

HISTOGENESIS OF HUMAN FETAL SPLEEN

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ABSTRACT

Introduction: Spleen is the organ of interest for research workers since a long time. Though, detailed study about embryonic development is there in the literature but microscopic structures of spleen at different stages in the fetal period is far less available. Hence, the present study attempted to find out the detailed histological changes of human fetal spleen during its development.

Materials and Methods: The study was carried out on 50 human foetuses of known gestational age in the department of Anatomy, MGM Medical College, Navi Mumbai. The sections of the spleen were studied after staining with Haematoxylin and Eosin stain and were observed under compound light microscope.

Observations and Results: At 12-14th gestational week of development there was irregular network of mesenchymal cells with thin capsule, reticular cells; lymphoblast's and few blood vessels were present. Increased haematopoietic cells including WBC's and RBC's were seen at 15th week. Vascularity increased at 16-17th week. At 18th week the tissue started showing division of red pulp and white pulp. Clear-cut division was seen after 20th week stage. From 22nd weeks onwards the structure was almost similar to that of adult spleen. Germinal centre was not seen up to 38th week of gestation.

Conclusion: The study provides a detailed knowledge about the histogenesis of human fetal spleen. It also suggests the functional status of spleen in fetal period.

KEY WORDS: Spleen, Histogenesis, White pulp, Red pulp, Reticular cells.

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INTRODUCTION

The spleen, the largest of the lymphoid organs appears at about 6th weeks of gestation as localized thickening of the coelomic epithelium of the dorsal mesogastrium near its cranial end. The proliferating cells invade the underlying angiogenetic mesenchyme which becomes condensed & vascularised. The process occurs simultaneously in several adjoining areas which soon fuse to form a lobulated spleen of dual origin i.e. from coelomic epithelium & from

mesenchyme of the dorsal mesogastrium [1].

The spleen is surrounded by a capsule and located in the pathway of the blood stream (splenic artery and vein). The substance of spleen, the splenic pulp, consists of red pulp and white pulp, so named because of their appearance in fresh tissue. The red pulp consists of a dense network of reticular fibres that contains numerous erythrocytes, lymphocytes, plasma cells, macrophages and other granulocytes. The main function of the red pulp

is to filter the blood. It removes antigens, microorganisms, platelets, and aged or abnormal erythrocytes from the blood. The white pulp is the immune component of the spleen and consists mainly of lymphatic tissue. Lymphatic cells that surrounds the central arteries of the white pulp are primarily T cells, whereas the lymphatic nodules contain mainly B cells. Antigen-presenting cells and macrophages reside within the white pulp [2, 3].

Though it is known that, all lymphoid organs in human including spleen shows variations in microscopic structure in adult but not many details are known about the microscopic structure of spleen at different stages of development in the prenatal period (from 10th to 38th gestational week (GW). Hence, the present study aims to find out the histogenesis and development of spleen in prenatal period.

MATERIALS AND METHODS

The present study was carried out on 50 normal human fetuses, aged between 10th to 38th gestational weeks. The normal fetuses were obtained from the Department of Obstetrics and Gynecology, MGM Medical College, Navi Mumbai and MGM Hospital, Kalomboli, Navi Mumbai, India. After permission from the Institutional Ethical Committee, MGMIHS the fetuses were collected in 10% formalin for carrying the present study. The age of fetuses was calculated from the obstetrical history, gross features, crown-rump lengths (CRL) and crown-heel length (CHL). The fetuses included the spontaneous abortion and still born fetuses. Cases with any anomaly or pathology were not included in the study.

The specimens were categorized into the following groups

Group I - 10th to 15th gestational weeks.

Group II - 16th to 20th gestational weeks.

Group III - 21st to 25th gestational weeks.

Group IV - 26th to 30th gestational weeks.

Group V - 31st gestational weeks onwards.

