

**Original Article**

## **MICROSCOPIC ANATOMY OF DEVELOPING SPLEEN: A CROSS-SECTIONAL STUDY FROM NORTH INDIA**

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### **ABSTRACT**

**Introduction:** Spleen is the largest lymphatic organ present in the upper part of the abdominal cavity. In fetal life it is a haemopoietic organ while it destroys blood in the postnatal life. It filters the blood from blood borne antigens and microorganisms. The present study was conducted to describe the detailed histological changes of human fetal spleen during its development.

**Materials and Methods:** The present study was a prospective, observational study conducted in the Department of Anatomy along with the Department of Obstetrics and Gynaecology at a tertiary care teaching hospital situated in Uttarakhand state from March 2021 to July 2023. Human fetuses between 13 to 40 weeks of gestational age and without any congenital malformations were collected after routine medical termination of pregnancies, still birth or intra uterine death for study.

**Results:** The microscopic anatomy of the spleen was observed at different gestational ages. During first trimester of pregnancy primary vascular system was seen. Formation of red pulp and white pulp was seen clear during second trimester of pregnancy. At the beginning of the third trimester, white pulp shows lymphatic nodule with eccentrically placed arteriole.

**Conclusions:** The detailed study of development of fetal spleen suggest the functional aspect of spleen in fetal life.

**Keywords:** Microscopic anatomy, Spleen, Histogenesis, Fetal autopsy, Embryology

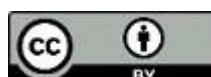
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## INTRODUCTION

Appearing at about the sixth week of gestation, the spleen is the largest lymphoid organ [1]. The lobulated spleen of dual origin develops simultaneously in several adjoining areas which soon fuse together. The spleen is capsulated and is situated in the pathway of the blood stream (splenic artery and vein). The spleen is enclosed by a dense connective tissue capsule from which trabeculae extend into the parenchyma of the organ [2]. Myofibroblasts are present in the connective tissue of the capsule and trabeculae [3,4]. These contractile cells also produce extracellular connective tissue fibers.

The substance of spleen known as the splenic pulp, is made up of red pulp and white pulp [5]. The red pulp consists of a dense network of reticular fibers 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 [6]. Mainly lymphatic tissue make up the white pulp and is the immune component of the spleen [7]. Lymphatic cells surrounding the central arteries of the white pulp are primarily T cells, while the lymphatic nodules contain mainly B cells. The spleen carries out many supporting roles in the body. It acts as filter for blood as part of the immune system, which helps fight infectious diseases. It also detects abnormal red blood cells.

The variations in the lymphoid tissue, including spleen are well documented in the adults but few studies have documented the variations in the structure of spleen at different stages of development in the prenatal period. The present study was conducted at a tertiary care center to study the macroscopic and histological development of the human spleen, by observing changes in the gross splenic morphology and histology in fetuses of different age groups during the prenatal period.

## MATERIALS AND METHODS

The present study was a prospective, observational study conducted in the Department of Anatomy at a tertiary care teaching hospital situated in Uttarakhand state from March 2021 to July 2023.

*Inclusion and exclusion criteria:* All fetuses ranging from the gestational age of 13 to 38 weeks, obtained from the Department of Gynaecology and Obstetrics of the tertiary care teaching hospital as a result of medical termination of pregnancy (MTP), intra-uterine death or still birth were included in the study. Fetuses showing any sign of congenital malformation were excluded from the study.

*Specimen collection and preservation:* The specimen/ fetus were collected from the Department of Obstetrics and Gynaecology after obtaining due written, informed consent

from the parents. These fetus specimen were collected within a window of one to two hours of the delivery. The gestational age was determined by reviewing the medical records available in the hospital and confirmation was done by taking the following measurements: crown-rump length, crown-heel length, length of the foot and the bi-parietal diameter. The weight of the fetus specimen was measured followed by injection of 10% formalin in the body cavities. Each fetus was then transferred and kept in a container filled with 10% formalin solution for seven to ten days.

*Specimen processing:* The abdomen of the fetus was opened and the spleen, the location and appearance of the spleen was observed and recorded. Careful dissection of the spleen was carried out [2,8,9] and the spleen tissue was further processed as per standard histological techniques. Briefly, the specimen underwent grossing, fixation, dehydration, clearing and impregnation. The spleen tissue was then embedded in paraffin blocks and sectioning was done to obtain slices of around 10-12 $\mu$ m thickness. Finally, staining of the slides was done using Haematoxylin and Eosin stain and the slides were observed under light microscope using 10x (low power) and 40x (high power) objective lenses.

