

The histological study of human fetal spleen

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Abstract

In the present observational study, thirty aborted human fetuses of 13-38 weeks gestational age with no obvious congenital abnormality were obtained. To study the histology of spleen in relation to the gestational age, spleen was procured after dissection of fetuses and slides were prepared with H & E staining. The findings were noted under the light microscope. On looking over the literature on development and the important functions served by the spleen as haemopoietic and lymphoid organ, the following study was undertaken. In the study, the mean body weight of fetuses was 130.83 at 14th week and 3150gm at the age of 38th week. The average crown rump length of fetuses at 14 weeks was 115mm and was 330mm at 38th week. Histologically, at 13th week of age there were large numbers of cells of irregular shape with large nucleus centrally. Few small thin walled vessels are seen. At 20th week of age, clearcut division of red and white pulp was seen. Our study may be regarded as a preliminary study including some detailed results. The histological findings acquired in our study will be beneficial for the evaluations about the fetal spleen.

Keywords: Human fetus, Spleen, Histology.

Introduction

The study of development of human being is always a subject of interest of many researchers.

Each and every organ of human body executes many important functions, helpful for the survival of human being in this world. Embryologists are very keen in knowing the development of every organ of human being.

One of the important among these is the spleen. The spleen is known to human beings since classical times. Histologically, the spleen is surrounded by a capsule composed of dense fibrous tissue, elastic fibers, and smooth muscle. The outermost layer of the splenic capsule is composed of mesothelial cells, which may not be evident on histologic section. Irregularly spaced trabeculae of smooth muscle and fibroelastic tissue emanate from the capsule into the splenic parenchyma. These trabeculae also contain blood and lymph vessels and nerves. The lymph vessels are efferent vessels through which lymphocytes migrate to the splenic lymph nodes. (Cesta MF).¹

As the spleen is playing important role in haematopoiesis and immunological functions, development of it is important to know.

To anyone looking over the literature on the development of spleen the necessity for more work on finer details of process is evident. The most extensive work has been done on the lower vertebrates and very few on human embryos. On looking over the literature on development and the important functions served by the spleen as haemopoietic and lymphoid organ, the following study is undertaken.

Aims and objectives

To study the histological and developmental changes in human fetal spleen at various gestational age of fetus.

Materials and Methods

Thirty aborted human fetuses of 13-38 weeks gestational age with no obvious congenital abnormality were obtained with the permission of Professor and Head of department of Obstetrics and Gynecology of our medical college and prior consent of the parents. The study was approved by the Ethical Committee of our college. These fetuses included spontaneous abortions and stillborns. Fetuses were obtained within 4-5 hours of birth to avoid post-mortem changes.

Materials used:

1. Double pan balance, chemical balance measuring scale and vernier caliper.
2. Permanent marker.
3. Plastic tag, scissors, scalpel and forceps.
4. Rubber gloves and filter paper.
5. Rotary microtome, reagents, stains, slides with coverslip.
6. Light microscope and digital camera

Measurements of External parameters:

Following parameters were measured:

1. The sex, gestational age was noted from the case paper.
2. The crown rump length: It was recorded by using thread and scale and measured in mm.

Fixation of fetuses: The fetuses were fixed by injecting 10% formalin locally into the various body cavities with the help of 10cc syringe and 20 number needle for better preservation.

Dissection, measurement and fixation of spleen:

1. The fetuses were carefully dissected by doing the window dissection to remove the spleen in one piece.
2. The spleen was weighed in chemical balance and recorded in grams.
3. The spleen was cut into pieces and fixed in 10% formalin for 24-48 hours.
4. After fixation, the paraffin wax blocks were prepared with the embedded splenic tissue in it.

Staining:

1. The slides were prepared with the proper H&E staining method and were labelled according to the gestational age.
2. The stained slides were observed under light microscope using 4X, 10X and 40X magnification and the findings were noted and compared with previous studies.



Fig. 1: Showing fetus of 24 weeks with materials used

Observations and Results

The present study was carried out over 30 human fetuses ranging from the 13th week to 38th week of gestational age. The gestational age of fetuses was determined with the help of case paper, obstetric history of mother and the ultrasonography. For the study purpose and for comparison with other workers, the fetuses were arranged in the 13 groups. When there was more than one fetus from a particular age group, average was calculated for each group.

To study the development and histological changes in the spleen in relation to gestational ages, following parameters were studied.

1. Body weight and crown rump length of fetus:

This data was tabulated (table-1)

Table 1: Showing average body weight (grams) and crown-rump length against weeks of gestation

Gestational age group (weeks)	Number of fetuses in the group	Body weight (gms)	Crown-rump length(mm)
14	3	130.83	115
16	2	200	122
18	2	300.25	150
20	2	420.50	172
22	2	610	185
24	3	802	202
26	2	1150	224
28	2	1290	270.5
30	3	1725	276
32	2	1924	295
34	2	2480	300
36	3	2910	324.5
38	2	3150	330

2. Histological study of spleen: Slides of the spleen of each gestational age were prepared and stained with haematoxylin-eosin and studied under light microscope.