The fetuses were fixed in 10% formalin for 10-15 days. The spleen was studied after the dissection of fetuses. The dissection was done according to Cunningham's text book of Anatomy 12th edition [4]. Then the spleens were kept in

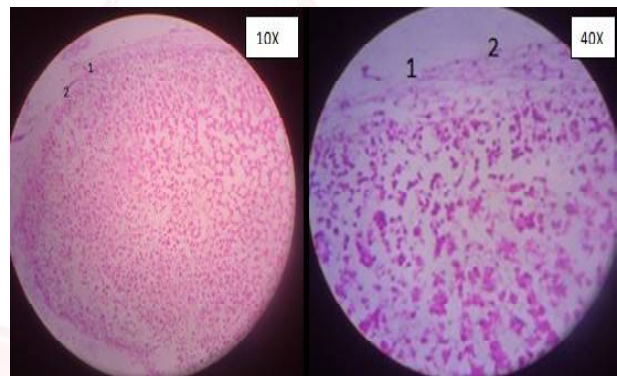
10% neutral buffered formalin (NBF) for 2 to 4 days and then processed for preparing paraffin sections. Serial sections of 5 µm thickness were cut with rotatory microtome. All the sections were studied under compound microscope and microphotography done with digital camera. All the digital images were stored and analyzed.

RESULTS

Observations done under 10X & 40X using compound light microscope.

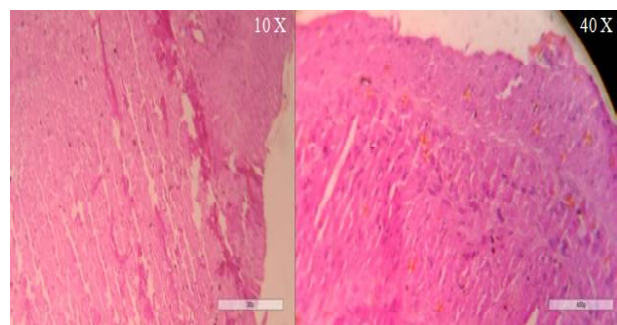
Group I – 10th to 15th GW: Spleen was covered by thin capsule made up of connective tissue. The capsule and the trabeculae appeared more cellular; fibroblasts were seen with minimal collagen fibres. The fibroblast was observed in the capsule.

Fig. 1: Fetal spleen at 10th GW shows 1- capsule, 2 – fibroblast (H & E stain).



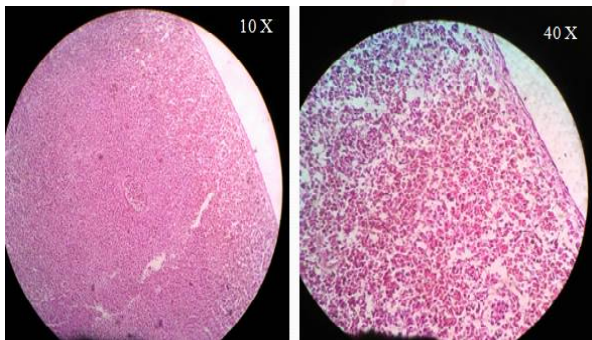
In the fetuses of 12th week, the spleen was covered with a thin capsule made up of connective tissue. The splenic interstitial tissue was predominated by collagen fibres. Fibroblasts and fibrocytes were observed among the connective tissue components. The fibroblasts cells were observed inside the capsule in the interstitial tissue, few blood vessels filled up with blood cells were observed. Blood cells were RBC's only. No central arterioles were seen till 12th week of gestation.

Fig. 2: Fetal spleen at 12th GW (H & E stain).



Fetal spleen at 14th weeks shows capsule and developing trabeculae in the interstitial tissue were seen. Reticular cells forming network are present and the reticular cells were comparatively increased in numbers but smaller in size. More number of blood vessels is seen (i.e. Spleen is more vascular). Numbers of haemopoietic cells are increased. Numbers of lymphocytes arranged in groups are seen but these groups are scattered. Clear-cut division of red pulp and white pulp is not evident.

Fig. 3: Fetal spleen at 14th GW (H & E stain).

