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Original Article

Histogenesis of spleen in human fetuses

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ARTICLE INFO

Article history:
Received 13 November 2013
Accepted 16 December 2013
Published online 3 January 2014

Keywords:
Reticular cell
White pulp
Red pulp
Stem cell
Haemopoiesis

ABSTRACT

Aims: The present study attempted to find out the histological changes of spleen during its development in human fetuses.
Methods: Spleen from 10th–40th GW fetuses were studied after staining with Hematoxylin and Eosin, and Masson's Trichrome stains.
Results: The spleen upto 13th GW was composed of collagen fibers with fibroblast cells, fibrocytes and reticular cells. From 14th GW onward RBC's were observed in the interstitial tissue with a few lymphocytes. Reticular cells could be detected till 20th GW fetuses. Some condensation of lymphocytes could be detected from 17th GW onward. The proper aggregation of lymphocytes could be observed from 24th GW onward, but the well defined lymphoid follicle or white pulp could be observed from 31st GW only. Sinusoids, capillaries with thin endothelial lining were observed from the earliest fetus of the present study. Blood vessels lined by interrupted endothelial cells could be detected from 15th GW fetuses. Hematopoietic cells were observed in the vascular lumen in 10th GW fetus. Nucleated red blood cells were observed throughout the gestational period.
Conclusion: During early development, spleen was composed of collagen fibers with fibroblast cells, fibrocytes and bigger reticular cells. Reticular cells could be detected till 20th GW fetuses. Condensation of lymphocytes could be detected from 17th GW onward, but the well defined lymphoid follicle or white pulp could be observed from 31st GW only. Even though immature RBCs and haemopoietic stem cells were detected, hemopoiesis in the spleen could not be ascertained in the present study.

1. Introduction

The spleen the largest of the lymphoid organ appears about 6th week 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 and vascularized. The process occurs simultaneously in several adjoining areas which soon fuse to form a lobulated spleen of dual origin (from coelomic epithelium and form mesenchyme of the dorsal mesogastrium).[1]

Mesenchymal cells in the body of the cellular mass differentiate into reticular cells that provide the splenic reticular stroma.[2] Vascular reticulum is well developed at 8–9

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0003-2778/$ – see front matter
http://dx.doi.org/10.1016/j.jasi.2013.12.002

weeks gestational age and contains immature reticulocyte and numerous closely spaced thin walled vascular loops.[1]

The specialization into red and white pulps seems to be depended upon the development and distribution of vascular channels, but there is no agreement as to which type is primary. The lymphoid tissue appears early but it is not until 6 month of fetal life that the splenic corpuscle form nodule around arteries.[3]

The spleen initially functions as a hematopoietic organ and until 14 weeks, it is strictly hematopoietic. Then it acquires its definitive lymphoid character.[4] The germinal center appears only after birth.[2]

2. Aims and Objectives

An attempt was made to find out the histogenesis of splenic tissues regarding its cellular and connective tissue components, appearance of red and white pulp during the fetal period. The findings of the present study were compared and discussed with the findings from the available literatures.

3. Materials and methods

Seventy four (74) fresh human fetuses without any gross abnormality, ranging from 10th to 40th gestational weeks (GWs) were collected from the RIMS Hospital, Department of Obstetrics & Gynecology, Imphal after seeking due permission from the concerned authority. Permission from the Institutional Ethics Committee, RIMS, Imphal was obtained to carry out the present study. The age of the fetuses were calculated from the obstetrical history, gross features and crown-rump lengths (CRL). The specimens were categorized into six age groups for easier study as, Group I (10th to 15th GW), Group II (16th to 20th GW), Group III (21st to 25th GW), Group IV (26th to 30th GW), Group V (31st to 35th GW), and Group VI (36th to 40th GW). Fetuses were fixed in 10% formalin for 10–15 days. Thereafter, specimen of spleen was collected. Sections of these specimens were fixed in the neutral buffer formalin for 10–15 days. After proper fixation and trimming, the tissue was processed for paraffin sections. Serial sections of 7 μm thickness were cut with Leika RM 2125 RT rotary microtome. Alternate sections were stained with Hematoxylin and Eosin (H&E) and Masson's Trichrome stains. All the sections were studied under trinocular compound light research microscope and microphotography was done with USB camera. All the digital microphotographs were stored and analyzed.

