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SPLEEN-A DEVELOPMENTAL & HISTOLOGICAL STUDY IN HUMAN FETUSES

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Abstract

Background: The study of development of human being is always a subject of interest of many researchers as human species is the unique species with unique features in the universe. This feature of human species forms the base of all the researches. Objective: To study the histological and developmental changes in human fetal spleen at various gestational age of fetus. **Material & 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. **Results:** The average crown rump length of fetuses at 14 weeks was 115mm, it was 202mm at 24th week and 276mm at 30th week. It was 330mm at 38th weeks. The relative weight at 14th week was at 0.12 gms and it slightly increased upto 20th week of gestation at 0.26 gms. After 20th week it increased to 0.30 gms in 24th week and later on it was more or less constant upto 38th week at about 0.34 gms. The mean splenic length at the age of 14th week was 9.40mm, which steadily increased upto 36.60mm at the 38th week. the length, width and thickness of the spleen showed the increase and the finding were correlating with the previous workers. **Conclusion:** We concluded that haemopoetic activity of spleen was seen from 14th week onwards and adult picture of spleen was seen from 22th week onwards.

INTRODUCTION

The spleen is also the largest secondary lymphoid organ containing about one-fourth of the body's lymphocytes and initiates immune responses to blood-borne antigens.[1] This function is charged to the white pulp which surrounds the central arterioles. The white pulp is composed of three subcompartments: the periarteriolar lymphoid sheath (PALS), the follicles, and the marginal zone. 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. Inspite of an extensive literature, one finds few recent papers dealing with the subject from the standpoint of the many interesting hematological problems involved. The most extensive work has been done on the lower vertebrates and very few on human embryos. Owing to lack of some of the most important stages and due to poor preservation of much of material, the work on the human fetus has contributed little toward the solution of many hematological problems. The investigators who have studied the problem of splenic development can be placed in two distinct groups. According to the first group, the spleen is of the mesodermal origin and according to second group, it is of endodermal origin. The first class of workers may be further divided into those who hold that rudiment of the organ developes from differentiation of the mesenchyme, and into those who maintain that the peritoneal epithelium also plays a part in the first stages of development.[2]

The chief exponents of the endodermal theory are Schenk, Gotte, Maurer, V. Kupffer, Woit, and Glas. These men, with the exception of Maurer and Gotte, believed there was a definite relation between the embryonic spleen and pancreas, and consequently considered the spleen as being dependent upon the endodermal cells which are given off from the primitive diverticulum of pancreas. The work of Maurer stands alone in maintaining a relation between the endodermal epithelium of intestinal tract and splenic rudiment.[2]

Fetal splenomegaly occurs in cases of congenital transplacental infections, hematological diseases,

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immunological diseases and lipidoses. On the contrary, a hypoplastic spleen is often found in cases of Di George syndrome and sickle cell disease. Thus, the measurement of the fetal spleen size should be a useful diagnostic tool in the detection of congenital splenic pathologies in utero.[3] On looking over the literature on development and the important functions served by the spleen as haemopoeitic and lymphoid organ, the following study is undertaken.

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.

Measurements of External Parameters

1. The sex, gestational age was noted from the case paper.
2. Body weight of fetus: The fetuses were weighed in double pan balance and were recorded in grams.
3. 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

