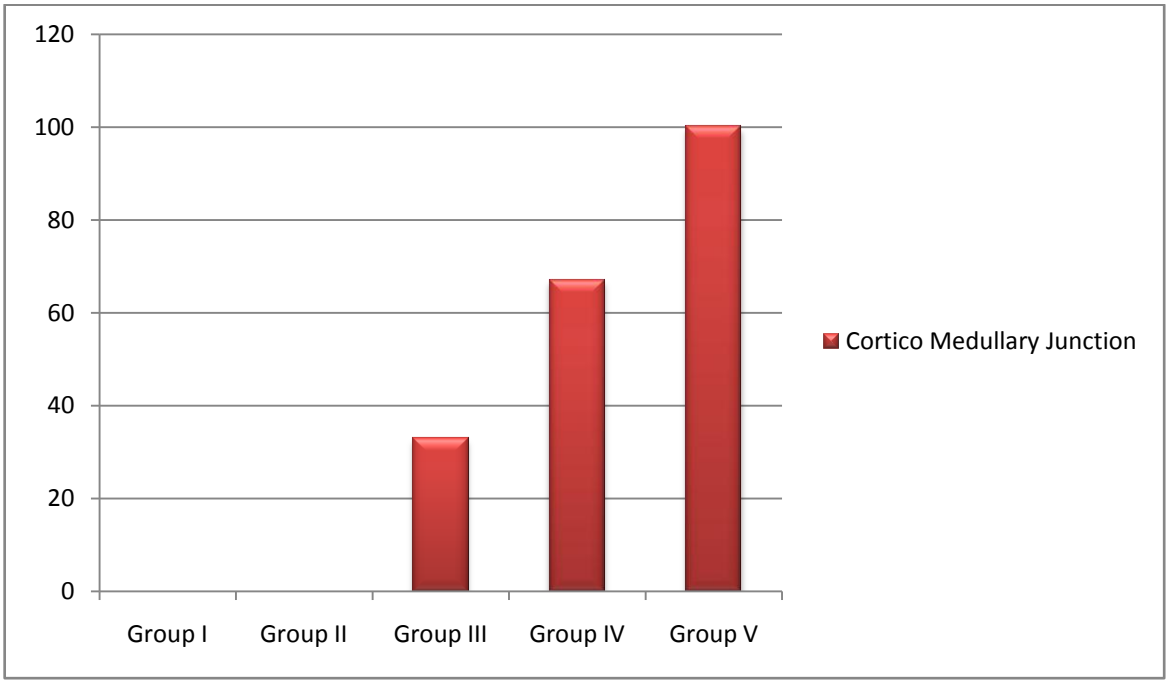


Table-9

Appearance of trabeculae in different gestational age groups

Group	Number of specimens	Number seen	Percentage
Group I	1	1	100%
Group II	3	2	67%
Group III	3	3	100%
Group IV	9	9	100%
Group V	4	4	100%

In the group I (9-11 weeks) the trabeculae was seen(100%) In the group II (12-14 weeks) the trabeculae noted in 2 specimens.(67%)In the group III (15-17weeks),the trabeculae was seen in all the specimens.(100%)In group IV (18-24 weeks), the trabeculae noted in all the specimens(100%). In the group V(25-31weeks) the trabeculae was visible in all the specimens(100%).

CHART –5

APPEARANCE OF TRABECULAE IN DIFFERENT GESTATIONAL AGE GROUPS

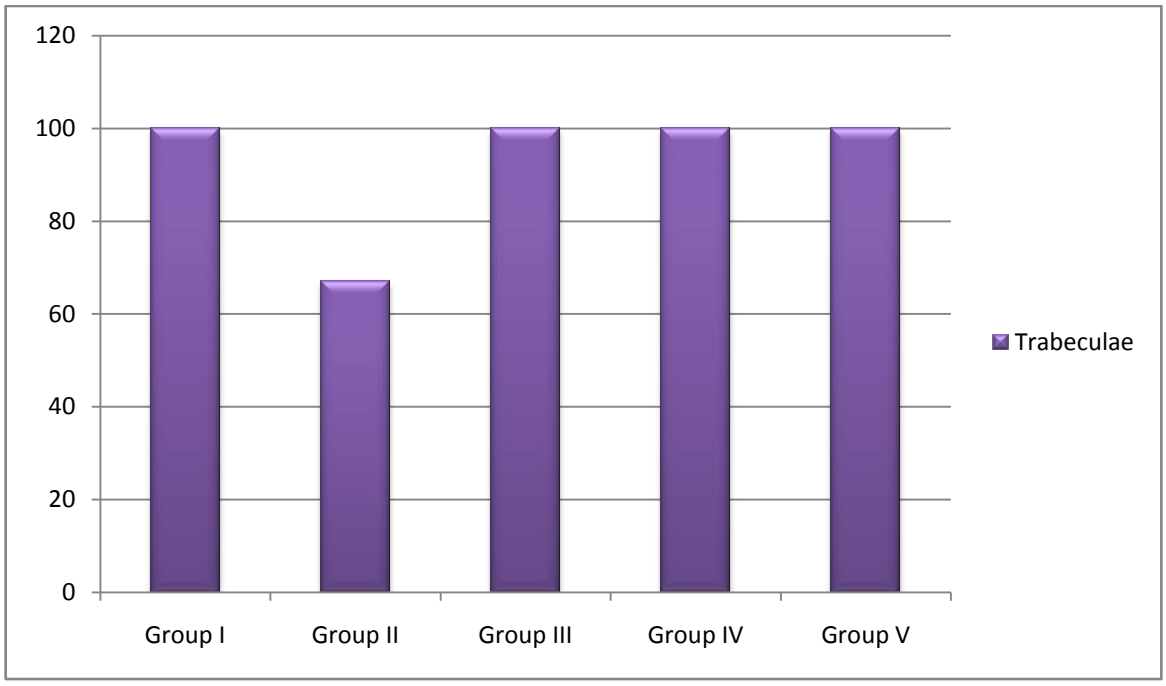


Table-10

Appearance of Hassall's corpuscle in different gestational age groups

Group	Number of specimens	Number seen	Percentage
Group I	1	0	0
sGroup II	3	0	0
Group III	3	2	67%
Group IV	9	9	100%
Group V	4	4	100%

In the group I (9-11 weeks) the Hassall's corpuscle was not seen.In the group II (12-14 weeks) the Hassall's corpuscle was not seen in any of the specimens.

In the group III (15-17 weeks), it was seen in 2 specimens.(67%)In group IV (18-24 weeks), the Hassall's corpuscle was seen in all the specimens(100%).

In the group V (25-31 weeks) , the Hassall's corpuscle was seen in all the specimens(100%).

CHART - 6

APPEARANCE OF HASSALL'S CORPUSCLE IN DIFFERENT GESTATIONAL AGE GROUPS

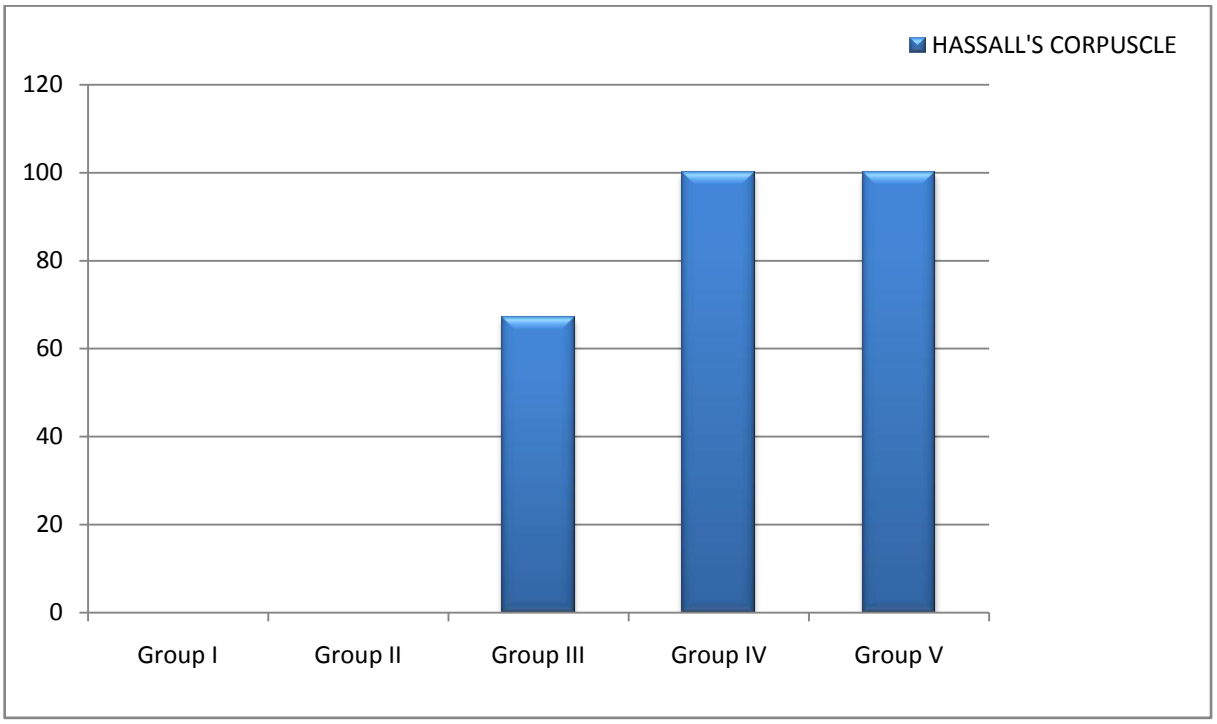


Table11

Appearance of Lobulation, cortex and medulla in different groups

Group	Lobulation	cortex	Medulla
Group I	0	0	0
Group II	33%	0	0
Group III	100%	33%	33%
Group IV	100%	100%	100%
Group V	100%	100%	100%

In Group I the lobulations ,cortex and medulla was not seen.No cortex and medulla were seen in group II while the lobulations were observed in 33%.

All the specimens in group III were lobulated(100%),while cortex and medulla recognizable in 33% .Lobulations,cortex and medulla were found in all the specimens which belong to group IV and group V(100%)