4. Results

4.1. *10th to 15th GW (Group I)*

At 10th GW, the splenic interstitial tissue was predominated by collagen fibers. Fibroblast and fibrocytes were observed among the connective tissue components.

Reticular cells, a population of cells of bigger size with round or oval in shape could be seen (Fig. 1a). They were big in size as compared to fibroblasts cells. The cytoplasm was granular with vesiculated nucleus (Fig. 1c). In the cytoplasm, some membrane bound multiple vesicles could be seen (Fig. 1b). Well defined small nuclei were observed in some of the cells. These cells were found surrounded by fibrous tissues with stellate like cells in some area. Within this surrounding, one or multiple mesenchymal like cell with scanty cytoplasm were found around many of the reticular cells (Fig. 1). Some of the reticular cells showed in the dividing stage (Fig. 1c).

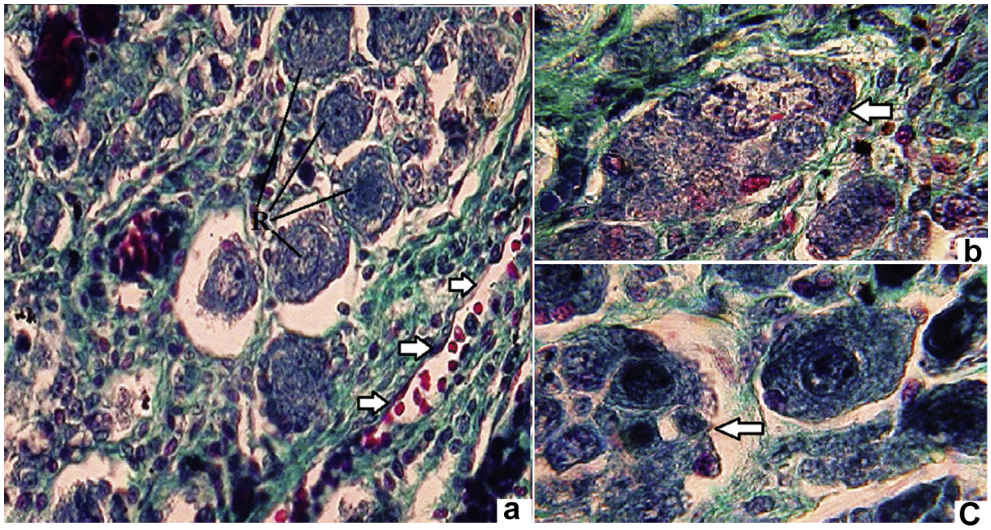

Fig. 1 – a) Photomicrograph of splenic tissue at 10th GW (Masson's Trichrome stain ×40) showing multiple reticular cells (R). Blood cells are seen inside developing blood vessels (arrow) only. b) Photomicrograph of spleen at 10th GW fetus (Masson's Trichrome, ×100) showing one reticular cell (arrow) dividing into smaller cell in size with well defined nucleus. c) Cytoarchitecture of spleen at 10th GW (Masson's Trichrome, ×100) showing multiple membrane bound vesicle inside the reticular cell (arrow) with nuclear chromatin.

In the interstitial tissue, few blood vessels filled up with blood cells were observed. Blood cells were almost RBCs only. No RBCs could be detected in the interstitial tissue. Haemopoietic cells with well defined dark stained oval nuclei and scanty cytoplasm could be detected inside the blood vessels (Fig. 2b). The capsule of the spleen was observed as fibrous tissue with fibroblast cells. It was lined by single layer of columnar cells (Fig. 2c).

In the fetus of 13th GW, sections showed increased in collagen fibers in the interstitial tissue. Reticular cells were now more oval in shaped as compared to earlier fetuses.

In fetus of 14th GW, the spleen section was observed to consist of more cellular component than the connective tissue component. Developing trabeculae were found in the interstitial tissue continued with the outer capsule of the spleen. The capsule was formed by thick fibrous tissue layer predominantly by collagen fibers lined by single flattened cells (Fig. 3a). The reticular cells were comparatively increased in numbers but smaller in size.

Now red blood cells were found abundant in the interstitial tissue some of which were clumped and others seen as scattered. Lymphocyte could be detected among the cellular component (Fig. 3a).