- The fetuses were carefully dissected by doing the window dissection to remove the spleen in one piece.
- Spleen was removed by cutting the blood vessels close to the hilum.
- The spleen was weighed and its external parameters such as length, width and thickness were noted. Weight of the spleen was measured in chemical balance and recorded in grams. Length, width and thickness of the spleen were measured by Vernier caliper in mm.
- Length of the spleen was determined as the distance between the two planes passing from the most protuberant points of the posterior and anterior extremities and vertical to longitudinal axis of the spleen.
- Width of spleen was determined as the distance between the two planes passing from the most protuberant points of the superior and inferior borders and parallel to longitudinal axis of spleen.
- Thickness of spleen was determined as the distance between the two planes passing from the most protuberant points of the visceral and diaphragmatic surfaces and parallel to the longitudinal axis of the spleen.
- The spleen was cut into pieces and fixed in 10% formalin for 24-48 hours.
- After fixation, the splenic tissue was kept under running water for 3 hours.
- The tissue was then placed in 70% alcohol for 2 and half hours, then 80% alcohol for another 2 and half hours and then in 90% alcohol for 2 hours.
- Then tissue was kept in absolute alcohol overnight.
- Then tissue was given 2 changes of clearing agent for 1 hour each.
- Then tissue was given 3 changes of paraffin wax for 1 hour each at 56-60 deg.
- The tissue was then embedded in glycerine smeared 'L' moulds.
- Label was fixed to inner side of mould.
- Fresh filtered paraffin wax was poured into the moulds about 1-2 cms thick and the tissue was inserted with its cutting surface facing down.
- Then the mould was filled to the brim. Any air bubbles formed were removed by hot spatula.
- After the wax was solidified, the block was removed from the mould and prepared for cutting.
- The individual blocks were fixed to the chucks by heating. These chucks were locked in place on the rotary microtome and thin ribbons of 5-8 µm were cut. The sections were gently lowered on the surface of water at 5-10˚c to remove the folds. Then the sections were lifted on albumin coated slides and kept for drying on a hot plate at 45-50˚C for 2 hours or more as necessary.

Staining

- Removal of paraffin wax was done by heating the slides and dipping into two changes of xylene of one or two minutes each.
- Removal of xylene was done by dipping the slides into two changes of absolute alcohol for one or two minutes each.
- Then the slides were kept for two minutes in 90% alcohol followed by two minutes in 70% alcohol.
- After this the slides were kept under running tap water for five minutes.
- Then they were stained with haematoxylin for 8-10 minutes and again kept under running tap water for five minutes. Excess stain was removed by dipping into acid alcohol for a second or two and again the slides were kept under tap water to regain the blue colour.
- The stain was checked under microscope.
- Then slides were rinsed in distilled water and the slides were then dipped into ascending grades of alcohol for dehydration.
- Then the slides were kept in eosin for 30 seconds to one minute to counterstain.

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- Then slides were kept in 95% alcohol for few seconds, then in 2 changes of absolute alcohol for 3 minutes.
- The slides were kept for clearing in xylene for one minute two times each.
- The slides were mounted with DPX and cover-slips applied. The slides kept at room temperature for some hours for firm adhesion of the cover-slip.
- The stained slides were observed under light microscope using 4X, 10X and 40X magnification and the findings were noted and compared with previous studies.
- For observational purpose and for comparison with other studies, the fetuses were arranged in 13 groups and mean of each parameter was calculated for each group.

RESULTS

The present study was carried out over 30 human fetuses ranging from the 13th week to 38th week of gestational age. 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.

Body weight and crown rump length of fetus:

It was almost a smooth curve, since there was gradual increase in weight from 13th week to 38th week of gestation. The average crown rump length of fetuses at 14 weeks was 115mm; it was 202mm at 24th week and 276mm at 30th week. It was 330mm at 38th weeks.

The average weight of 14th week of gestation was 0.15gms. The spleen weight increased at very slow rate upto 30th week and was 4.14 gms. After 30th week the weight increased with fast rate upto 38th week of gestation. At 38th week spleen weight was 10.65 gms.

Relative Weight of Spleen

The percentage relative weight of spleen was measured in different weeks of gestation by the formula as follows:

The % relative weight of spleen= weight of spleen ×100

Body weight

The average relative weight of spleen was recorded.