CHART- 7

APPEARANCE OF LOBULATION, CORTEX AND MEDULLA IN DIFFERENT GROUPS

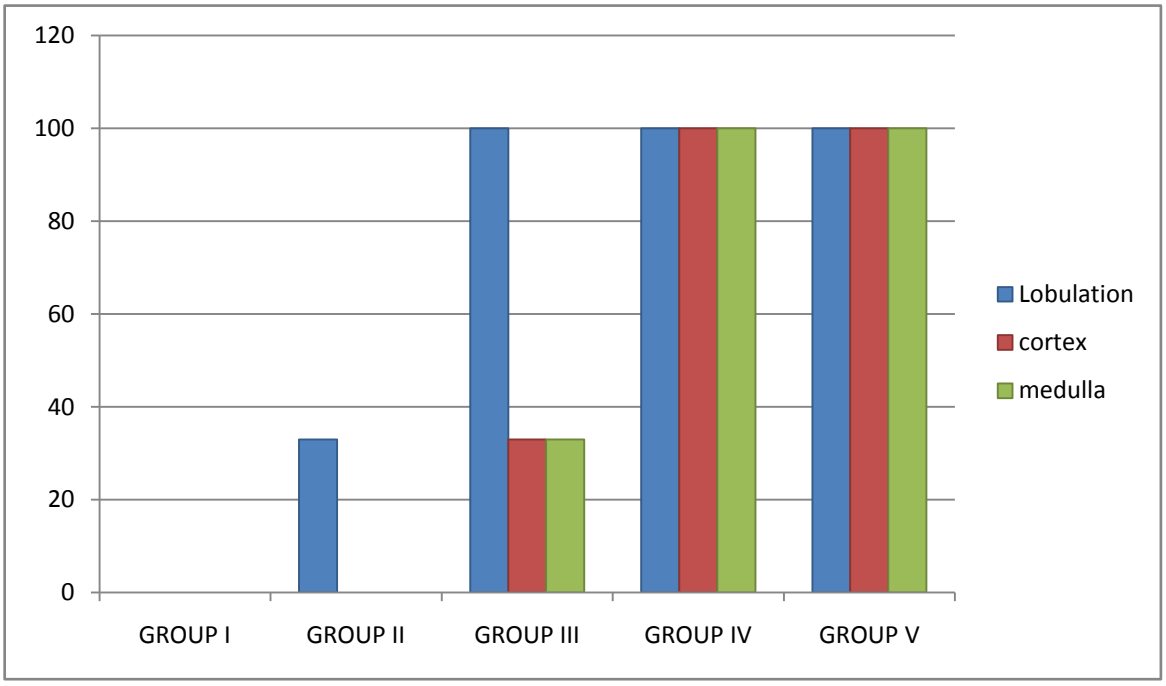


Table-12

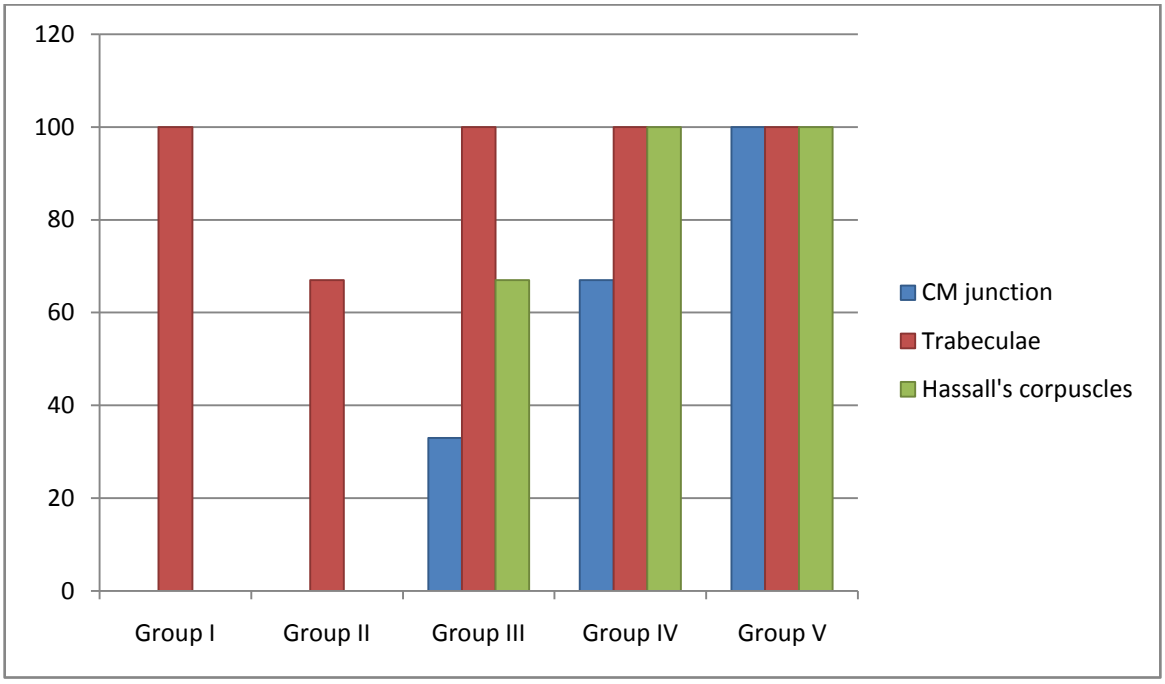
Appearance of corticomedullary junction,trabeculae and Hassall's corpuscles in different groups

Group	Corticomedullary Junction	Trabeculae	Hassall's corpuscles
Group I	0	100%	0
Group II	0	66%	0
Group III	33%	100%	67%
Group IV	67%	100%	100%
Group V	100%	100%	100%

The corticomedullary junction and Hassall's corpuscles were not seen, while the trabeculae was seen in group I(100%).In group II, the corticomedullary junction and the Hassall's corpuscles were not seen but the trabeculae was noted in 66%.The trabeculae was found in 100%,the corticomedullary junction in 33% and the Hassall's corpuscles in 67% in group III. The corticomedullary junction was seen in 67%, while the trabeculae and Hassall's corpuscles were seen in 100% of group IV specimens .In group V all the components were 100%.

CHART -8

APPEARANCE OF CORTICOMEDULLARY JUNCTION, TRABECULAE AND HASSALL'S CORPUSCLES IN DIFFERENT GROUPS



DIFFERENTIAL STAINING

1.Reticulin Staining

In the present study, reticulin stain is used to demonstrate the reticular fibres in fetal thymus , which form the supporting framework of the gland appearing black, while the collagen and cytoplasm appear purplish grey.Photo17 which was taken from sample number 1(24 weeks), which belongs to group IV shows the reticular fibres which appear black.Lobulations and trabeculae are seen well. Blood vessels and Hassall's corpuscles were seen in photo 18 which belongs to 31weeks .(group V, sample no.2)

2. Masson's Trichrome staining

In this study two samples were selected ,sample number 9 and 5 which belonged to gestational ages 26 weeks (group V) and 21 weeks (group IV) respectively and stained. Photo 19 highlights , the blood vessel in close relation to connective tissue trabeculae and the Hassall's corpuscle .The parenchyma made up of reticular fibers and collagen appear blue.Hassall's corpuscles appear reddish brown .In photo 20,Hassall's corpuscle and trabeculae which appear in blue colour is seen.

3. Van Gieson's stain -

In the present study ,sample number 14 which belonged to group V of 25 weeks gestation was stained .The lobules appear yellow in colour due to the cytoplasm of lymphocyte which take up the picric acid present in the stain.(photo 21)

4.Periodic acid-

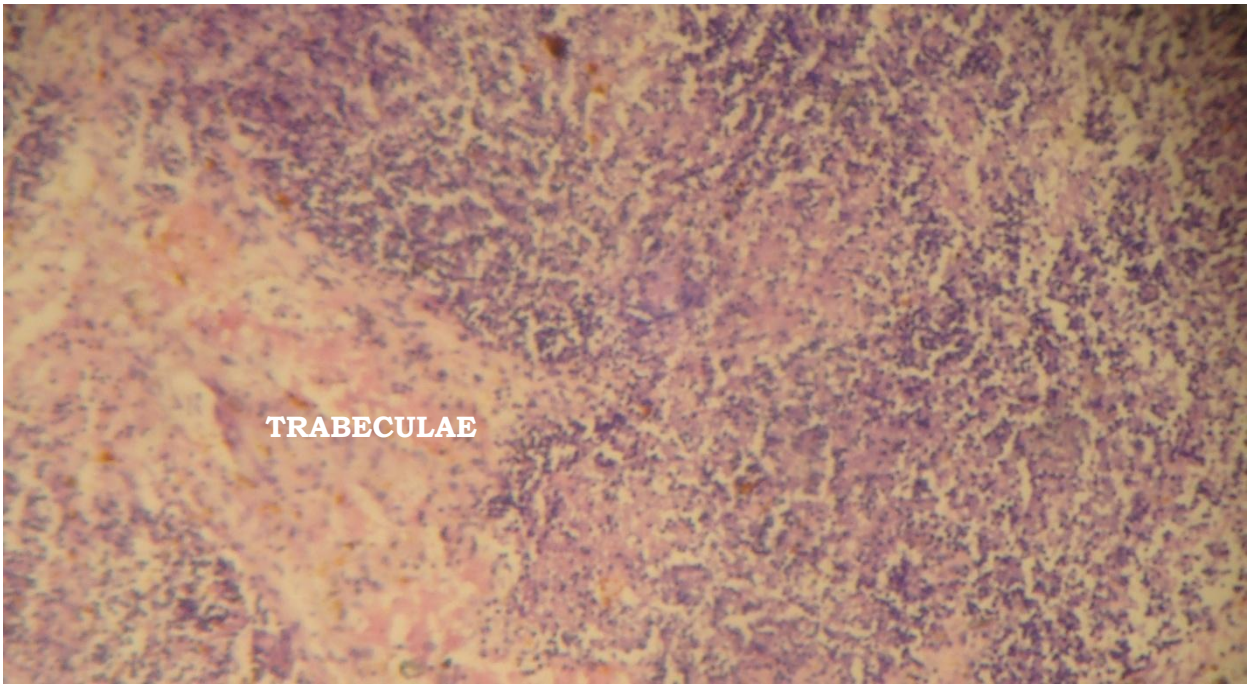
Schiff stain - In this study ,sample number 4 of 19 weeks fetus belonging to group IV was stained. The Hassall's corpuscle take up the stain and found to be PAS positive.(photo 22).

IMMUNOHISTOCHEMISTRY STAINING

Immunohistochemistry staining was done for 3 slides ,belonging to group IIgroup III and groupV ,with S 100 marker to demonstrate the proportion of Interdigitating cells(IDC) in the thymus. Observations were made about staining pattern-regular or irregular and the intensity of staining described as1+(mild) to 2+(moderate).The sample no;19 and 13 did not show any staining pattern, while the sample no;2 showed,regular staining pattern ,with the intensity of staining 2+.(photo23).

Photo-1

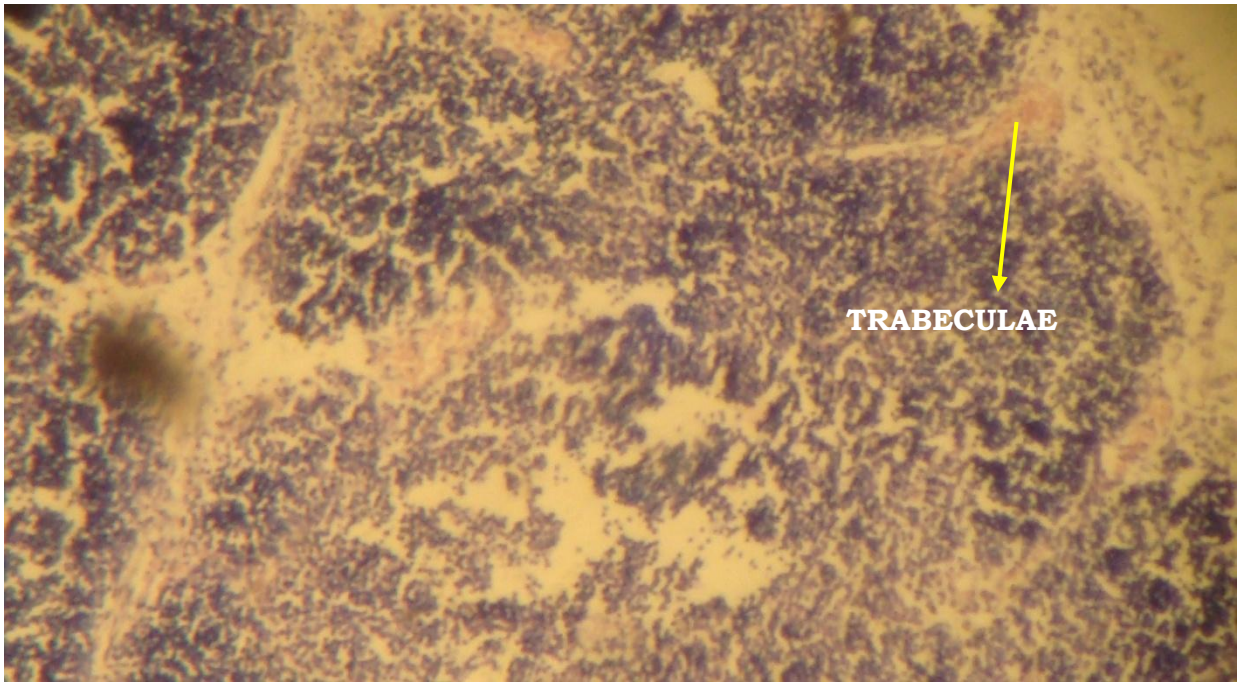
No Lobulation, no corticomedullary differentiation at 10 weeks (Group I)