Blood vessels were seen inside the trabeculae with ill defined lumen containing blood cells predominantly RBCs. Some sinusoids were also seen lined by interrupted endothelial cells.

At 15th GW, the connective tissues were well organized with more number of RBC's (Fig. 3b).

4.2. *16th to 20th GW (Group II)*

The spleen had mixed population of cellular and connective tissue component.

Blood vessels and sinusoids were seen more in plenty. The cellular component now consisted of red blood cells as well as lymphocyte. Lymphocyte number increased as the age of the fetus advanced. Aggregation of lymphocytes could be detected in at the periphery of arteriole. It became more prominent by 17th GW, though well defined dense aggregation could not be detected in this group (Fig. 3c).

Reticular cells of comparatively small in size and less in number could be detected till 20th GW. More amount of connective tissue was observed around the reticular cells in comparison to earlier group.

The capsule of the spleen section had bundles of collagen fiber with fibroblast cells as well as fibrocytes.

4.3. *21st to 25th GW (Group III)*

The red blood cells were found more inside the vessels and few in the interstitial tissue. The numbers of lymphocyte aggregations as well as density of aggregations were found to be increased by 24th GW (Fig. 4a). Those aggregations were associated with artery. In some area, the trabecular artery was found to be continuous within the connective tissue framework as thin discontinuous endothelial lined lumen.

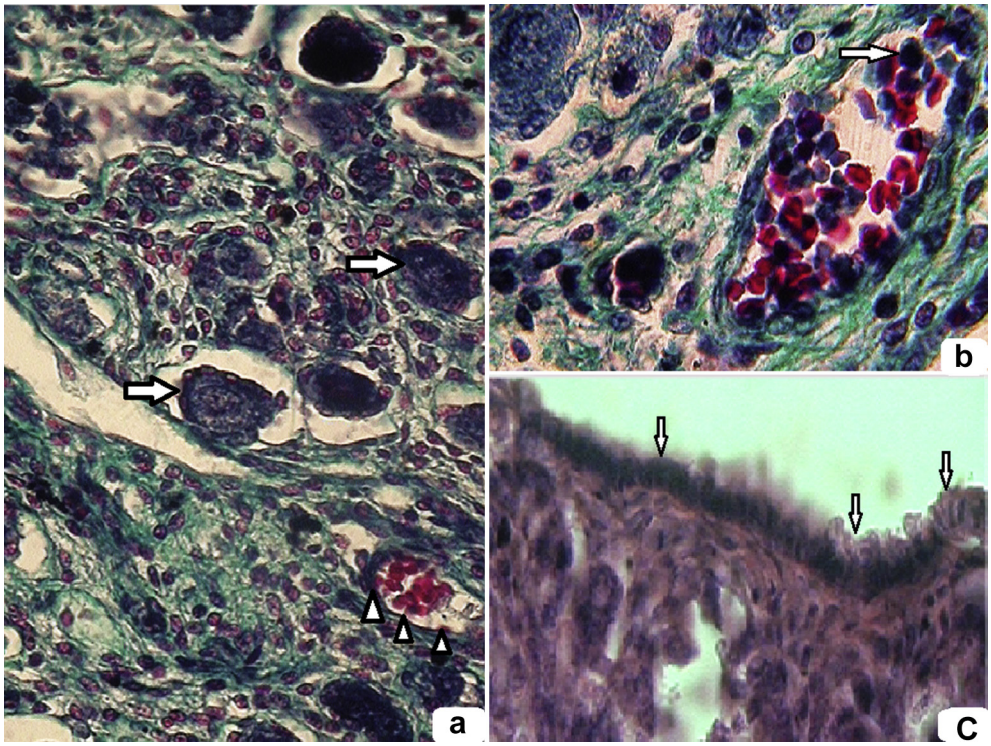

Fig. 2 – a) Cytoarchitecture of spleen at 13th GW (Masson's Trichrome, ×40) showing reticular cells (arrow) more oval shaped as compared to earlier fetus. No RBCs in the interstitial tissue could be detected. Blood vessels (arrow head). b) Photomicrograph of spleen at 10th GW fetus (Masson's Trichrome, ×100) showing nucleated RBCs with Haemopoietic cell (arrow) inside the developing vessel (arrow head). c) Photomicrograph showing splenic capsule at 10th GW (H&E, ×40) with fibroblast (Fb) & fibrocyte (Fc), lined outside by columnar epithelium (arrow).