Table1: Showing mean spleen weight and mean relative weight of spleen in different weeks of gestation

Gestationalage (wks)	Absolute spleenweight(gms)	Percentage relativeweightofspleen (gms)
14	0.15	0.12
16	0.48	0.24
18	0.75	0.25
20	1.09	0.26
22	1.64	0.27
24	2.40	0.30
26	2.87	0.25
28	3.35	0.26
30	4.14	0.24
32	5.38	0.28
34	7.192	0.29
36	9.312	0.32
38	10.65	0.34

It was seen that the relative weight at 14th week was at 0.12 gms and it was slightly increased upto 20th week of gestation at 0.26 gms. After 20th week it was increased to 0.30 gms in 24th week and later on it was more or less constant upto 38th week at about 0.34 gms.

Length of the spleen

Table 2: showing the gestational age (in weeks) and the mean length of the spleen (in mm)

Gestational age (in weeks)	Length of spleen (mm)
14	9.40
16	12.50
18	13.5
20	17.5
22	20.5
24	21.25
26	25
28	23.17
30	24.80
32	29.6
34	31.50
36	30.83

ISSN (O): 2687-5365; ISSN (P): 2753-6556

38	36.60

The mean splenic length at the age of 14th week was 9.40mm, which steadily increased upto 36.60mm at the 38th week. For the statistical analysis, the study group of 30 fetuses was divided in the 3 groups and mean length and standard deviations of each group was tabulated in table-3.

Table 3: Showing the mean splenic length and standard deviation of the groups

Group	N	Mean splenic length	Standard deviation
13-25week	15	15.78	4.729
26-37week	10	27.48	3.572
38-40weeks	5	36.63	0.278

Width of spleen

At the 14th week of gestational age the average width was 5.60mm. After that there was increase in width rapidly upto the age of 22nd week of gestation. At 22nd week, width was 13.65mm and from onwards the increase in width was slow and steady, reaching upto 26.2mm at the age of 38th week.4The study groups were formed for the statistical analysis and the mean width of spleen and standard deviation were tabulated in table-4.

Table 4: showing mean width and standard deviation in relation to different age groups

Group	N	Mean splenic width	Standard deviation
13-25week	15	9.90	3.392
26-37week	10	18.77	2.926
38-40weeks	5	26.43	0.494

At the age of 14th week of gestation the average thickness was 4.10mm. The thickness increased rapidly upto 12.30mm at the age of 26th week and after that slowly and steadily upto 16.10mm at the age of 38th week.

Table 5: Showing the average thickness of spleen (mm) at different gestational age

Gestational age (in weeks)	Average thickness of spleen(mm)
14	4.10
16	5.20
18	5.65
20	6.85
22	8.75
24	8.78
26	12.30
28	12.50
30	13.10
32	14.02
34	16.00
36	16.30
38	16.10

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 13th week of age

- There were large numbers of cells of irregular shape with large nucleus centrally.
- The cells were seemed to be attached with each other, forming the network.
- Few small thin walled vessels are seen.

At 14th week of age

- Histological structure is more or less similar to previous age.
- Capsule was seen.
- Reticular cells forming network were present.
- More number of blood vessels were present.
- Numbers of hemopoietic cells were increased.
- Lymphocytes were seen in groups, but these groups were scattered.

At 15th week of age

- Capsule was thickened and prominent.
- Reticular cells forming fibers were seen and were forming the network.
- Number of blood vessels was increased.
- Haemopietic cells are seen in large numbers.
    - Majority of WBC seen were lymphoblasts, arranged in groups.
- These groups are scattered randomly all around.

At 16th week of age

- Connective tissue elements and reticular fibers were increased.
- These connective tissue were seemed to be supporting the sinusoids.
- Vascularity was increased as the number of blood vessels was increased.

At 17th week of age

- The features of this age were similar to the previous age.

At 18th week of age

ISSN (O): 2687-5365; ISSN (P): 2753-6556

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

At 19th week of age

- Features were similar to those of previous age.

At 20th week of age

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

From 22nd to 38th week of age

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

DISCUSSION

Development and growth involves change in size, shape, weight and structure. This is accompanied by attaining full functional status at the end of developmental period.

While studying the development of spleen in antenatal period applying the same principles stated above, the study of size and weight of spleen at different stages and their proportion to body weight were discussed first.