At 14th week of age:

1. Capsule was seen.
2. Reticular cells forming network were present.

3. More number of blood vessels were present.
4. Numbers of hemopoietic cells were increased.
5. Lymphoblasts were seen in groups, but these groups were scattered.

At 16th week of age:

1. Capsule was thickened and prominent.

2. Reticular cells forming fibers were seen and were forming the network.
3. Haemopoietic cells are seen in large numbers.
4. Majority of WBC seen were lymphocytes, arranged in groups.
5. These groups are scattered randomly all around.
6. These connective tissue were seemed to be supporting the sinusoids.
7. Vascularity was increased as the number of blood vessels was increased.

At 18th week of age:

1. The vascularity was increased further.
2. Capsule along with few trabeculae was prominently seen.
3. Lymphocytes were arranged in groups.
4. At the margin of these groups, sinusoids and large number of RBC's were noted.
5. The division of white and red pulp was noted firstly in this age.
6. But the lymphocytes were less compactly arranged and their association with arteriole was not prominently seen.

At 20th week of age:

1. Capsule was seen prominently.
2. Trabeculae were seen easily.
3. Large number of blood vessels were noted.
4. Clearcut division of red and white pulp was seen.
5. White pulp consisted of compactly arranged lymphocytes surrounding the arteriole as the periarteriolar sheath.
6. In between the white pulp, red pulp can be seen consisting of sinusoids and RBCs.

From 22nd to 38th week of age:

1. Structure was similar to the adult spleen.
2. Thickened well defined capsule was seen.
3. Number of trabeculae were present.
4. Spleen showed rich vascularity showing large number of arterioles.
5. Division of red and white pulp was seen prominently.
6. In white pulp, lymphocytes were compactly arranged and arterioles were eccentric in position.

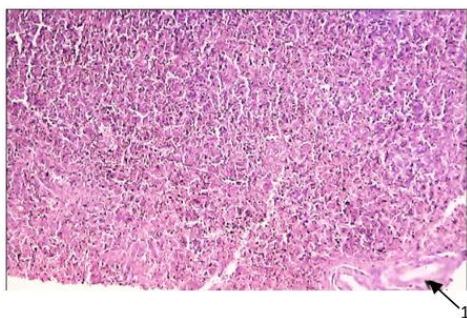


Fig. 2: Slide of spleen of age-14th week (10x) showing blood vessel (1)

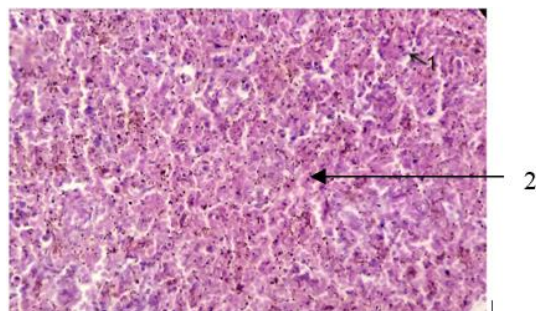


Fig. 3: Slide of spleen of age-14th week (40x) showing lymphoblasts(1) and Reticular cells(2)

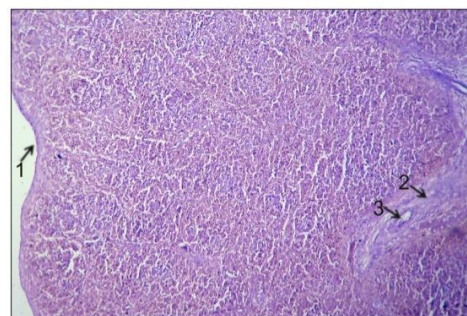


Fig. 4: Slide of spleen of age- 16th week fetus (10x) showing capsule(1), trabeculae(2) and trabecular vessels (3)

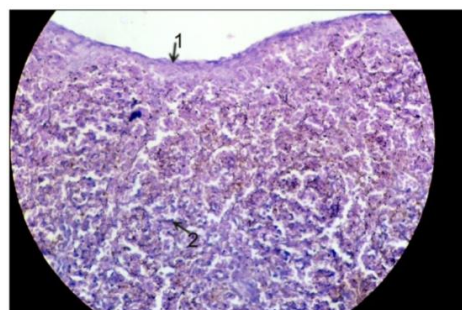


Fig. 5: Slide of spleen of age- 16th week fetus (40x) showing capsule(1), lymphocytes(2)