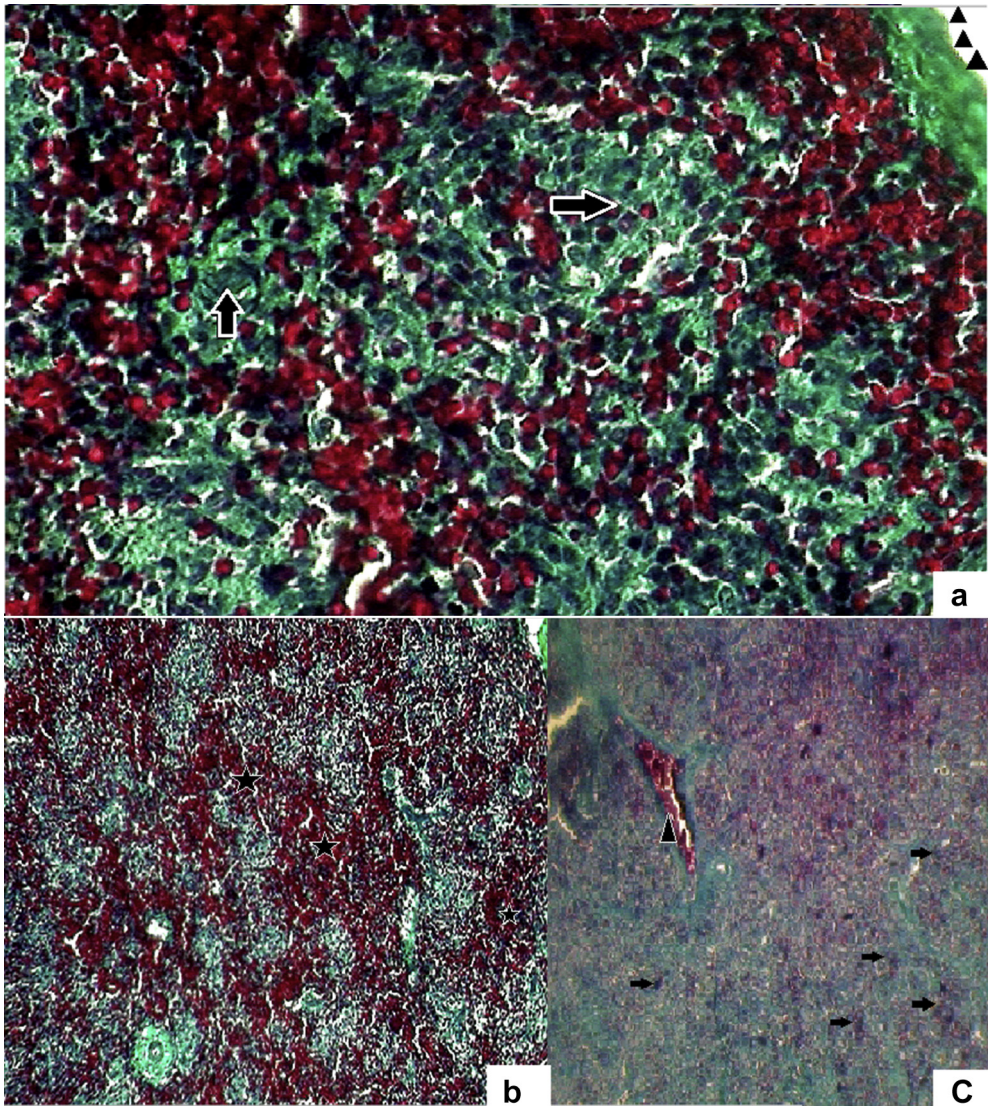

Fig. 3 – a) Cytoarchitecture of spleen at 14th GW (Masson's Trichrome, ×40) showing blood cells in the interstitial space with mostly RBCs and few lymphocytes. Developing trabeculae (arrow) with blood vessels in the connective tissue area and capsule (arrow head) is now thicker with more collagen. b) Photomicrograph of spleen at 15th GW (Masson's Trichrome, ×10) showing increased infiltration of blood cells (star) in the interstitial tissue. c) Cytoarchitecture of spleen at 17th GW (Masson's Trichrome, ×10) showing some area of aggregation of lymphocyte (arrow) in the interstitial tissue area. Trabeculae (arrow head) with blood vessels are also seen.