S.NO.20 40x magnification

Photo-2

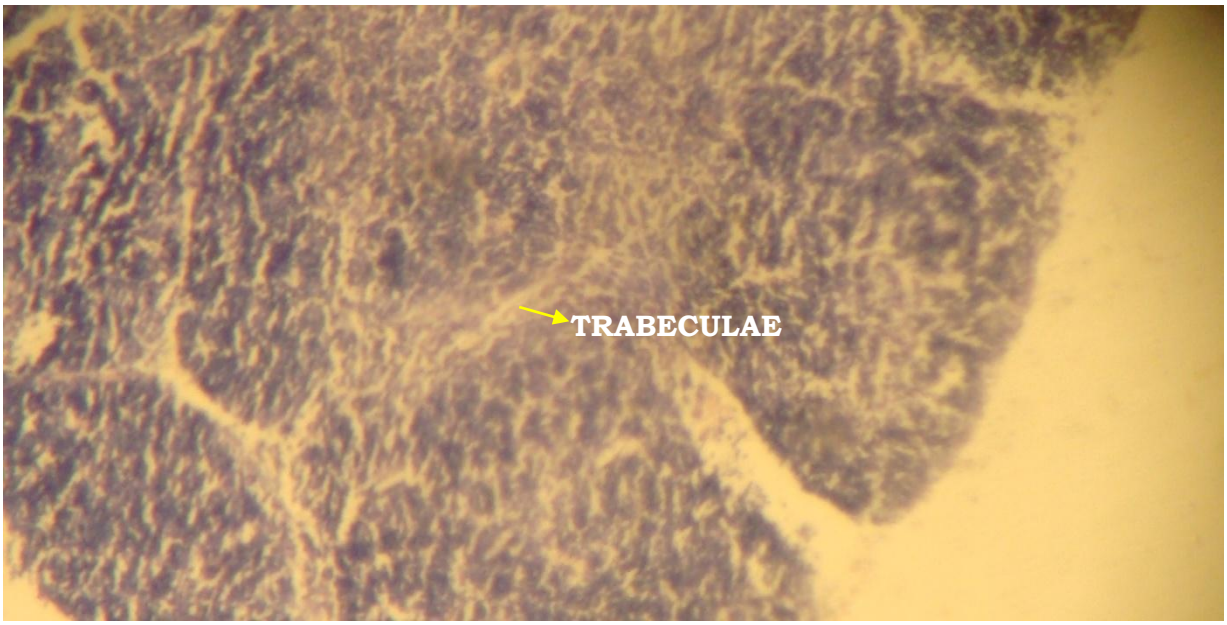
Lobules begin to appear at 12 weeks (Group II)



S.NO.19 40x magnification

Photo-3

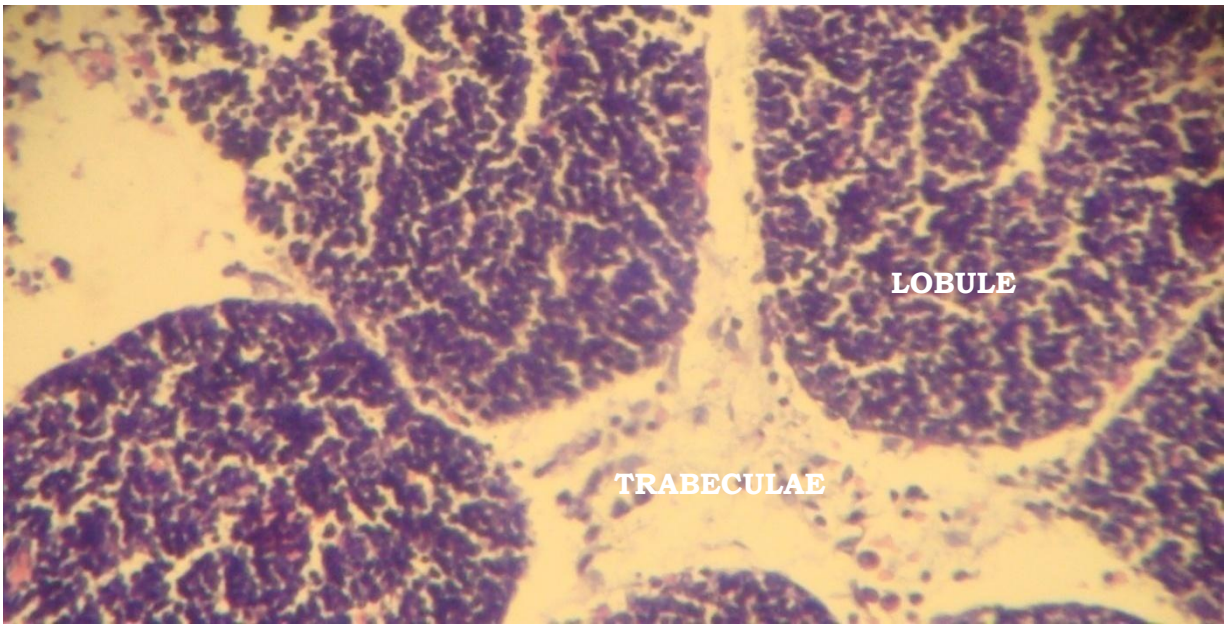
Poorly formed lobules at 13 weeks (Group II)



S.NO.6 40x magnification

Photo-4

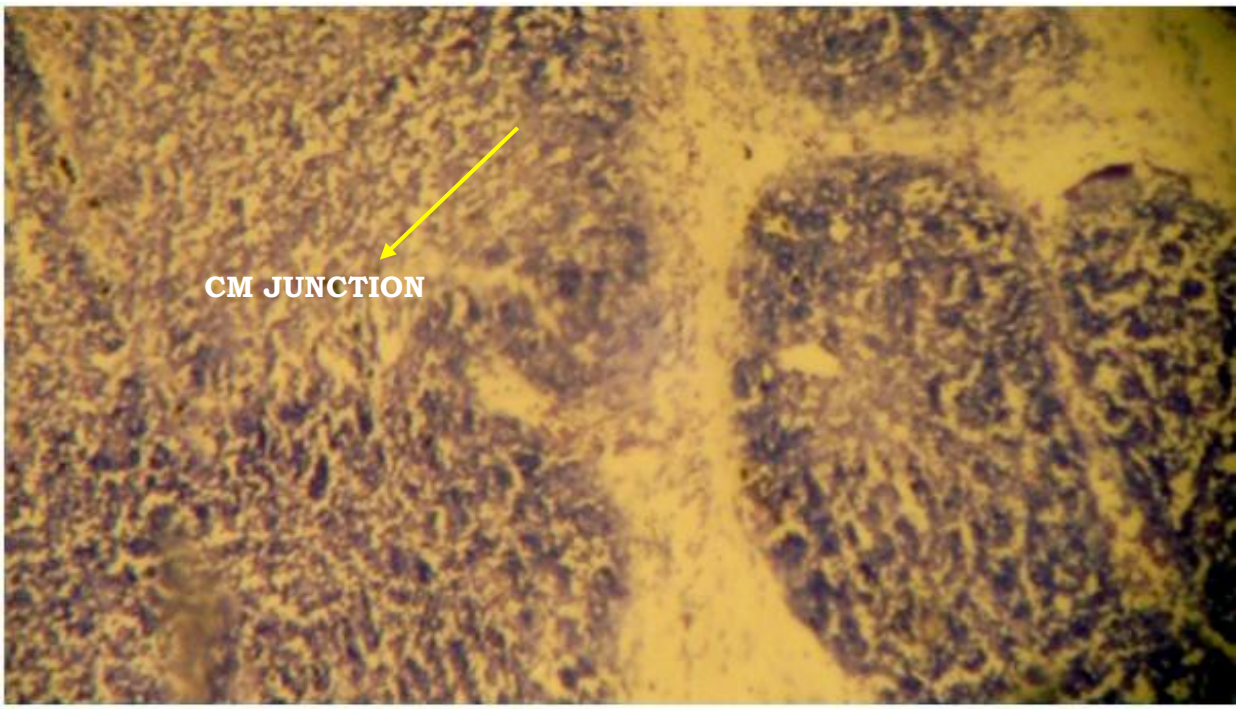
Lobulations seen, no corticomedullary differentiation at 14 weeks (Group II)



S.NO.8 100x magnification

Photo no- 5

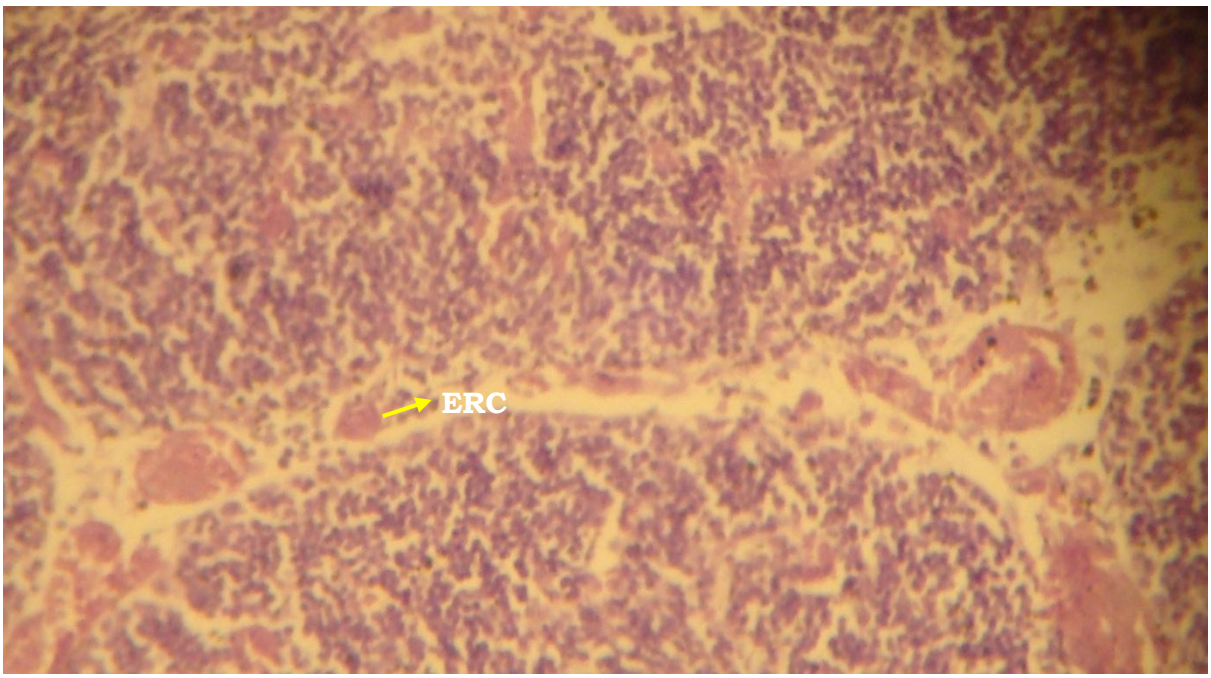
Lobulations seen , corticomedullary differentiation ill defined at 15 weeks (Group III)



