e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(8); 731-735

Original Research Article

Histological Study of Fetal Spleen at Different Gestational Ages

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Received: 18-06-2023 / Revised: 11-07-2023 / Accepted: 14-08-2023
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Conflict of interest: Nil

Abstract:

Background and Objectives: The spleen is a largest collection of lymphoid tissue with peculiar anatomical and physiological features. Spleen plays an important role in fetal hematopoiesis and immunomodulation. The aim of the study is to perform detailed histological analysis of human fetal splenic specimens of various gestational ages and compare the findings with earlier studies. The aim of the study is to perform detailed histological analysis of human fetal splenic specimens of various gestational ages and compare the findings with earlier studies.

Material and Methods: The present study included 40 fetal cadaveric spleen and morphometric features i.e., weight was measured and the sections of the spleen were stained with Haematoxylin and Eosin stain and were observed under compound light microscope.

Results: Study the histology of spleens of prenatal group showed the well-defined red pulp, venous sinusoids and diffusely spread lymphocytes at 18 weeks of gestation and the organization of lymphoid follicles was noticedat 24-28 weeks. At 32 weeks well defined white pulp was observed and the microscopic architecture of the spleen was similar to the histology of adult spleen.

Conclusion: A detailed knowledge of splenic morphometric dimensions, Histological is crucial in deciphering the role of spleen in fetal development and fetal wellbeing.

Keywords: spleen, hematopoietic, microscopic.

Introduction

The spleen is a largest secondary lymphoid organ which plays an important role in fetal hematopoiesis and immunomodulation. The spleen arises from a mesenchymal proliferation in dorsal mesentery or mesogastrium during 5th week of gestation. [1] The spleen assumes its definitive morphological structure during the 3rd month, the size gradually increasesduring the fetal period as mentioned by Sir Henry Gray in 1854 in the book "The structure and the use of spleen". [2] Spleen by tissue nature belongs to the lympho- reticular organs but unlike the 'white' lymph nodes it is not included in the lymph but in the blood circulation [3]. In the antenatal period, the spleenplays an important role in the immunomodulation, acts as a primary haemopoietic centre until late in thefetal period, also plays and important role in apoptotic cell clearance, immune tolerance, efficient removal of blood-borne microorganisms, lymphocytedifferentiation and activation. These functions are carried out by the two main components of the spleen, the white pulp (including the marginal zone) and the red pulp, which are vastly different in their microscopic architecture, vascular organization, and cellular composition [5,6]. The white pulp is subdivided into periarteriolar lymphoid sheath (PALS),the follicles, and the marginal zone. The antenatal period the histogenesis of spleen occurs in three stages : 1. Preliminary stage also calledthe "primary vascular reticulum," lasts up to the 14th gestational week. The primordium of the spleen appears in the embryos of size 6-7 mm in the 5thgestational week, and the hematopoietic cells were observed in the vascular lumen in 10th week [7].

Numerous erythrocytes, normoblasts and macrophages are seen among a network of mesenchymal cells and argyrophilic fibers.

Stage of transformation: The characteristic structure of the organ becomes established duringthis stage beginning with the 15th gestational week. Splenic lobules begin to form during the 15th to 17th gestational week.

Stage of lymphoid colonization : The development of the white pulp is correlated with the stage of lymphoid colonization around the centralarteriole occurs during the 18- 20 weeks. Around the 23rd week the aggregation of primary follicles is discernible at the periphery of the PALS and by 30-33 weeks distinct red and white pulp will be formed [4,7]. However, there is limited

research regarding the histology and histogenesis of spleen at different gestational ages which justifies the purpose of this study.

Objectives

The aim of the study is to perform detailed histological analysis of human fetal splenic specimens of various gestational ages and compare the findings with earlier studies.