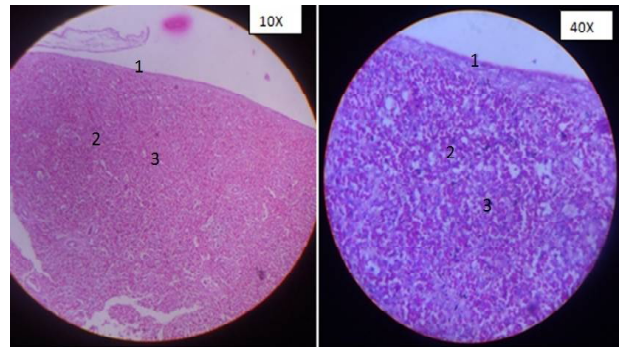


Group II – 16th to 20th GW: In the foetuses of 16th gestational weeks, the spleen shows increase in the amount of connective tissue. The reticular fibres, sinusoidal spaces and numbers of blood vessels are also increased. The spleen shows more vascularity at this stage. Large numbers of hemopoietic cells are seen similar to that of previous stage. The spleen of 17th GW shows mixed population of cellular and connective tissue components. Blood vessels and splenic sinusoids were seen more in plenty. The cellular component consists of red blood cells as well as lymphocytes. Lymphocytes aggregations start to become more prominent at this stage and could be detected at the periphery of arteriole, though well defined dense aggregation couldn't be established.

At 20th Gestational weeks, the spleen shows clear-cut capsule surrounding the spleen having bundles of collagen fibres with fibroblast cells as well as fibrocytes was observed. The trabeculae with well differentiated blood vessels were dispersed in the substance. The number of blood vessels is increased. The lymphocyte aggregations started differentiating around the central arteriole forming the periarteriolar lymphatic sheath (PALS) by 20th weeks. Reticular cells of comparatively small in size and less in number could be detected. More

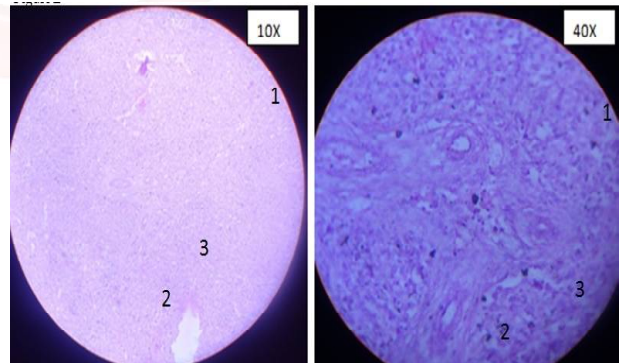
amount of connective tissue was observed in comparison to the earlier group.

Fig. 4: Fetal spleen at 16th to 20th GW (H & E stain). Fetal spleen shows 1- Capsule, 2- immature RBCs, 3- lymphocytes



Group III – 21st to 25th GW: Lymphoid aggregation with centrally placed arteriole was seen. Red pulp with RBCs and blood vessels was clearly defined. The red blood cells were found more inside the blood vessels and few in the interstitial tissue. The numbers of lymphatic aggregations as well as density of aggregations were found to be increased by 24th and 25th week. Those aggregations were associated with artery. From 22nd weeks onwards the structure was almost similar to that of adult spleen.

Fig. 5: Fetal spleen at 21st to 25th GW (H & E stain). Fetal spleen shows 1- capsule, 2- red pulp with RBCs, 3- lymphoid aggregation.



Group IV – 26th to 30th GW: Capsule with trabeculae seen. Distinct white pulp showing lymphatic nodules with peripherally placed central arteriole. Distinct red pulp containing RBCs, and sinusoids seen.

Group V – 31st GW onwards: At 31st gestational week, numerous sinusoids observed in the red pulp. The reticular framework was differentiated. The capsule appeared predominantly fibrous than cellular. Aggregation of lymphocytes in lymphoid follicles was denser and white pulp was well defined.

Fig. 6: 26th to 30th GW (H & E stain).