S.NO.10 40x Magnification

Photo no - 6

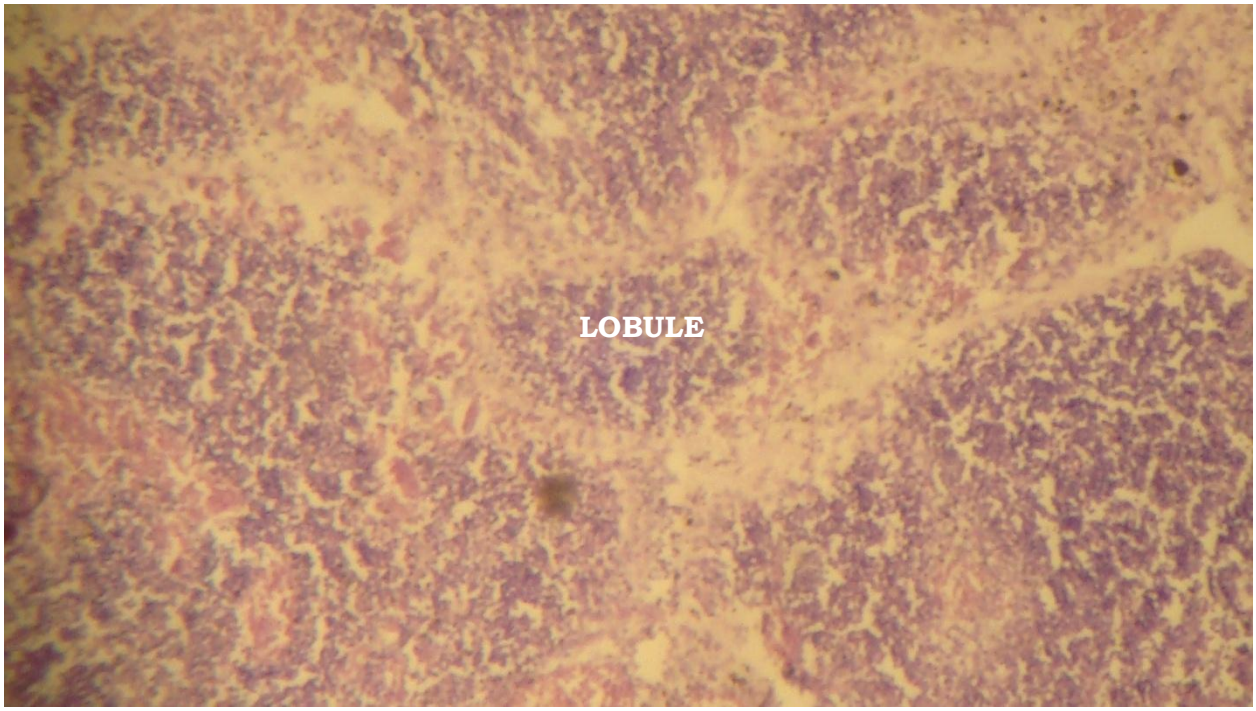
Epithelial reticular cells seen in trabeculae at 18weeks (Group IV)



S.NO: 7 40x Magnification

Photo no - 7

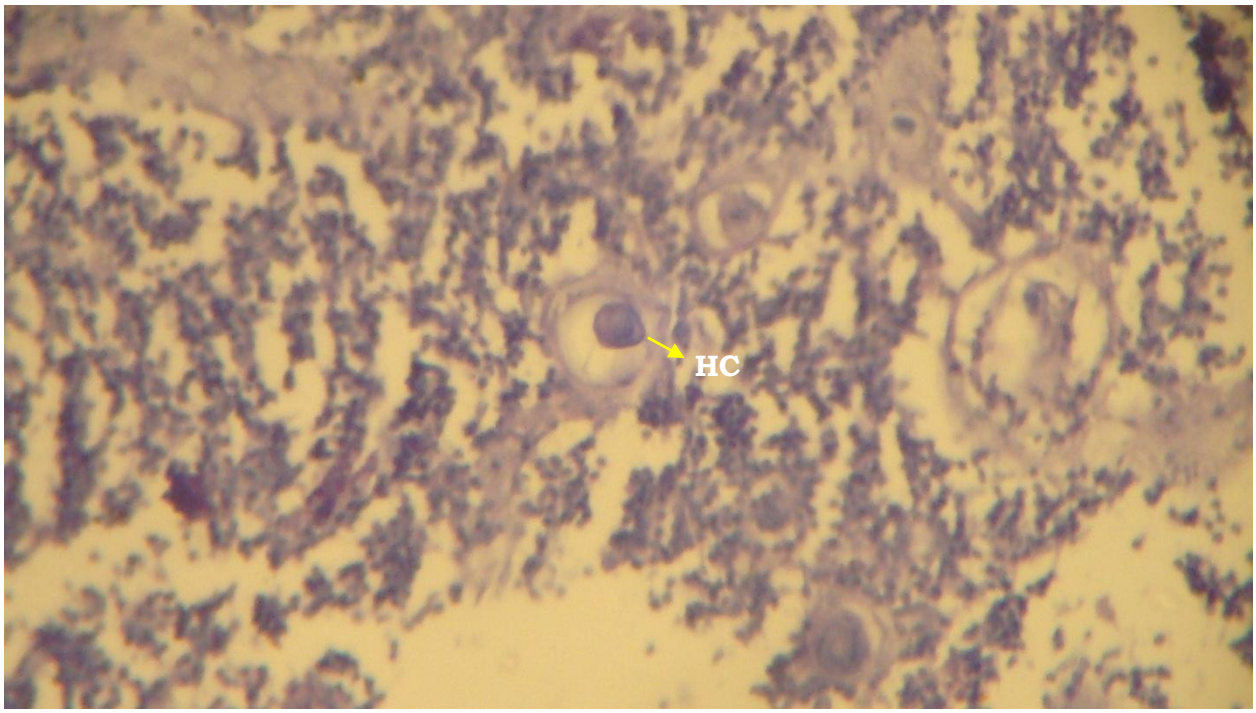
Well formed lobules and blood vessels seen at 18 weeks (Group IV)



S.NO.16 40x magnification

Photo no -8

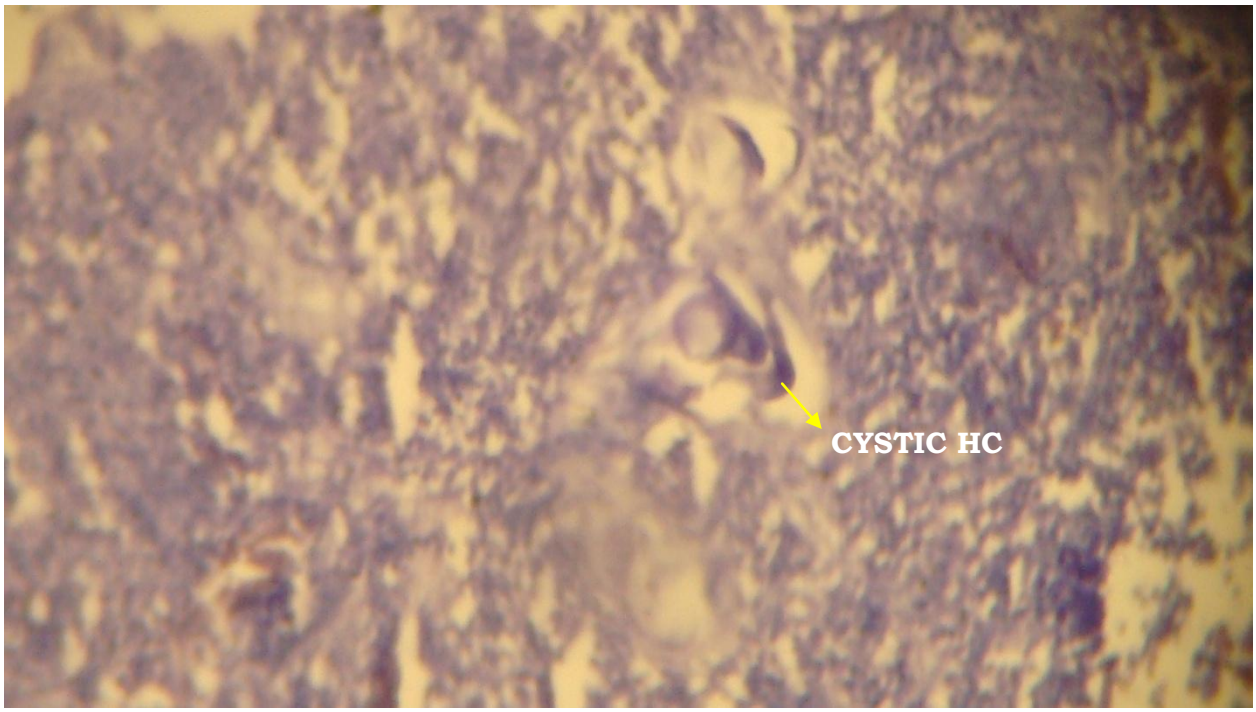
Many Hassall's corpuscles seen at 19 weeks (Group IV)



S.NO.4 100x Magnification

Photo no -9

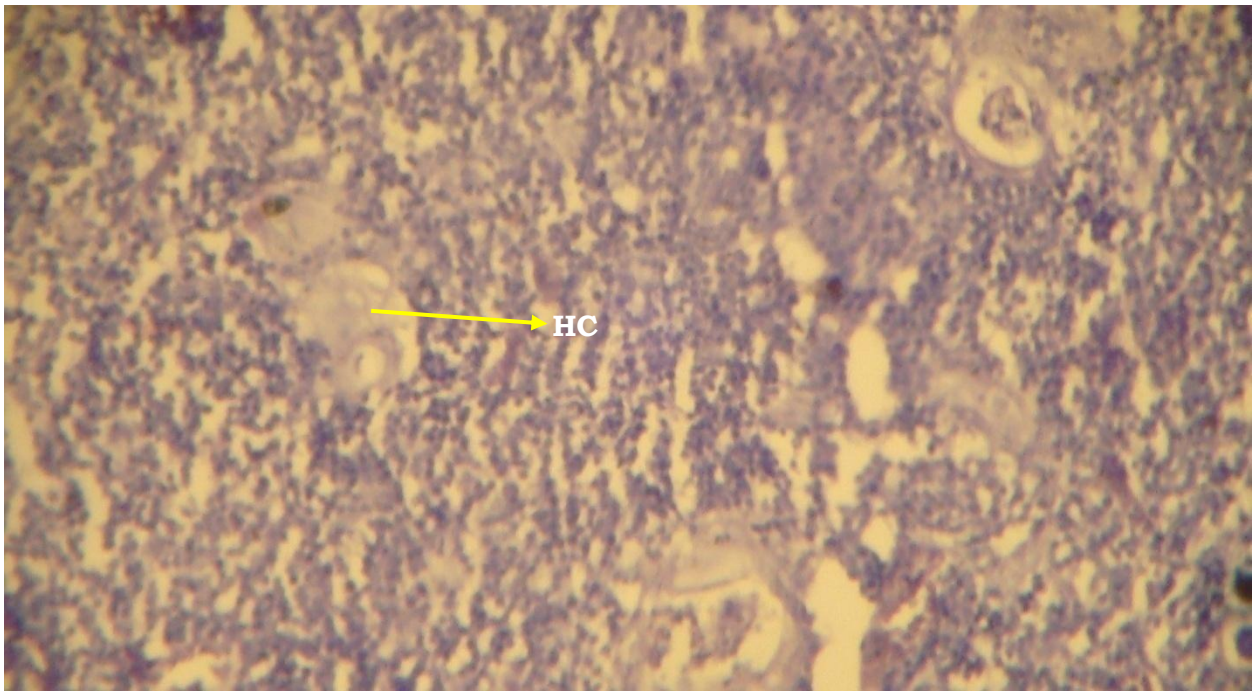
Many Hassall's corpuscles seen at 20 weeks (Group IV)



S.NO.12 40x Magnification

Photo no -10

Many developing Hassall's corpuscles seen at 20 weeks (Group IV)