Material and methods

The study is a prospective study conducted in the department of Anatomy, Patna Medical College and hospital Patna Bihar. Study duration of Two Years. Medically terminated fetuses of both sexes and relevant obstetric data were collected from Government Maternity Hospital, Patna, which includes 40 dead aborted fetuses of both sexes ranging from 16 weeks. of gestation to term. In the department of anatomy the collected fetuses were observed for congenital anomalies and they were preserved in 10% formalin. After one week of preservation, the abdomen was opened by using routine dissection method and the morphological observations were made insitu. Later spleens were removed by using routine dissection method and outer surface of fetal spleens were dried with blotting paper For microscopic observation, the antenatal specimenswere broadly categorized into 5 groups.Representative samples from each group were subjected to routine histological processing for H & E and reticulin stains. The sections were observed under 10x and 40x objective piece of microscope and representative fields were photographed by using photomicrographic equipment and the results were analyzed.

1. Haematoxylin and Eosin staining.
2. Reticulin stain to demonstrate reticular fibres.

The collected data was subjected to statistical analysis by computing the mean of each parameter with respect to the gestational age–wise groups by using SPSS 20 version.

Results

The Crown-rump length of all the fetuses were initially measured and fetal gestational age is calculated in weeks and based on the age in weeks the fetal specimens were categorized into 5 groups i.e., 16-20 weeks, 21- 24 weeks, 25-28 weeks, 29-32 weeks and 33 weeks to Term. The largest group was fetuses with gestational age 16-20 weeks with 10 specimens. The gender-wise distribution was 57.5% and 42.5 % for male and female groups respectively.

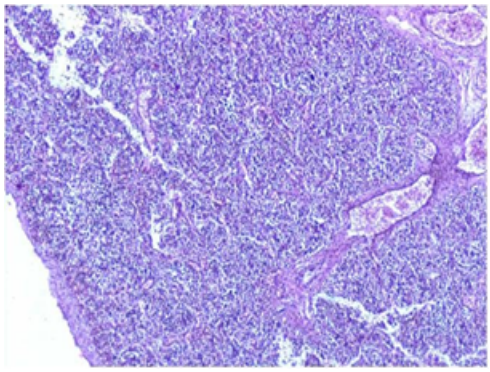
Table 1 : Gestational age/ Gender wise distribution of prenatal

group				
Group	Gestationalage (weeks)	Male (%)	Female (%)	Total (%)
A	16-20 Weeks	6 (26)	4 (23.5)	10(25)
B	21-24 Weeks	3(13)	2(11.7)	5(12.5)
C	25-28 Weeks	3(13)	6(35.7)	9(22.5)
D	29-32 Weeks	6 (26)	2(11.7)	8(20)
E	33 – Term	5 (22)	3(17.6)	8(20)
	Total	23 (100 %)	17(100 %)	40 (100)

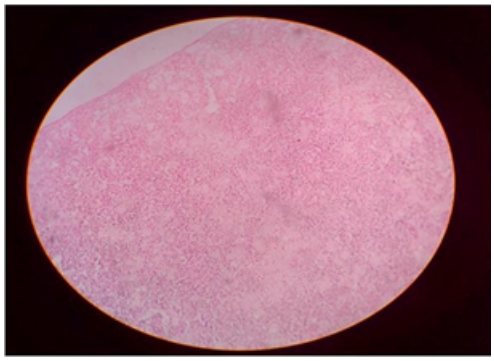
Histology of spleens

In the study the earliest specimen observed was that of fetal spleen of 16 weeks gestational age. The observations made in prenatal group were as follows.