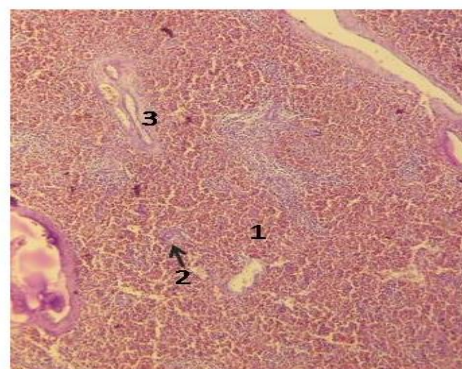


Fig. 6: Slide of spleen of age- 20th week fetus (10x) showing red pulp(1), white pulp(2) and blood vessels(3)

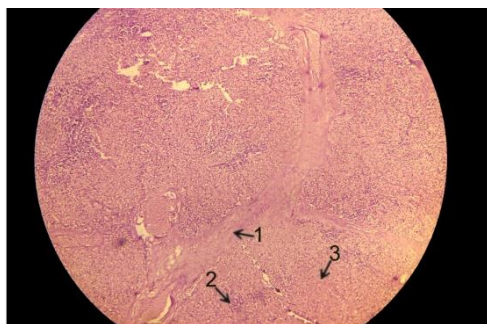


Fig. 7: Slide of spleen of age- 34th week fetus (10x) showing trabeculae(1), white pulp(2) and red pulp(3)

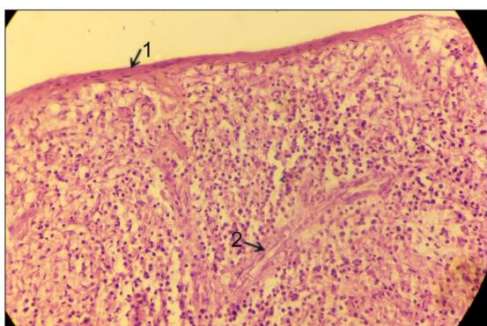


Fig. 8: Slide of spleen of age- 38th week fetus (40x) showing capsule (1), trabeculae(2)

Discussion

The microscopic structure of spleen at different ages in antenatal period was discussed which gave an idea how the final structure was attained through this developmental period.

The following parameters of the developing human fetus were studied in the present study:

1. Body weight of fetus
2. Crown rump length
3. Microscopic structure of spleen at different gestational age.

All the parameters were compared with the gestational age of fetus.

1. **Body weight of fetus:** The findings of the present study about body weight showed gradual increase from 14th week to 38th weeks of gestation (table-1). When the findings of present study were compared with those of different scientists (table-10), it was found that, the body weight reported by **Arey²(1954)**, **Gruenwald et al³ (1960)**, **Schulz et al⁴(1962)** and **Potter et al⁵ (1976)** were less than the present study. However, the findings of the present study were comparable with the findings reported by **Parulekar⁶(1995)**. The body weight upto 28th week of gestation was found to be greater than reported by **Hamilton et al⁷ (1962)** but it was comparable thereafter upto the 38th week of gestation.

Table 2: Showing comparison of fetal body weight (gms) of present study with other workers

Gestational age in weeks	Arey 1954	Gruenwald 1960	Hamilton 1962	Schulz 1962	Potter & Craig 1976	Parulekar 1995	Present study
14	-	-	-	-	38	110	130.83
16	105	-	73	-	73	200	200
18	-	-	-	-	161	320	300.25
20	310	-	200	316	227	460	420.50
22	-	-	-	-	348	630	610
24	640	638	530	748	361	820	802
26	-	845	-	-	394	1000	1150
28	1080	1020	845	1127.5	-	1300	1290
30	-	1230	-	-	1431	1700	1725
32	1670	1488	1940	1748	1900	2100	1924
34	-	1858	-	-	2348	2500	2480
36	2400	2165	3025	2448	2555	2900	2910
38	-	2678	-	-	2879	3400	3150

2. **Crown rump length:** The crown rump length of the fetus at different gestational age was also measured in mms. The crown rump length at 14 week gestation was 115mm whereas it was 330mm at 38 week. The findings of the present study were tabulated in table-3 for comparison with crown rump findings of the previous workers.

From the table it was seen that the values of crown rump length given by **Moore et al,⁸ Potter et al⁵ (1976)** and **Hamilton et al⁷ (1962)** were more or less similar to the findings of the present study.

Table 3: Showing the comparison of crown rump length of fetus in mms at different gestational age with previous workers

Gestational age (weeks)	Moore	Hamilton 1962	Potter and Craig 1976	Present study
14	120	-	-	115
16	140	61-100	-	122
18	160	-	-	150
20	190	101-200	-	172
22	210	-	-	185
24	230	151-200	209	202
26	250	-	234	224
28	270	201-260	254	270.5
30	280	-	271	276
32	300	261-320	284	295
34	-	-	298	300
36	340	321-390	324	324.5
38	360	391-450	334	330

3. Microscopic structure of spleen: Developmental changes in the histology of spleen were compared with the findings of different scientists.