4.4. *26th to 30th GW (Group IV)*

Number of lymphocyte in the red pulp area appeared to be increased from the earlier group. Aggregations of lymphocytes were also found to be increased in number, however clear line of demarcation from the surrounding was not visible. Reticuloendothelial cell in the wall of sinusoids could be detected (Fig. 4b).

4.5. *31st to 35th GW (Group V)*

Aggregation of lymphocytes in the white pulp area were found to be more dense and white pulp (lymphoid follicle) was well defined from the surrounding in comparison to earlier group of fetuses (Fig. 4c). Lymphocyte in the red pulp area found to be decreased in comparison to earlier group.

4.6. *36th to 40th GW (Group VI)*

In the term size fetuses of the present series, the splenic tissue appeared as homogenous with well defined multiple white pulp component in between (Fig. 5a).

Lymphocytes were densely aggregated in the interstitial tissue area as white pulp. Some fibroblast with fine threads of collagen fibers could be found in the white pulp. Some red blood cells were observed among the white pulp cells towards the periphery. The white pulp was clearly visible and well defined from the surrounding tissue. Sometimes the central arteries were observed multiple in number. Sinusoidal spaces

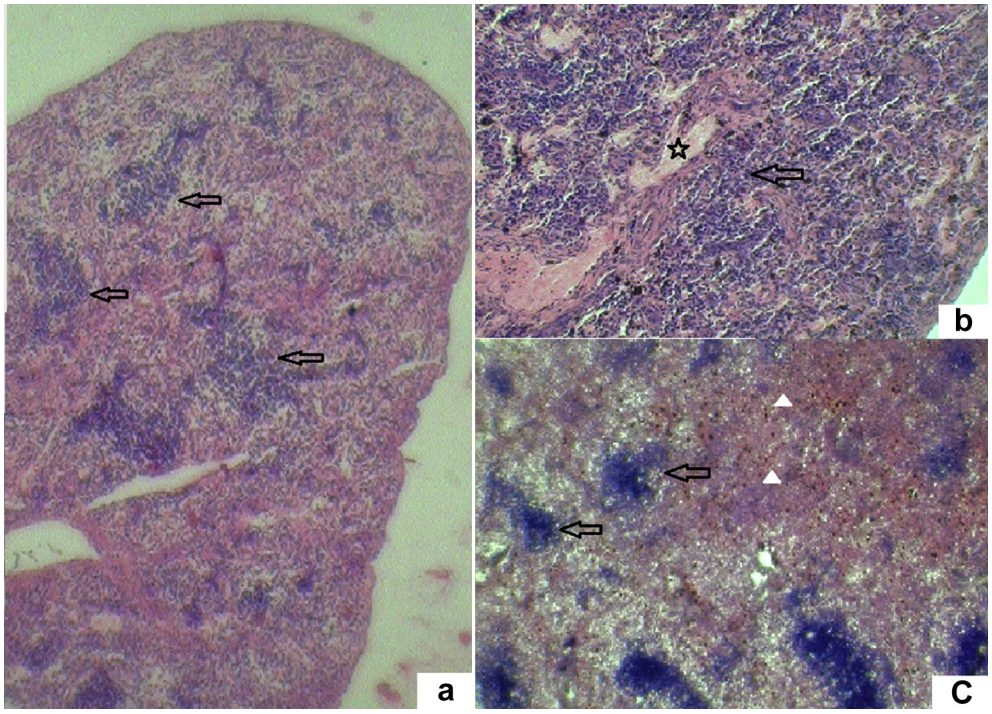

Fig. 4 – a) Cytoarchitecture of spleen at 24th GW (H&E stain, ×10) showing increased well defined aggregation of lymphocyte (arrow) in the interstitial tissue. b) Cytoarchitecture of spleen at 26th GW fetus (H&E stain, ×10) showing increased number of aggregation of lymphocytes (arrow) and sinusoids (star). c) Cytoarchitecture of spleen at 33rd GW (H&E stain, ×10) fetus showing well defined aggregation of lymphocytes (white pulp, arrow) and blood cells in the sinusoids and interstitial tissue (red pulp, arrow head).

were observed filled with red blood cells however endothelial lining could not be detected (Fig. 5b, c).