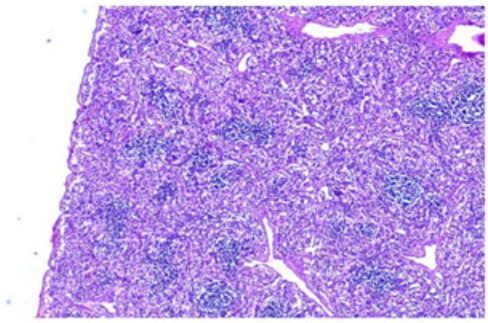
Group A (16-20 weeks): Fetal spleen sections showed thickened capsule and dilated sinusoids filled with blood cellular elements, diffuse aggregations of lymphoblasts. Well-defined lymphoid follicles central arterioles and white pulp were conspicuously absent. Megakaryocytes seen with evidence of extramedullary hematopoiesis. Group B (20-24 weeks): The spleen sections showed distinct fibrous capsule, accumulation of lymphoid follicles around arteriole. aggregation of lymphocytes to form lymphoid follicles was noticed. Sinusoids, capillaries with thin endothelial lining were observed. Reticular network, Sinusoids and blood vessels increased in number. Extramedullary hematopoiesis was noticed. Cleardemarcation between red pulp wand white pulp was absent. Group C& D (25-32 weeks): The spleen sections showed white pulp formation in the form of well-defined lymphoid follicles and trabeculae were present. There was a well-defined evolution of Red pulp with RBC. Periarteriolarlymphoid aggregation was noticed. Well-developedlymphoid follicles with central arteriole were seen. Group E (33 weeks – Term): The spleen sections showed well fibrous capsule, numerous sinusoids, aggregation of the lymphoid follicles with an eccentric arteriole. Increased size of the white pulp was noticed. Well-differentiated Malpighian corpuscles were present. In this group at term the splenic tissue showed well defined white pulp with dense aggregation of lymphocytes with clear demarcation from the surrounding tissue. Whitepulp was sparsely associated with collagen fibers. Thered pulp showed collagen network of fibers incontinuance with trabeculae. It also showed sinusoids filled with RBC, occasional fibroblasts and the microscopic structure at term resembled that of adultspleen architecture.



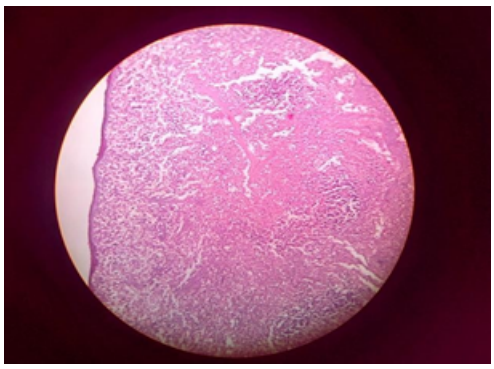
18 weeks human fetal spleen section showing thickened capsule and dilated sinusoids filled with blood cellular elements, aggregations oflymphoblasts are seen H&E stain, 40X



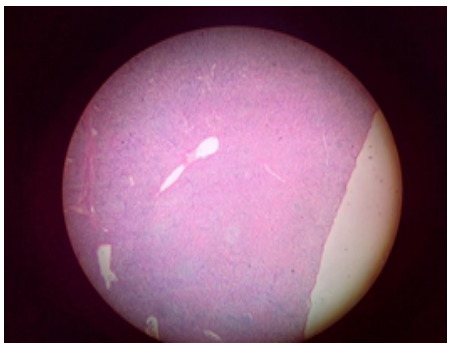
20 weeks human fetal spleen section showing fibrous capsule and dilated sinusoids filled with blood cellular elements. White pulp and malpighian bodies are absent H&E stain, 40X.



28 weeks human fetal spleen showing whitepulp in the form of follicles, with trabeculae, peri arteriolar lymphoid aggregation is also seen H&E stain, 40X.



35 weeks human fetal spleen showing trabeculae, periarteriolar aggregation of lymphoid cells, well differentiated white pulp seen H&E stain, 40X.



38 weeks human fetal spleen showing whitepulp in form of lymphoid follicles, arteriole and redpulp H&E stain, 40X.

spleen was 1.98 gm. The splenic weight ranged from 0.1 gm to 4.9 gm which gradually increased with gestational age significantly till 38 weeks of gestation.