S.NO.12 40x Magnification

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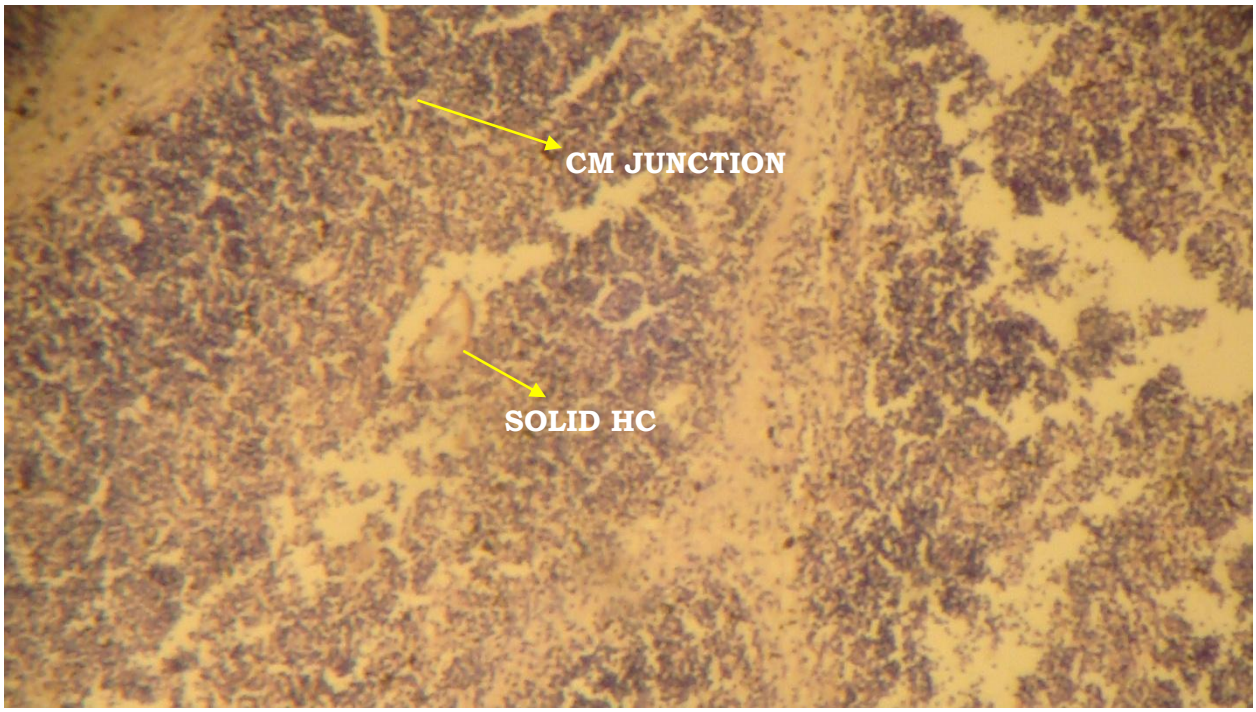

S.NO.5 40x Magnification

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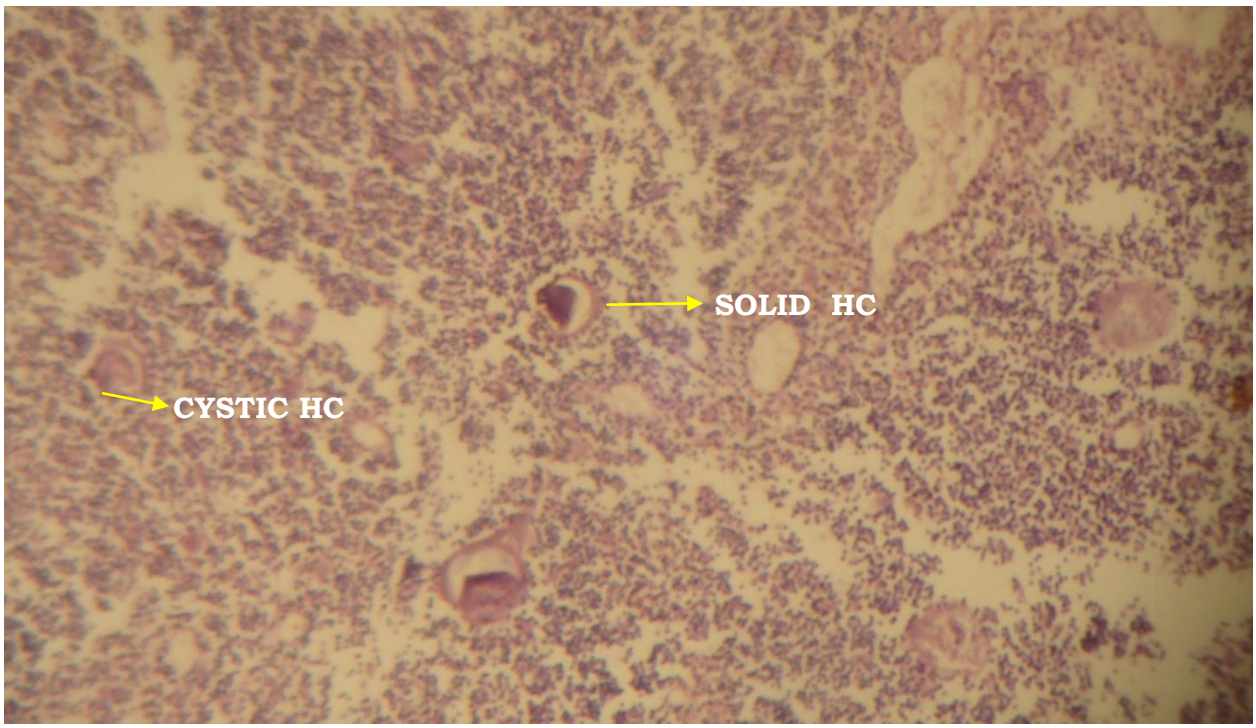
Solid and cystic type of Hassall's corpuscle seen at 24 weeks (Group IV)



S.NO.1 40x Magnification

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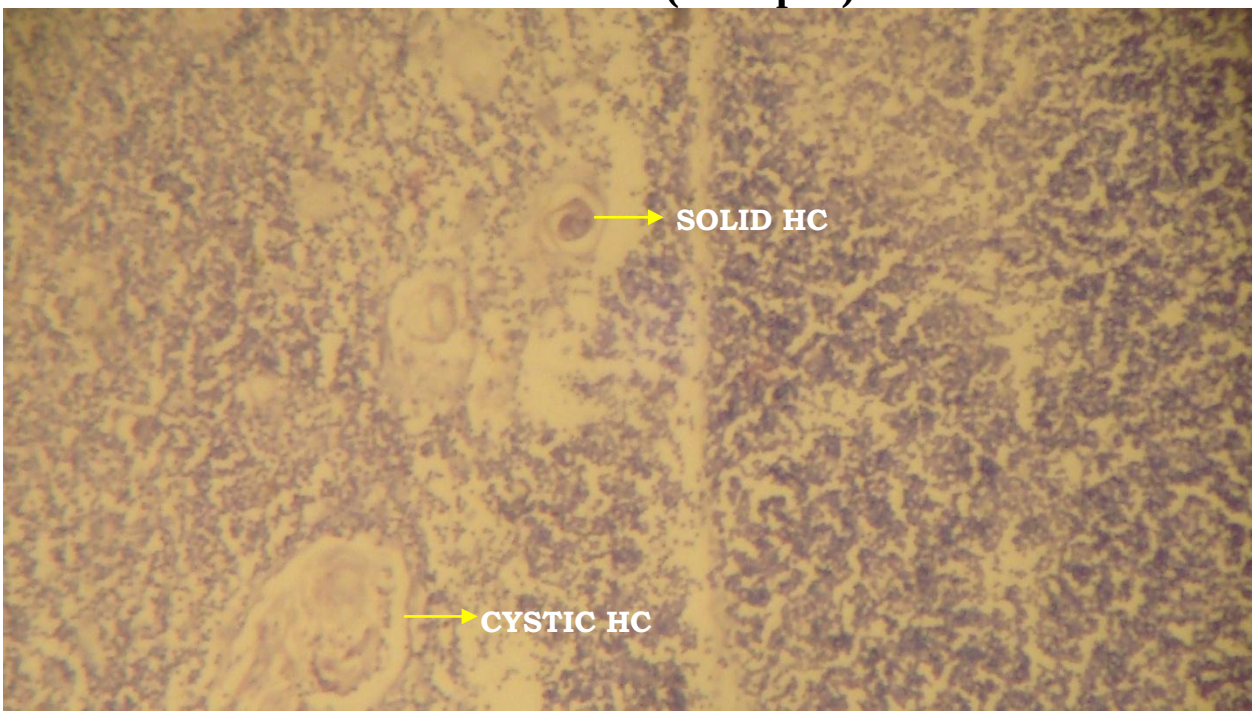
Solid and cystic type of Hassall's corpuscle seen at 25 weeks (Group V)



S.NO.14 40x magnification

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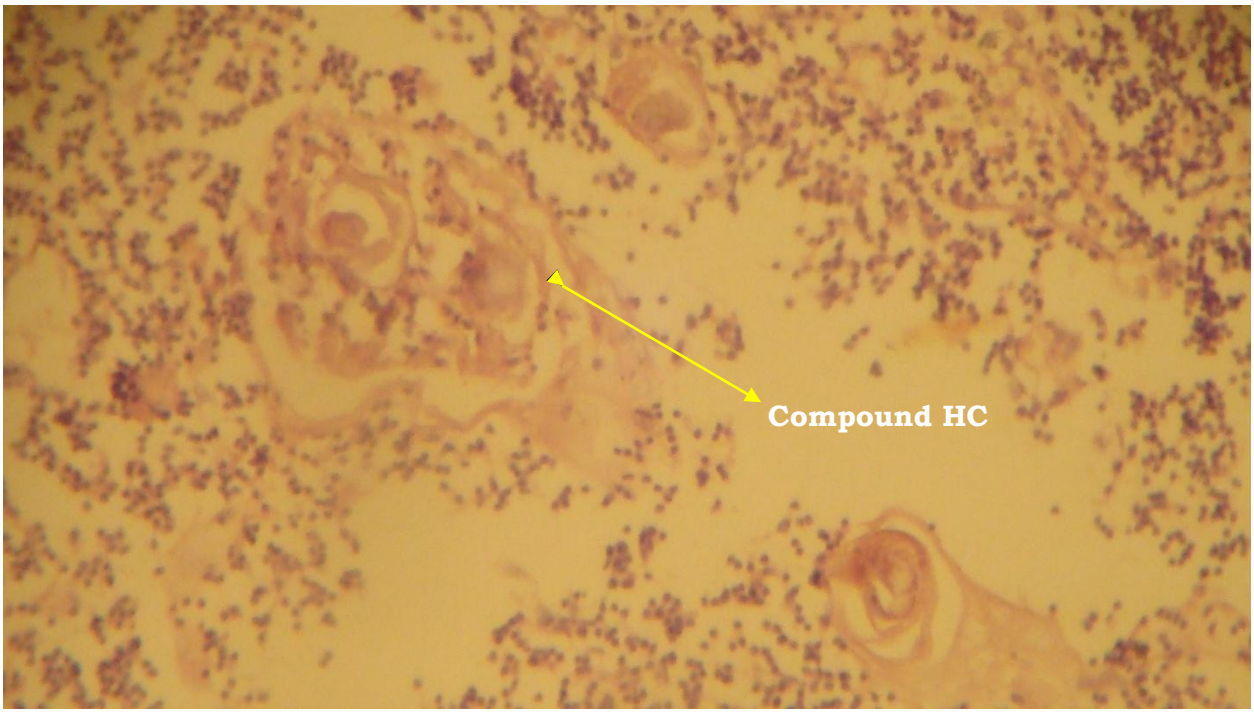
Well formed compound type of Hassall's corpuscles seen at 26 weeks (Group V)