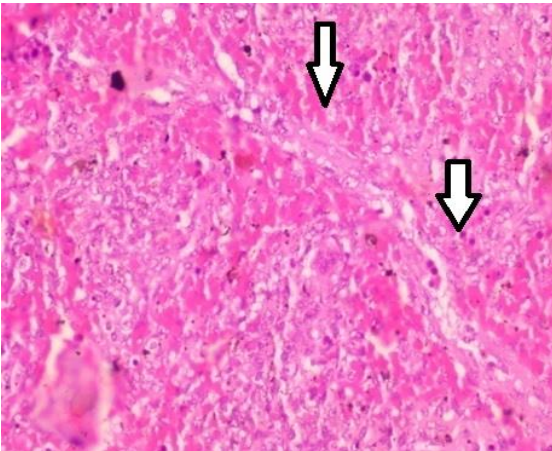
*Ethical considerations:* The study was conducted as per the ethical guidelines for biomedical research on human Informed consent was taken from all cases.

subjects as given by the Central Ethics Committee on Human Research (CECHR) of the Indian Council of Medical Research (ICMR) and the 'Declaration of Helsinki' revision of 2013 after obtaining due ethical clearance from the Institute ethics committee.

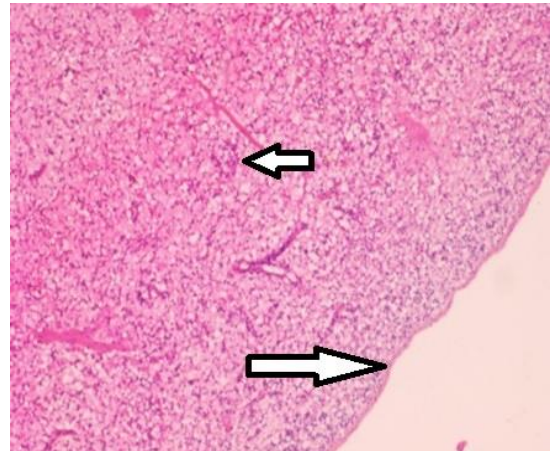
## **RESULTS**

The study was conducted on 60 fetuses received in the department of anatomy. So, a total of 60 fetal spleen were included in the study. Thirty four spleen belonged to fetus between 13 to 20 weeks of gestation, 17 had a gestational age of 21 to 30 weeks while nine had a gestational age of 31 to 40 weeks. The following microscopic features at different gestational ages were observed in these fetuses.

*Group 1: 13-20 weeks of gestation:* At 13 weeks, Spleen was covered by thin capsule made up of connective tissue. Fibroblast were observed in the capsule. Splenic interstitial tissue was dominated by collagen fibers. More reticular fibers, sinusoidal spaces, and blood vessels appeared. Development of trabeculae begins within the capsule. Number of haemopoietic cells were increased. Numbers of lymphocytes arranged in groups are seen but they were scattered. At 18 weeks, lymphoid aggregation with centrally placed arteriole was seen. At the margins of these groups, sinusoids and large number of RBCs were observed. At 20 weeks, division of Red and white pulp appeared. Fig. 1 is a



**Fig. 1. Photomicrograph of spleen at 14 weeks showing lymphoblasts and reticular system**



**Fig. 2. Photomicrograph of spleen at 20 weeks showing well-developed capsule along with trabecular vessels**

histological image of a spleen at 14 weeks showing lymphoblasts and reticular system.

*Group 2: 21-30 weeks of gestation:* Capsule and trabeculae were seen easily and they become thick. Red pulp and white pulp were seen clearly. Large number of blood vessels were noted. Red pulp containing RBCs and sinusoids were seen. At 30 weeks, white pulp showing lymphatic nodules with eccentrically placed arteriole were seen. Fig. 2 is a histological image of a spleen at 20 weeks showing well-developed capsule along with trabecular vessels.

*Group 3: 31-40 weeks of gestation:* At 31-35 weeks, numerous sinusoids were appeared in red pulp, denser lymphocytic aggregation in lymphoid follicles were seen. Well defined white pulp was seen. Primary lymphoid follicles with germinal centers were also present. At 37 weeks, hemopoietic activity is completely absent. At 38-40 weeks, fetal

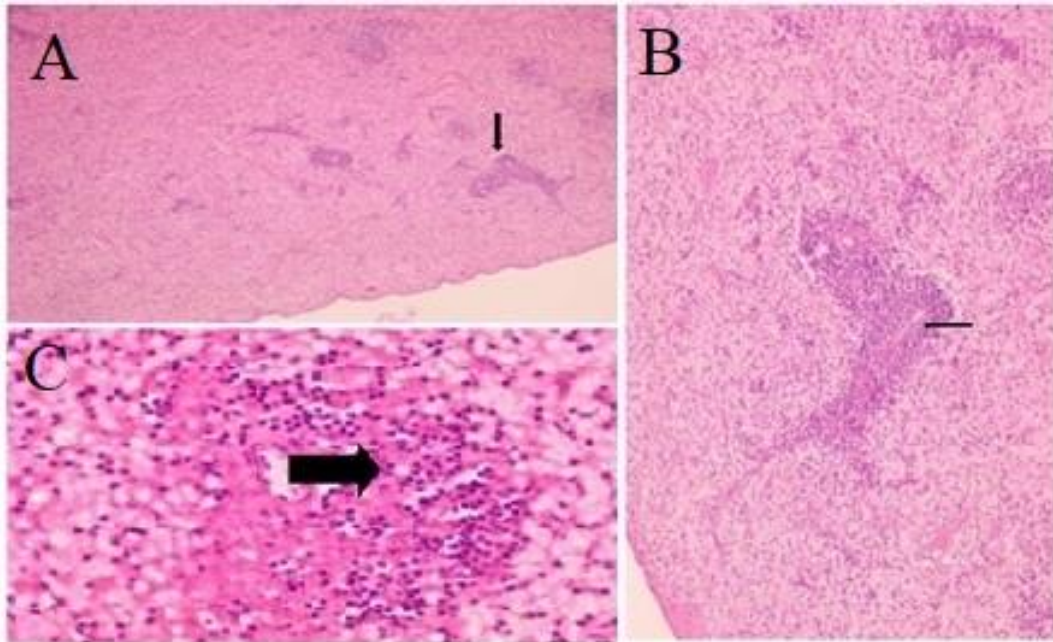
spleen resembled that of an adult spleen. Figure 3 is a histological image of a spleen at 38 weeks showing well-developed red and white pulp with eccentrically placed arterioles.