Discussion

In the present study the average weight of the prenatal

Showing comparison of fetal body weight (gms) of present study with literature					
Gestational age (wks.)	**Present study**	**Parulekar et al [8] [1995]**	**Gruenwald [9] [1960]**	**Schultz et al[10] [1962]**	**Ajit holkunde et al [11]**
16 weeks	310	200			200
20 weeks	545	460		316	420
24 weeks	870	820	630	748	802
28 weeks	1270	1300	1020	1127	1290
32 weeks	1809.5	2100	1488	1748	1924
36 weeks	2446	2900	2165	2448	2910

The findings regarding the weight of the spleen in relation to gestational age were in agreement with the observations of Gruenwald P & Minh HN [9] (1960) and with the descriptions of Sir Henry Gray [2] (1854) till 32 weeks of gestation and not in agreement afterwards. On comparison of findings of study with existent literature, the body weight in relation to gestational age was in agreement with findings of Parulekar et al., and was more when compared to the findings of Gruenwald *et al*.

Histology of fetal spleen:

Vellguth *et al.* proposed three stages of spleen development from 14th-24th week of gestation namely- preliminary stage of splenic primordium [from 14th week], Transformation stage of development of splenic architecture [15-18 weeks], followed by Lymphoid colonization & development ofwhite pulp [18-24 weeks], According to Vellguth et al, at 14 weeks connective tissue, fibroblasts, vascular endothelium are formed and there was increase in blood vessels and mesenchyme. Venous sinuses are not formed.Splenic lobules begin to form in 15 th week, and fully differentiated by 17th week. The lobules consisted a central artery, with red pulp forming at the periphery.The differentiation of the red pulp is closely correlated with the development of the venous system. An accumulation of lymphocytes around the central arteries (PALS) can be recognized during the 19th and 20th week of gestation which marked the formation of white pulp. Around 23rd week the primary follicles were arranged at the periphery of the PALS. In the present study earliest, fetal spleen was of 16 weeks which showed dilated sinusoids, aggregation of lymphoblasts with evidence of extramedullary hematopoiesis. At 20-24 weeks, formation of lymphoid follicles was observed. At 30 weeks, white pulp formation in the form of well-defined lymphoid follicles and trabeculae was noticed. At 33 weeks well differentiated white pulp, red pulp and well-formed Malpighian corpuscles were present. According to Radhika et al [13] lymphocytic aggregation started by 11 th week. 20th week marked the development of lymphoid follicles and by 32 weeks well developed lymphoid follicles with central arteriole was observed. Mature lymphoid follicles with peripherally placed arteriole were noticed at 36 weeks of gestation. These findings are almost in agreement with the study. According to Rajeev Mukhia et al [2016] vasculature, connective tissue were formed and sinusoids were seen at 16-20 weeks. At 20th Gestational week, the spleen shows clear-cut capsule surrounding the spleen with bundles of collagen fibres & fibroblasts. The lymphocyte aggregations started differentiating around the central arteriole forming the periarteriolar lymphatic sheath (PALS) was noticed by 20 weeks. Distinct white pulp showinglymphatic nodules with peripherally placed central arteriole was observed at 26 -30 weeks. At the 23rd Gestational weeks, the structure of spleen resembles to the adult spleen.

Above findings were in concordance with the observations of study. The salient Histological features noticed in the present study were.

At the 16th week:

1. Thickened capsule, dilated sinusoids, lymphoblasts and few blood vessels were seen.
2. Evidence of extramedullary hematopoiesis was noted.

At 20-24 weeks: aggregation of lymphocytes to form lymphoid follicles around an arteriole was noticed.

At 25-32 weeks: well-defined white pulp, Red pulp, periarteriolar lymphoid sheath [PALS], well developed lymphoid follicles with central arteriole were seen.

After 33 weeks - Well differentiated Malpighian corpuscles were present, structure resembled that of adult spleen. These findings were in agreement with the observations of Mrinmoy pal et al. [15], Merida- Velasco JA [16].

Conclusion

The current study was conducted to analyze the histological development of spleen at various gestational ages. We hope a detailed knowledge of Histological may provide an insight in understanding the role of spleen in fetal development and fetal wellbeing. However further research directed at molecular level and immunobiology is required in elucidating the actual role of spleen in human beings in immunity & autoimmunity.

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