S.NO.9 100x magnification

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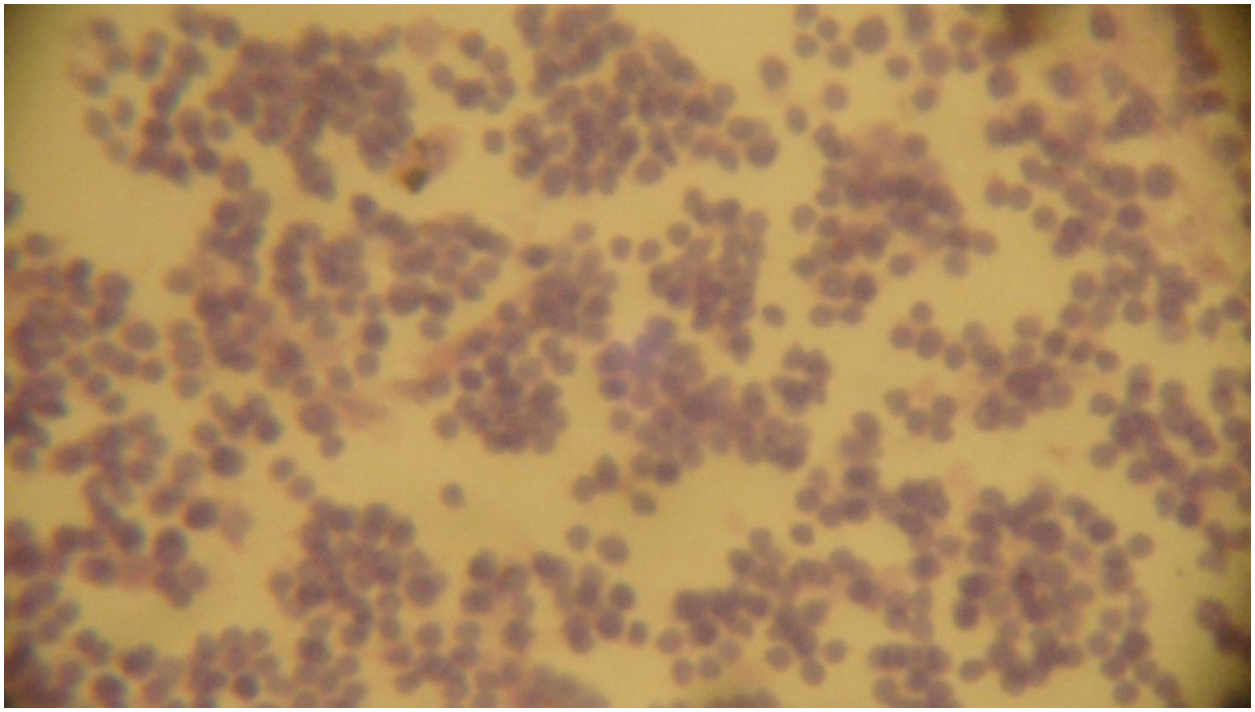

S.NO 15 400x magnification

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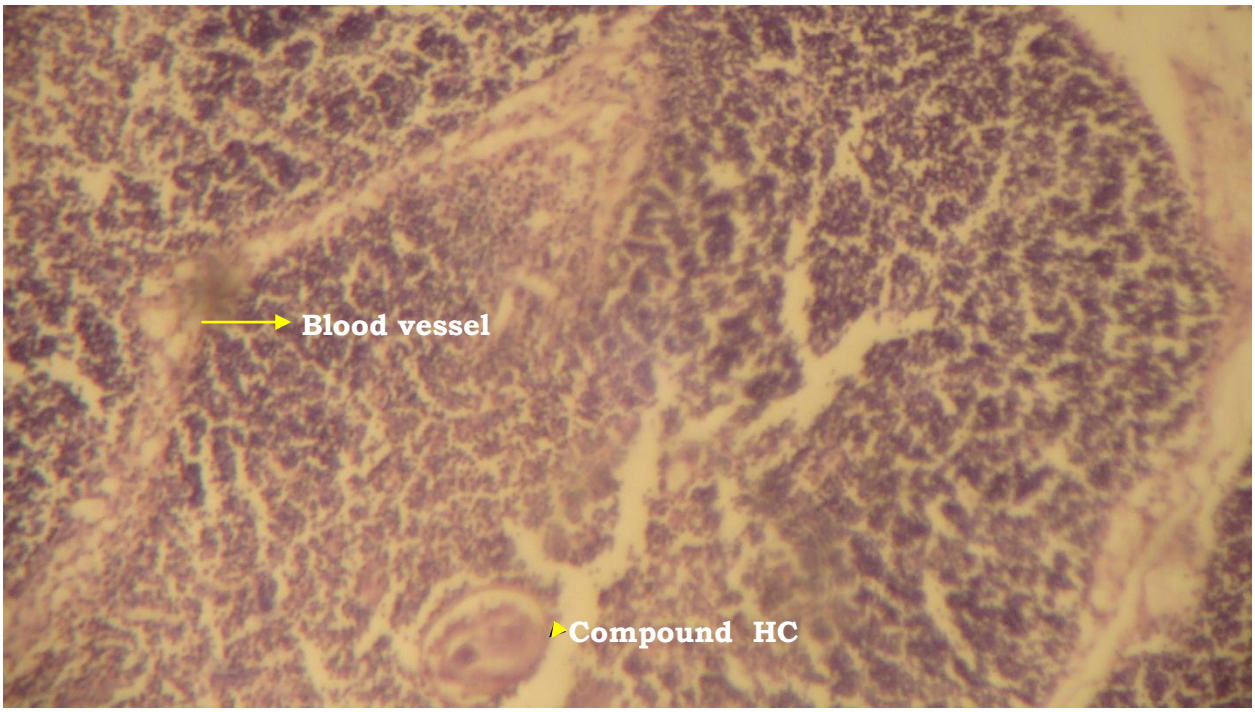

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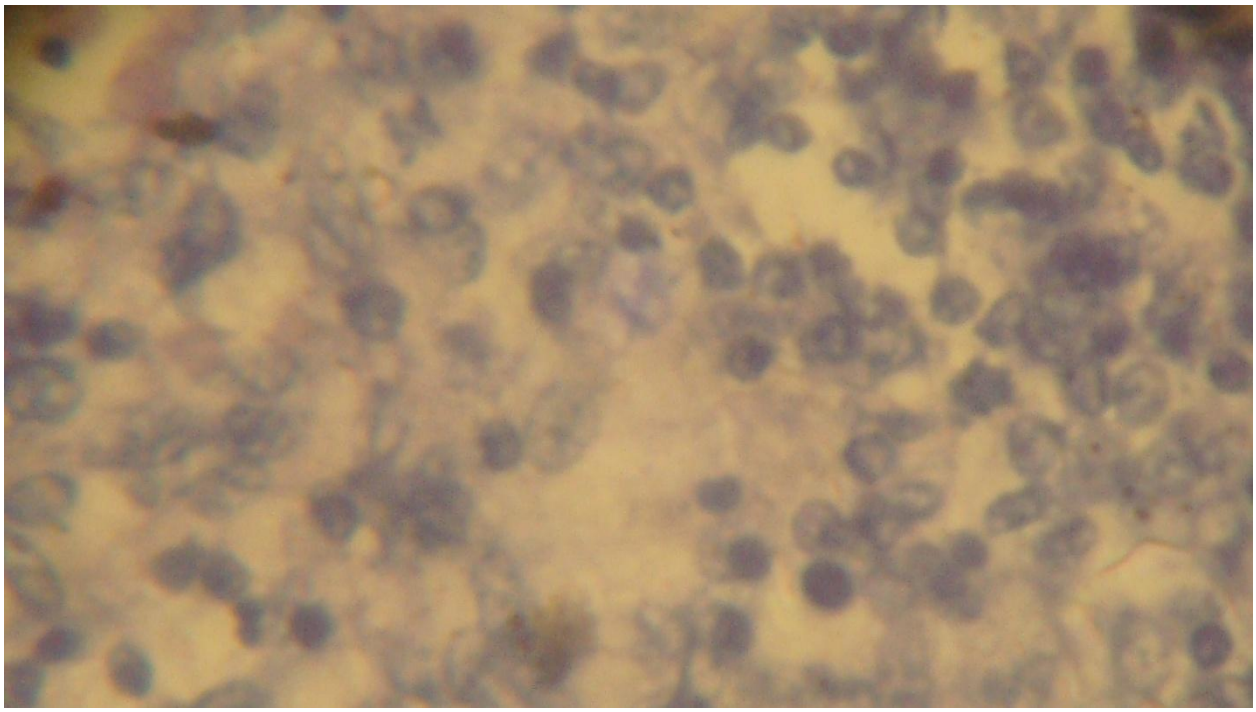
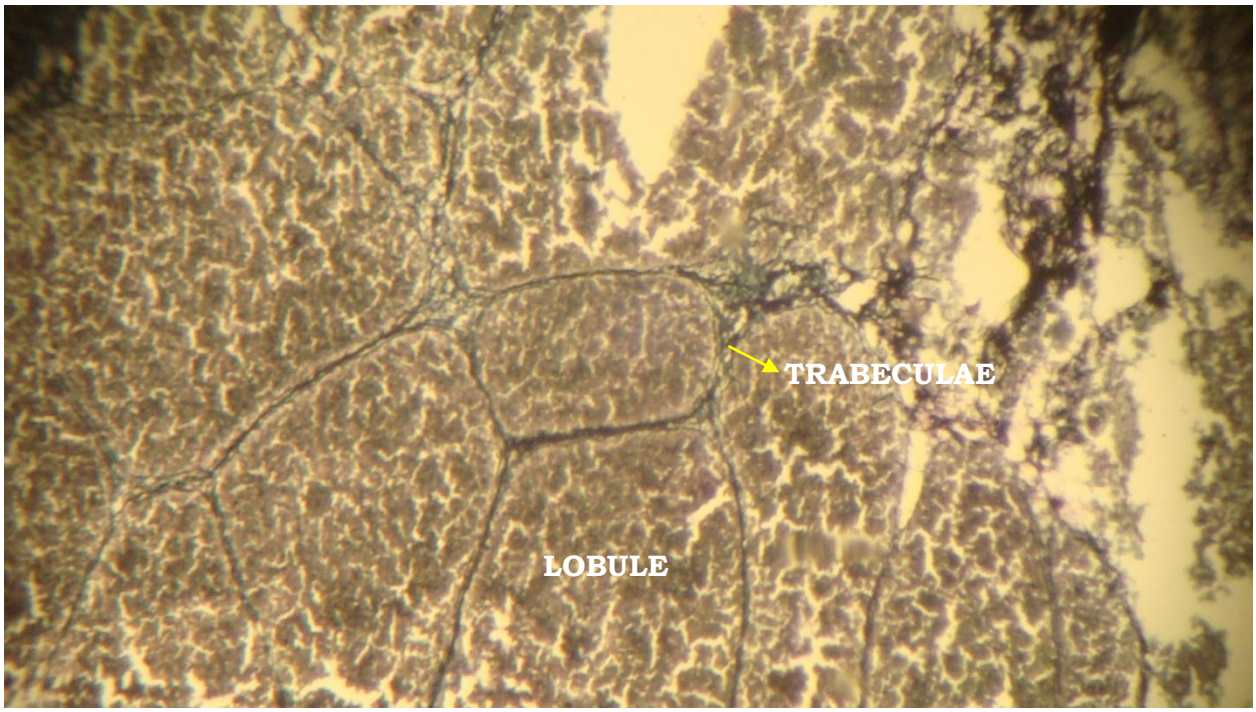
Macrophages are seen -18 weeks (Group IV)



S.NO.16 400x Magnification

Photo no -18

RETICULIN STAIN-Lobulations well formed at 24 weeks (Group IV)