According to **Hamilton et al⁷** (1978), mesenchymal cells of splenic condensation multiply rapidly at 4th – 5th month of gestation and differentiate into erythroblasts myeloblasts, monocytes, megakaryocytes and further erythroblasts. In present study, at the 13th -14th week of age, mesenchymal condensation was seen and after this age, differentiation was seen into blood cells.

However, **Copenhaver et al⁹** (1978) stated that fetal spleen participated in development of lymphocytes, erythrocytes and granular leucocytes and erythropoietic function ceased at about 8th month.

While in the present study, we have observed the haemopoetic cells throughout, all the stages studied, i.e. from 13th to 36th weeks of gestation.

Van Furth et al¹⁰ (1965) observed that the white pulp of spleen showed lymphoid blast cells having larger chromatin and poor nucleus having nucleolus. Older fetuses showed increase in number of lymphocytes. Germinal centres were not observed. The red pulp showed distinct cytoplasmic pyrinophilic cells having cytoplasm with round, chromatin nucleus.

According to **Potter et al⁵** (1976) spleen was made up of only connective tissue and reticular cells during first two trimesters. At birth the appearance of spleen is fairly similar to the adult spleen except the less amount of connective tissue. White pulp consisted of lymphoid cells, arranged in sheath like manner around all major branches of splenic artery. Red pulp of spleen consisted of endothelium lined sinusoids, which were much prominent at birth. In our study, connective tissue

framework was seemed to be formed from 13th week onwards.

Hamilton et al⁷ (1978) stated that in early stages splenic condensation becomes arranged into anastomosing trabeculae. Trabecular columns produced reticular fibres which became connective tissue framework of spleen during histological changes splenic artery comes to open in spaces called sinusoids which are devoid of endothelial lining. Some lining cells of sinusoids became specialized to form a part of reticuloendothelial system.

Copenhaver et al⁹ (1978) stated that white pulp showed enlargement but definite nodules did not form until late fetal stage. Germinal centres did not appear until after birth. Same findings were noted in present study.

Vellguth et al¹¹ (1985) studied the development of human spleen from 14th to 24th week of age. He stated three stages in the development.

1. Developmental stage of the primary vascular reticulum- from 14th week of age
2. Transformation stage with formation of lobules- from 15th to 18th week of age
3. Developmental stage of lymphoid colonization- from 18th to 24th week of age

In preliminary stage, connective tissue fibres and vascular system was started developing. Reticular cells were seen and the number of blood vessels increased. At 14th week, arteries have a continuous endothelial wall connected by desmosomes. Venous sinuses are very rare at this stage. In 13th and 14th week of age, mesenchymal condensation forming the connective tissue and the developing blood vessels were noted which was consistent with **Vellguth et al.¹¹**

In transformation stage, from 15th week, splenic lobules began to form in the primary vascular reticulum. In the present study, the blood vessels

seemed to be developing in the trabeculae but distinct lobules were not seen at this stage.

In developmental stage, from 18th week, there was lymphoid colonization around the arterioles. White pulp was recognizable during the 19th and 20th week of age. Same was noted in our study. Between 20th to 24th, remarkable growth in the white pulp was seen while red pulp seemed to cease its extension. In 24th week, white pulp occupied about 50% of organ volume, which was in contradiction to the proportion in the postnatal spleen, where red pulp made up more than 80% of volume. These findings indicate alternative proliferation phases of red and white pulp.

Conclusion

Thirty fetuses of different age groups were used in the present study. The body weight and crown-rump length were recorded. The mean value was calculated for different stages of gestation. To study the histology of spleen, sections of spleen at different gestational ages were taken and stained with H&E. The slides were observed under light microscope.

In the present study, body weight showed gradual increase from 13th to 38th week of gestation. The mean body weight of fetuses was 130.83gms at the age of 14th week, 802gms at the age of 24nd week and 3150gms at the age of 38th week.

Histological development of the spleen

In the present study, following findings were noted

At 13th-14th week:

1. Thin capsule, reticular cells, lymphoblasts and few blood vessels were seen.
2. Irregular network of mesenchymal cells were present at this stage.

At 20th week:

1. Red and white pulp were seen prominently.
2. Lymphocytes were compactly arranged surrounding the arterioles.

At 22nd to 38th week:

1. Structure was exactly similar with that of adult spleen.

Hence our study showed the developmental and histological changes in the human fetal spleen at various gestational age of fetus. From this, we concluded that haemopoetic activity of spleen was seen from 14th week onwards and adult picture of spleen was seen from 22th week onwards.

Our study may be regarded as a preliminary study including some detailed results. We hope that the histological findings acquired in our study will be beneficial for the evaluations about the fetal spleen.

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