The red pulp area was composed of thin collagen fibers continuous with the collagen fibers of trabeculae and capsule. The interstices of the red pulp consisted of fibroblast, fibrocytes & blood cells. Sinusoids were seen in the red pulp area with blood cells.

5. Discussion

At the beginning of the fetal stage of development, the cells of the splenic condensation become arranged into anastomosing trabeculae with some of the mesenchymal elements isolated as free cells in the trabecular meshes.[2,5,6] The trabecular columns produce reticular fibers which becomes the connective tissue framework of the spleen.[6] In the present study till 13th GW, the splenic tissue was composed of collagen fibers, fibroblasts, fibrocytes and blood vessels with sinusoids. From 14th GW, developing trabeculae were found in the interstitial tissue and these trabeculae continued with the outer capsule of the spleen.

Spleen first consists of large mesenchymal cells.[5] Mesenchymal cells differentiate into reticular cells that provide the splenic reticular stroma.[2] At 38-mm CRL fetus, reticular cells had short broad processes and interstitium was quite limited. The reticulum continued to open with advancing age until; in the 57-mm fetus it had long slender processes and formed a reticulum with voluminous interstices.[7]

In the present study, Reticular cells, a population of cells of bigger size was observed among the connective tissue component till 20th GW fetuses. Decrease in size and observations on reticular cells in dividing stage might indicate that this cell may give rise to interstitial cell. Again, the membrane bound vesicles found inside the cell may be secretory vesicle for the formation of reticular fibers. However, it is not possible to ascertain from this study under light microscope and without histochemistry.

The primary vascular reticulum is well developed at 8–9 weeks, and contains numerous closely spaced thin-walled vascular loops which last up to the 14th gestational week.[1,8] A rich vasculature of closed thin-walled loop was present in the 38-mm fetus. There after an open vascular pattern, most pronounced in the 50- and 57-mm CRL fetuses were observed. Though splenic pulp in simple form, it accomplished the fundamental function of the spleen: a filter and modifier of blood.[7] Similarly, in the present study, thin wall vessels were observed from 10th GW. RBCs were observed inside the blood vessels only till 13th GW, which could be due to closed vascular circulation in the organ of these fetuses, (Figs. 1a and 2a, b). From 14th GW, blood cells mostly RBCs and few lymphocytes could be seen in the interstitial spaces of the splenic tissue (Fig. 3a), which might be due to open vascular pattern as it was described by Weiss L.[7]

The development of white pulp is correlated with the stage of lymphoid colonization within the spleen. An accumulation of lymphocytes around the central arteries can be recognized during the 19th and 20th GW. Around the 23rd GW the