S.NO.1 100x Magnification

Photo no -19
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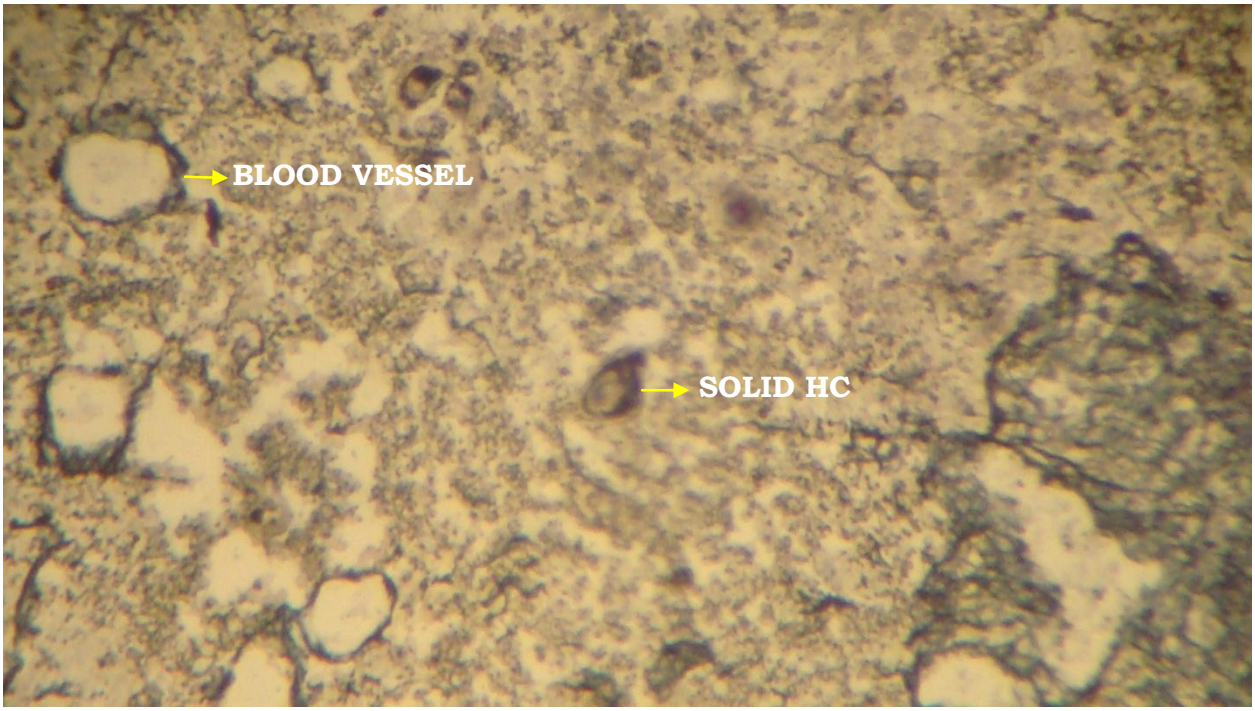

S.NO.2 400x Magnification

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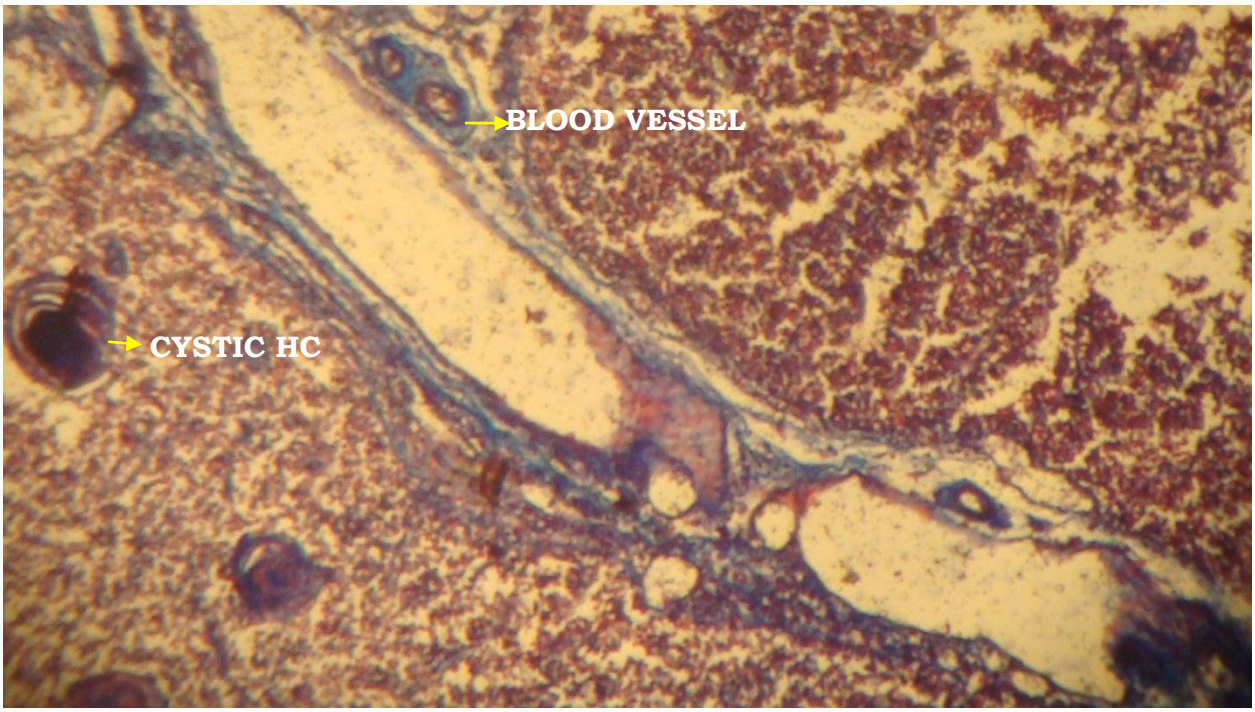

S.NO.9 400x Magnification

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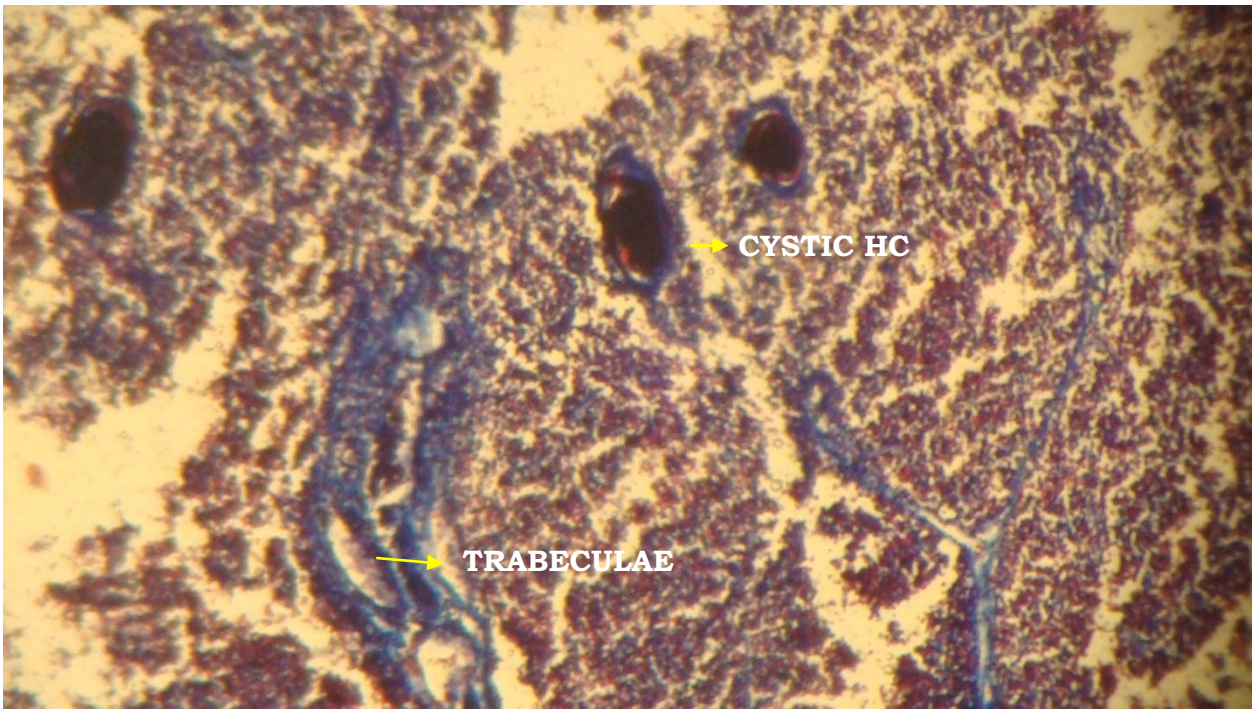

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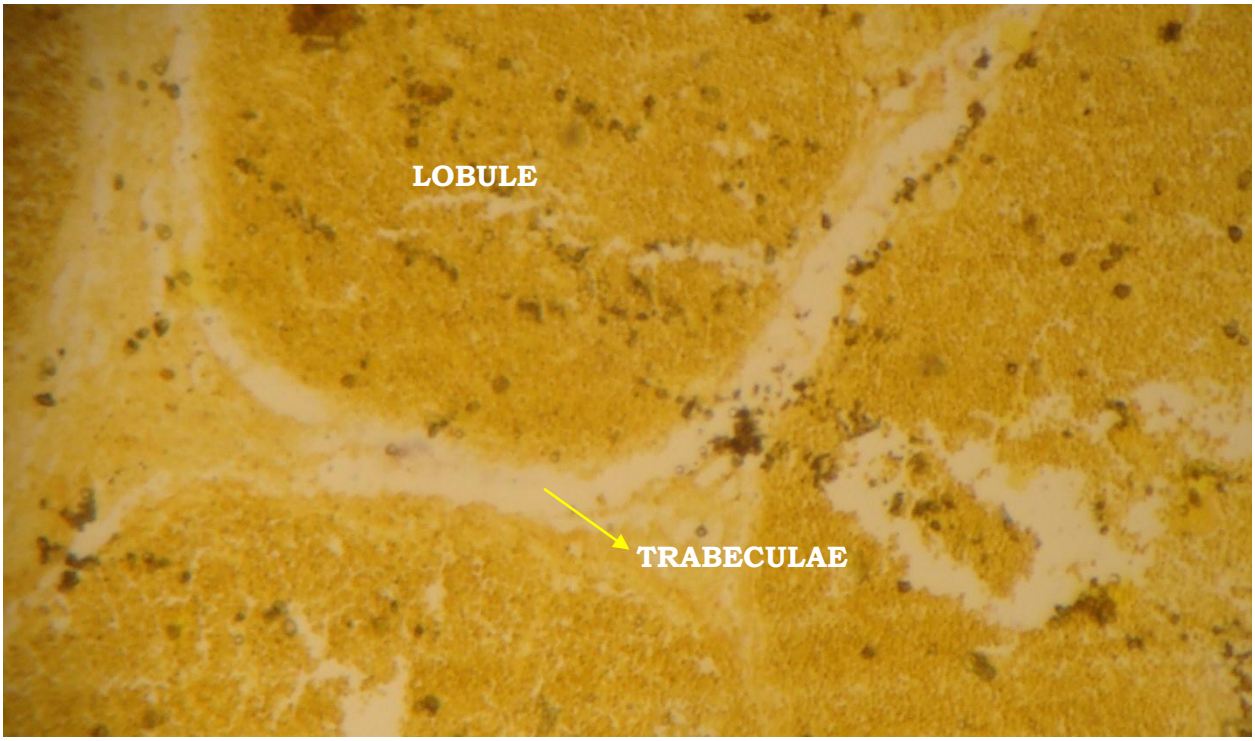