Fetal spleen shows 1- capsule, 2 – red pulp containing RBCs & sinusoids, 3- white pulp showing lymphatic nodules.

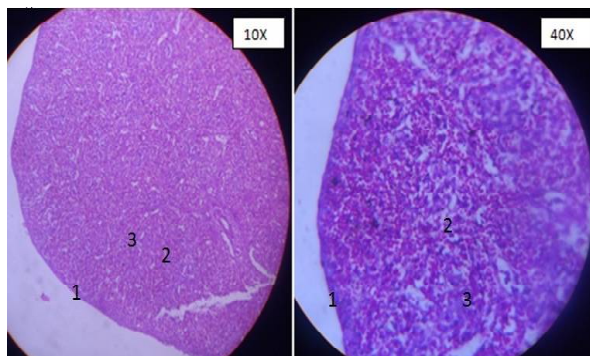
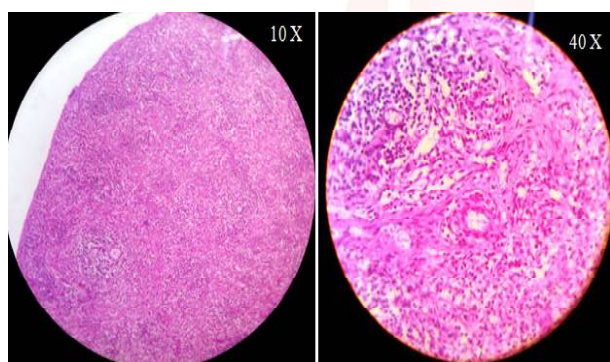
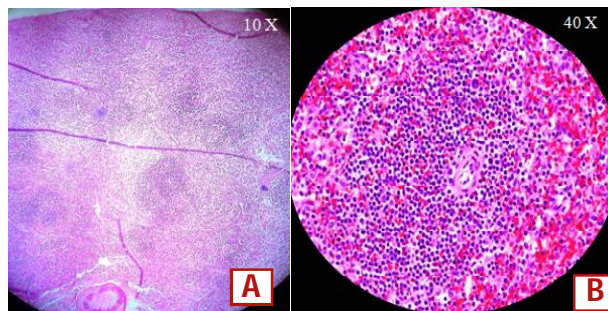


Fig. 7: Fetal spleen at 31st GW (H & E stain).



Fetal spleen at 34th gestational week, splenic tissue shows thickened and well defined capsule with large number of trabeculae present. Central arteriole was seen at the periphery of the lymphoid follicle. Numerous sinusoids were observed in the red pulp. Splenic capsule with numerous primary lymphoid follicles without germinal centers. Fetal spleen at 38th gestational week shows primary lymphoid follicle. Germinal centers are not seen. The splenic tissue appeared homogenous with well defined multiple white pulp component. The white pulp (densely aggregated lymphocytes) area was clearly visible and well defined from the surrounding tissue. Some fibroblast and thin collagen fibres were also observed in the white pulp. Sinusoidal spaces were observed and filled with red blood cells. The red pulp area was composed of thin collagen fibres continue with the collagen fibres of trabeculae and capsule. Fibroblasts, fibrocytes and sinusoids containing few blood cells were also observed in the red pulp. Structure at this stage is almost similar to the adult spleen.

Fig. 8: Fetal spleen at 34th (A) and 38th (B) GW (H & E stain).



DISCUSSION

According to Vellguth et al, at 14th gestational week numerous erythrocytes, normoblasts and macrophages are seen among a network of mesenchymal cells. Splenic lobules begin to form during the 15th to 17th week of gestation. They consist of a central artery, surrounded by a sheath of lightly stained cells which resemble myofibroblasts. The development of the white pulp starts around the 18th gestation week. An accumulation of lymphocytes around the central arteries was recognized during the 20th week of gestation. Around 23rd week the primary follicles were arranged at the periphery of the PALS [5]. In the present study by 12th week, the spleen was covered with a thin capsule made up of connective tissue. The splenic interstitial tissue was predominated by collagen fibres. Fibroblasts and fibrocytes were observed among the connective tissue components. The fibroblasts cells were observed inside the capsule in the interstitial tissue, few blood vessels filled up with blood cells were observed. Blood cells were RBC's only. No central arterioles were seen till 12th week of gestation. The distinct red and white pulps were observed by 22nd week of gestation.