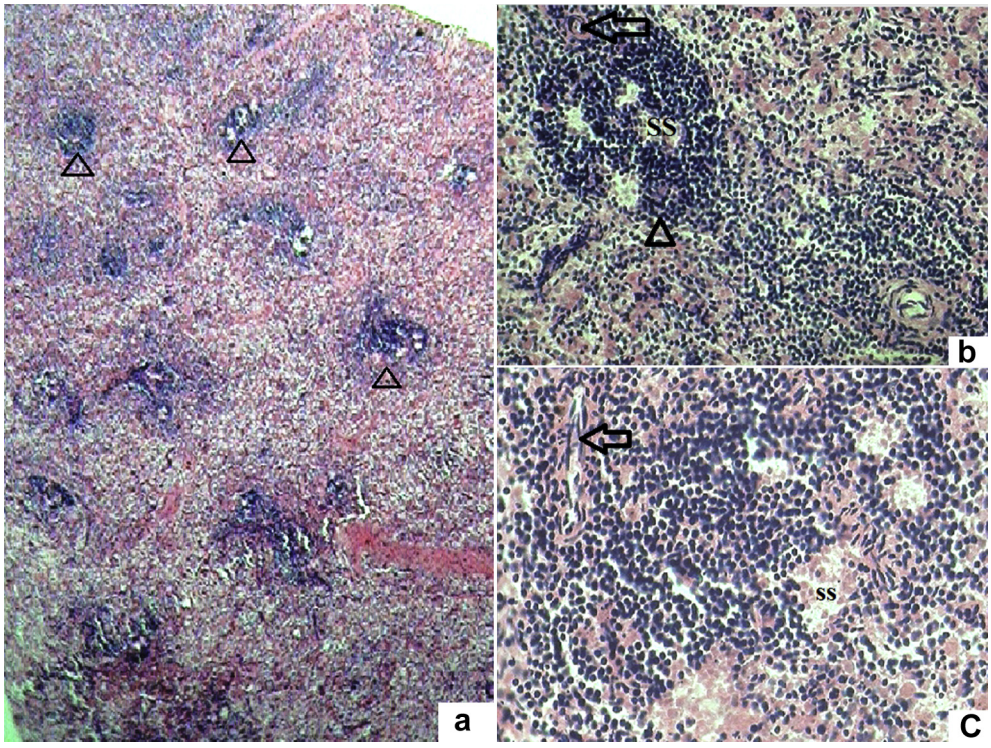

Fig. 5 – a) Cytoarchitecture of spleen at 38th GW fetus (H&E stain, ×10) showing aggregation of lymphocytes as white pulp (arrow head). b) Same specimen (H&E stain, ×40) showing white pulp (arrow head) with well developed central artery (arrow) lined by endothelium and multiple sinusoidal spaces (ss) inside without any endothelial lining. c) Cytoarchitecture of spleen at 38th GW fetus (H&E stain, ×100) showing white pulp with endothelial lined artery (arrow) and multiple sinusoidal spaces (ss) without any endothelial lining filled up with RBCs.

assemblage of primary follicles is discernible at the periphery of the periarteriolar lymphatic sheath (PALS).[5,8] In the present study, aggregation of lymphocytes could be detected in at the periphery of arteriole by 17th GW but dense aggregation of lymphocyte as pulp formation associated with the artery could be seen from 24th GW only (Fig. 4a). From 26th to 30th GW, aggregations of lymphocytes were increased in number as the gestational week advanced however clear line of demarcation was not visible. From 31st GW onward till term fetuses, aggregations of lymphocytes were found to be denser and were well defined from the surrounding structure (Fig. 4c).

It had been mentioned that germinal center started to appear in varying number from the 15 days after birth onward.[2,9] Similarly in the present study no germinal center was observed in any of the splenic section of the present study also.

At the periphery of splenic lobules the red pulp forms. Initially mobile cells are distributed throughout the reticulum. Soon they begin to accumulate in the venous sinuses, which develops from the lacunae among the reticular network and come into contact with venous system. The endothelial wall of these sinuses remains discontinuous; confirming the theory of open vascularization of spleen.[8] The present study also had the similar finding with discontinuous endothelial lining in some areas. Comparatively distinct red pulp was observed with the proper formation of white pulp.

The capsule in early fetal life consisted of a simple layer of irregularly cuboidal cells having microvillus and cilia on the peritoneal surface.[1,7] The capsule rested on a well defined basal lamina.[7] Similarly in the present study, the capsule was lined by simple columnar epithelium upto 13th GW (Fig. 2c). From 14th GW onward it was lined outside by single layer of flattened epithelial cells (Fig. 3a).

Hematopoietic stem cells migrate in to the spleen primordia during the first trimester of pregnancy.[10] Until 14 weeks, spleen is hematopoietic during the preliminary stage of its development.[4] The spleen function as a hematopoietic center until late fetal life; however it retains its potential for blood cell formation even in adult life.[11] According to some authors the spleen is not a significant organ of hematopoiesis in the human fetus but that immature hematopoietic cells found there merely reflect trapping of circulating precursors in the fetal blood.[12] In the present study, hematopoietic cells could be detected in 10th GW (Fig. 2b). Some of the RBCs were nucleated showing their different stages of maturation. Even though immature RBCs and haemopoietic stem cells were detected, haemopoiesis in the spleen could not be ascertained in the present study.

6. 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. Reticular cells could be detected till 20th GW fetuses. These reticular cells may be the source of reticular fibers and other interstitial cells. Condensation of lymphocytes could be detected from 17th GW onward, but the well defined lymphoid follicle or white pulp could be observed from 31st GW only. Even though immature RBCs and haemopoietic stem cells were detected, haemopoiesis in the spleen could not be ascertained in the present study.

Conflicts of interest

All authors have none to declare.

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