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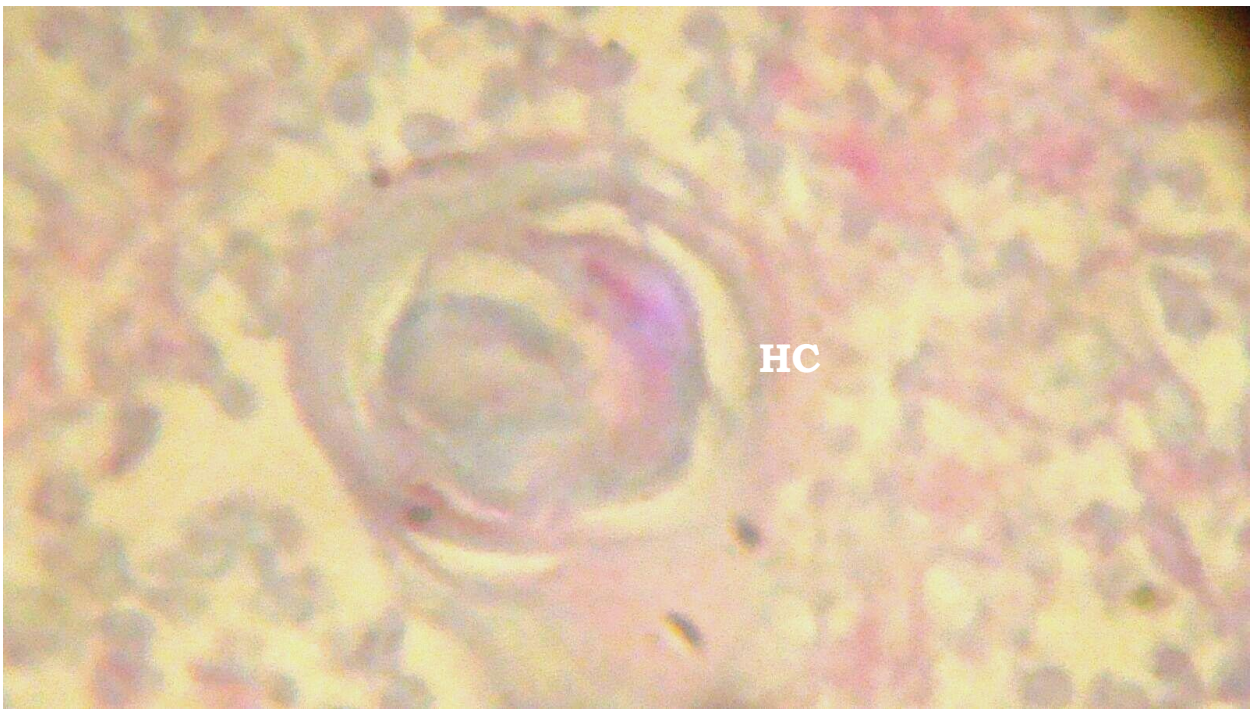

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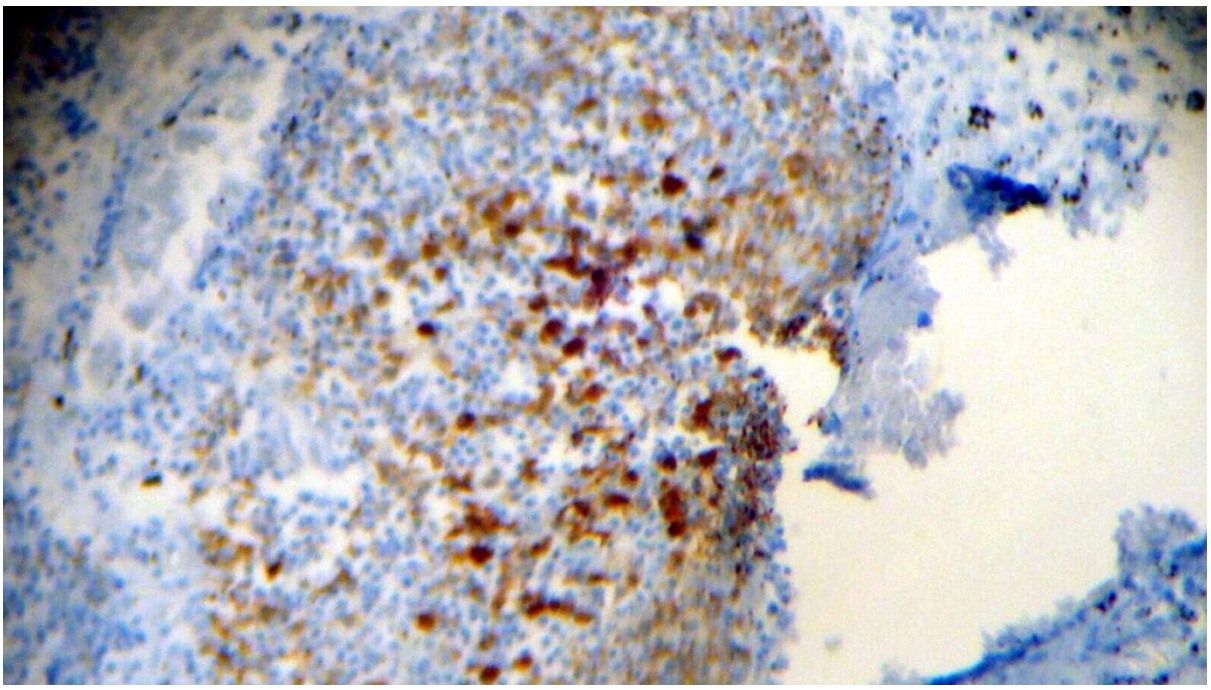

S.NO.2 100x Magnification

DISCUSSION

In the present study the development of human fetal thymus is studied under the following histological parameters.

1. Lymphocytes:

The time at which the lymphocytes were present in the thymus varies in different studies. It appears by 8th week according to Williams and Standring S et al and Romanes GJ .Reported to appear by 9th week according to HaarJL, Hamilton and Mossman ,Von Gaudecker,Ritter and Lampert,Ajita et al,prabhavathy, Bashir khan and Sanobar sheikh.

In the present study the lymphocytes were observed from 10th week which have round purple nuclei with basophilic cytoplasm. It could not be ascertained whether lymphocytic infiltration occurs from 8th week onwards, since the present study examined fetuses from 10 weeks.

2. Lobulation:

The epithelial cells grow as thumb like protrusions into the mass of mesenchyme, which later forms a thin capsule around the organ. In the region between epithelial protrusions, the mesenchyme remains and forms thin incomplete septa, to give the lobular appearance.

Ghali et al, Bashir khan and Sanobar sheikh reported lobulation at 10th week, while Haar et al reported appearance of Lobulation at 12th week.

In the study conducted by Ajita et al, Prabhavathy Lobulations started at 9th week and completed by 12th week. According to Vijayalakshmi et al Lobulations appeared by 16th week.

In the present study Lobulation started by 12th week and completed by 15th week.

3**. Cortex and Medulla**

The cells of Lymphatic series are more concentrated towards the borders of each lobule .Hence, at the periphery of the lobules lymphocytes are numerous and densely packed forming darkly stained cortex, whereas the medulla is lightly stained due to less number of lymphocytes.

The Cortico medullary differentiation noted in the embryos of 40mm crown-rump length by Hamilton and Mossman ,Ghali et al by 11th week,Hayward, Arey and Muller – Hermelink et al by 12th week,Haar, Lobach & Haynes, Prabhavathy by 14th week.Ajita et al reported that the differentiation started at 9th week and more distinct at 12 to 14 weeks.According to Bashir khan and Sanobar sheikh the differentiation started at 12th week and more distinct by 14th week.Vijayalakshmi et al reported at 16th week.

In the present study the cortico medullary differentiation started at 15th week more distinct by 18th week. This coincides with the study of Vijayalakshmi et al

4. Blood Vessels

According to Haar, Hamilton and Mossman, Ajita et al. Bashir khan and Sanobar sheikh vascularization started at 9^{th} week, medullary vessels seen at 12^{th} week.

Vascularity was reported by Williams et al by 10^{th} week, by Ghali et al at 11^{th} week.

In the present study blood vessels were seen by 10^{th} week in the trabeculae. Since the fetus prior to 10^{th} week was not examined, it could not be ascertained whether blood vessels were present at early stages.

5. Macrophages

Haynes reported macrophages by 10^{th} week, while the appearance of macrophages was reported at 12^{th} week by Ajita et al. Stranding S et al , Bashir khan and Sanobar sheikh reported its appearance at 14^{th} week.

In the present study macrophages were seen from 12^{th} week, which coincides with the study of Ajita et al.

6. Epithelial Reticular cells

Williams et al, Hamilton and Mossman, Von Gaudecker, Muller-Hermelink and Standring S et al have described the appearance of epithelial reticular cells by 8^{th} week.Ajita et al observed the cells at 9^{th} week.Haywar,

Arey and Bashir khan et al reported at 10th week.Vijayalakshmi et al reported the cells at 12th week of gestation.

In the present study the epithelial cells were observed at 10th week. Since the fetus prior to 10th week was not examined, it could not be ascertained whether the epithelial cells were present at an earlier stage.

7. Hassall's corpuscles:

The time of appearance of Hassall's corpuscles varies in different studies; Fawcett, Hamilton and Mossman reported its appearance as early as 8th week,Gilhus et al from 9th week,Williams and Stranding S et al and Arey at 10th week. Ghali et al at , Sawant, Bashir khan et al at and Varga et al at reported its appearance by 11th ,12th and 13th week respectively.

Ajita et al and Krishnamurthy et al noted its appearance by 15th week,Lobach and Haynes reported it between 15th and 16th week while Liberti et al noted at 16th week.According to Bodey and Kaiser Hassall's corpuscles develop in the 2nd part of 3rd lunar month.Vijayalakshmi et al reported it at 18th week of gestation

In the present study the Hassall's corpuscle was observed from 15th week onwards,which coincides with the study of Ajita et al.

Growth of Hassall's corpuscles

Bodey and Kaiser reported that the greatest development was between 6^{th} and 10^{th} lunar month.Liberti et al mentioned that the mean area of Hassall's corpuscle increased with the fetal age with greatest difference between 16^{th}-19^{th} week and 20^{th}-23^{rd} week.Ajita et al and Krishnamurthy et al observed the increase in number and size during 17^{th}- 24^{th} weeks.Bashir khan et al reported the growth to occur during 18^{th}- 24^{th} week.

In the present study the number and size of the Hassall's corpuscle increased during 18^{th}- 24^{th} week ,which coincides with the study of Bashir khan et al.

Immunohistochemistry

The immunoreactive - interdigitating cells (IDCs) cells are distributed mainly in the medulla with some scattered elements in the cortex. The immunocytochemistry for S-100 visualizes the precise distribution and extension of the IDCs under the light microscope and indicates that the IDCs form no structural networks such as those established by the thymic epithelial cells.

CONCLUSION

The present study concludes that in the histogenesis of human fetal thymus, significant cellular events like lobulation ,corticomedullary differentiation and the appearance of Hassall's corpuscle all takes place between 15^{th} and 18^{th} week of gestational age. Thereafter the microscopic growth and maturity takes place in the form of increase in size of lobules , blood vessels and increase in size and number of Hassall's corpuscle.

Hence the period of gestation between 15 and 18 weeks is critical for the development of fetal thymus .Any insult occurring to the developing thymus in the form of radiation or drugs can affect its histogenesis leading to impaired immunity.

As the above observations were made in a very limited sample size statistical significance could not be determined. This precluded the quantitative study of fetal thymic cellular components in relation to gestational age and hence an attempt was made for qualitative study.

The clinical implication of this study is to provide the basis for more accurate interpretation of the histogenesis of fetal thymic cellular components in relation to gestational age .Individuals in whom there is a persistence of myoid cell beyond 28 weeks of gestation, suggest they are prone for myasthenia gravis.

Immunohistochemical study for demonstrating the Interdigitating cells (IDC) are the sites where the Human Immunodeficiency Virus(HIV) can remain latent, and gets activated by CD40 ligation or by the presence of T-Helper cells. They play a role in transport of HIV .The virus derived from infected IDCs carry T cell specific factor that make them highly infectious.

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Specimen number	Gestational Age in weeks	group	Lobulation	cortex	medulla	Cortico medullary junction	trabeculae	Blood vessel	Hassall's corpuscles
1	24	IV	+	+	+	+	+	+	+
2	31	V	+	+	+	+	+	+	++
3	20	IV	+	+	+	+	+	+	+
4	19	IV	+	+	+		+	+	+
5	21	IV	+	+	+	+	+	+	+
6	13	II					+	+	
7	18	IV	+	+	+		+	+	+
8	14	II	+				+	+	
9	26	V	+	+	+	+	+	+	++
10	15	III	+				+	+	
11	19	IV	+	+	+	+	+	+	+
12	20	IV	+	+	+	+	+	+	+
13	16	III	+	+	+	+	+	+	+
14	25	V	+	+	+	+	+	+	+
15	27	V	+	+	+	+	+	+	++
16	18	IV	+	+	+		+	+	+
17	16	III	+				+	+	+
18	19	IV	+	+	+	+	+	+	+
19	12	II					+	+	
20	10	I					+	+	