Pal et al used Haematoxylin and Eosin and Masson's Trichrome stain to describe the connective tissue components. The spleen up to 13th week was composed of collagen fibers with fibroblast cells, fibrocytes and reticular cells. From 14th week onward RBCs were observed in the interstitial tissue with few lymphocytes. Reticular cells could be detected till 20th week of gestation. The proper aggregation of lymphocytes could be observed from 24th week onwards, but the well-defined lymphoid follicle or white pulp could be observed from 31st

week [6]. In the present study the lymphocyte aggregations started differentiating around the central arteriole forming the periarteriolar lymphatic sheath (PALS) by 20 weeks. Reticular cells of comparatively small in size and less in number could be detected. The reticular framework was well differentiated by 32nd weeks. According to Radhika et al, by 11th week only lymphocytic aggregation was seen. By 20th week developing lymphoid follicles observed. By 32nd weeks well developed lymphoid follicles with central arteriole was observed [7]. At 36th weeks mature lymphoid follicles with peripherally placed arteriole was seen. The above findings are almost in agreement with the present study. According to Satoh, the spleen of the 17th gestational week showed, alpha-smooth muscle actin positive reticulum cells which were scattered around the arterioles. From the 23rd week, they increased in number and began to form a reticular framework. An accumulation of T and B lymphocytes occurred within the framework, and a primitive white pulp was observed around the arterioles [8]. In the present study, the spleen of 17th GW shows mixed population of cellular and connective tissue components. Blood vessels and splenic sinusoids were seen more in plenty. The cellular component consists of red blood cells as well as lymphocytes. Lymphocytes aggregations start to become more prominent at this stage and could be detected at the periphery of arteriole, though well defined dense aggregation couldn't be established. At 20th Gestational weeks, the spleen shows clear-cut capsule surrounding the spleen having bundles of collagen fibres with fibroblast cells as well as fibrocytes was observed. The trabeculae with well differentiated blood vessels were dispersed in the substance. The number of blood vessels is increased. The lymphocyte aggregations started differentiating around the central arteriole forming the periarteriolar lymphatic sheath (PALS) by 20th weeks. Reticular cells of comparatively small in size and less in number could be detected. At 23rd Gestational weeks, the structure of spleen resembles to the adult spleen. Capsule of spleen is thickened and well defined. Numbers of trabeculae is present. The red pulp was distinctly observed containing the

RBC's and sinusoids and the white pulp containing lymphoid follicles were observed by 23rd week.

It had been mentioned that germinal centre started to appear in varying number from the 15 days after birth onward [9, 10]. Similarly in the present study no germinal centre was observed in any of the splenic section up to 38th week of gestation.

The present study is a preliminary attempt to explain the histogenesis of the human spleen at different gestational age. A detailed description of the development of parenchymal and vascular components is essential in understanding the normal development of spleen.

CONCLUSION

The present study was carried out to find out the histological changes of the spleen during its development in the human fetuses. During early development, spleen was composed of collagen fibers with fibroblast cells, fibrocytes and bigger reticular cells. The spleen of 17th gestational week shows mixed population of cellular and connective tissue components. The lymphocyte aggregations started differentiating around the central arteriole forming the periarteriolar lymphatic sheath (PALS) by 20th weeks. Reticular cells of comparatively small in size and less in number could be detected. The reticular framework was well differentiated by 32nd weeks. Hence, we concluded that the splenic capsule, framework of trabeculae, and the cellularity of white pulp and red pulp showed variations in the different age groups. The present study also provides a detailed knowledge about the histogenesis of human fetal spleen. It also suggests the functional status of spleen in fetal period.

Conflicts of Interests: None

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