## DISCUSSION

In a study by Mukhia et al [10] 10-15 gestational week the spleen was covered by thin capsule made up of connective tissue. Splenic tissue dominated by collagen fibers. Fibroblast cells observed inside the capsule. Blood cells were RBC'S only. At 14 weeks, capsule along with developing trabeculae were seen in interstitial tissue. Reticular cells forming network were present, but they were smaller in size, lymphocytes were present in scattered groups. At 16-20 weeks, spleen shows mixed population of cellular connective tissue components.

Blood vessels and splenic sinusoids were more in number lymphocytes aggregation become prominent and could be detected at





**Fig. 3. Photomicrograph of spleen at 38 weeks showing well-developed red and white pulp with eccentrically placed arterioles under 4x objective (A), 10x objective (B) and 40x objective (C)**

periphery of arteriole. At 20 weeks, the lymphocyte aggregations started differentiating around the central arteriole forming the peri-arteriolar lymphatic sheath. At 22 weeks, lymphoid aggregation with centrally placed arteriole was seen. At 31 gestational weeks, aggregation of lymphocytes in lymphoid follicle was denser and white pulp was defined.

In the present study, at 13 week, the spleen was covered by thin capsule consist of connective tissue, fibroblast and fibrocyte is present, development of trabeculae begins, hemopoietic cells increases. Thomas et al [11] used hematoxylin and eosin stain to describe microscopic structure of spleen in fetus at 15 weeks, and observed that the

trabeculae arose from the capsule by this time. Arterioles present with no aggregation of lymphocytes. At 16 weeks, arteries were seen within trabeculae at 21 weeks lymphocytic differentiation starts around arteriole.

In the present study from 21 weeks onwards capsule within trabeculae seen prominently. Red and white pulp was distinct. Lymphoid aggregation were present around arterioles forming a peri-arteriolar lymphatic sheath. In a study by Holkundre et al [12], by the end of 13 weeks of gestation, a thin capsule was developed, reticular cells and lymphoblast were seen. By 20 weeks, red and white pulp were seen prominently and lymphocytes were compactly arranged surrounding arterioles. At 36 weeks, onwards pattern of trabeculization

was seen, division of red and white pulp were seen prominent. Lymphocytes were compactly arranged, and arterioles were eccentric in position. The above findings are consistent with the results of the present study.

Sandhya et al [13] studied the histogenesis of human fetal spleen at different gestational ages. According to their study the spleen is lined by capsule at 12 weeks. Central arteriole starts appearing at 17 weeks, and venous sinuses are present with scattered hematopoietic cells. Lymphocytes were seen around central arteriole. Around 18-24

weeks, capsule was thicker, trabeculae were seen, and central arteriole were surrounded by few lymphocytes. At 20 weeks, red and white pulp was seen at 24 weeks reticular fibers were seen around white pulp. At 24-30 weeks germinal center was seen at 28th weeks eccentrically placed central arteriole was seen. At 30 -36 weeks, at 31 weeks capsule was thick, white pulp was well defined with germinal center. 36-40 weeks, the fetal spleen resembled that of an adult spleen, the hemopoietic activity is completely stopped. A few studies reporting the histogenesis of spleen at various gestational age are summarized in table 1.

Author	n	Capsule	Lymphocyte aggregation	Lymphoid follicle	Central arteriole	Resemblance with adult spleen
Souza <sup>[14]</sup>	15	T1	T2	20-23 wk	30 wk	36 wk
Mukhia <sup>[10]</sup>	50	12-14 wk	-	18 wk	22 wk	38 wk
Thomas <sup>[11]</sup>	34	10-15 wk	20 wk	21-30 wk	31-40 wk	-
Holkunde <sup>[12]</sup>	30	13-14 wk	-	20 wk	22 wk	38 wk
Yatagirj <sup>[13]</sup>	100	12 wk	-	18-24 wk	24-30 wk	38-40 wk
Haldar <sup>[2]</sup>	9	12 wk	T2	-	-	36 wk

**Table 1. Previous studies reporting histogenesis of spleen**

**CONCLUSION**

The present study sheds light on the histological and developmental changes in the human spleen at different ages of gestation. Splenic histogenesis is a multistep process which depends upon the gestational age. Various structural and functional abnormalities later in life are thus dependent on any deviation from normal development.

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