The microscopic structure of spleen at different ages in antenatal period was then 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. Average weight & relative percentage of the spleen in relation to body weight of fetus.
4. Length, width, thickness of spleen.
5. Microscopic structure of spleen at different gestational age.

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

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 Arey,[4] (1954), and Potter et al.[5] (1976) were less than the present study. However, the findings of the present study were comparable with the findings reported by Parulekar,[6](1995). The body weight upto 28th week of gestation was found to be greater than reported by Hamilton et al,[7] (1962) but it was comparable thereafter upto the 38th week of gestation.

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-11 for comparison with crown rump findings of the previous workers.

It was seenthat the values of crown rump length given by Potter et al,[5] (1976) and Hamilton et a,[7] (1962) were more or less similar to the findings of the present study.

The exact figures of relative weight of spleen were not quoted in the previous studies. However, from the data presented in these studies, the

Comparison of relative weight of spleen showed that the findings of the present study were more or less similar to the findings recorded by Gruenwald et al,[8] (1960) and Potter et al,[5] (1976).

Length of the spleen

For comparison with other workers, the length of the spleen was arranged in groups.The table showed that the mean length of the spleen of the present study was more or less comparable with that of Ungor B et al.[35]

Thickness of the spleen: The thickness of the spleen of present study was more or less similar with those reported by the Ungor B et al.[3]

Microscopic structure of spleen

Developmental changes in the histology of spleen were compared with the findings of different scientists.

In the present study, it was seen that at 13th to 14th week stage of development there were mesenchymal cells forming irregular network. Thin capsule, reticular cells, lymphoblasts and few blood vessels were present.

At 15th week blood vessels and haemopoetic cells were increased in number. WBCs mainly contained lymphoblast cells which were present in groups, scattered randomly all around the tissue.

At 16th to 17th week stage reticular fibres supporting sinusoidal spaces were increased and spleen showed rich vascularity at this stage.

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At 18th to 19th week lymphocytes aggregated in groups. At these stages the first indication of division into red pulp was seen. However, lymphocytes were less compactly arranged and clearcut association with arterioles was not evident.
At 20th week clear-cut division of spleen into red pulp and white pulp was evident. White pulp consisted of compactly arranged lymphocytes surrounding arterioles.
At 22nd to 38th week of age, structure was similar to the adult spleen, thick well developed capsule, number of trabeculae, rich vascularity. Clear cut division of red and white pulp was evident. Developing RBCs were seen. White pulps consisting of lymphocytes around arterioles were seen. Arterioles showed an eccentric position in white pulp.
According to Hamilton et al,[7](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 al,[9] (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 al,[10] (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. These findings coincided with our results.
According to Potter et al,[11] (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 al,[7] (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 al,[9] (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.
According to Williams PL et al,[12] (1980) spleen appeared about 6th week of gestation. The proliferating cells became condensed and vascularised. Vascular reticulum became well developed at 8th to 9th week with numerous, closely spaced, thin walled loops. Differentiation of arteries, veins, capillaries and sinusoids occurred by 11th to 12th week.
Vellguth et al,[13] (1985) studied the development of human spleen from 14th to 24th week of age. He stated three stages in the development.

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

CONCLUSION

ABHA is one of the elementary components of Ayushman Bharat Digital Mission (ABDM) which could help in bridging the health disparities among the people especially underprivileged sections of population, who, because of their socio-economic and geographical disadvantage have a very poor access to health care. However, the intended goals of Ayushman Bharat Health Account (ABHA) can't be realized until all sections of the population are not made fully aware about the programme. The study reveals huge existent knowledge gaps among the underprivileged sections of the population with regard to the Ayushman Bharat Health Account (ABHA) and, hence, emphasizes the need towards their early sensitization through the proper dissemination of the necessary information. Once the public is abreast about the pros and cons of the digital change in health sector, they will develop a positive attitude, which can be transformed into practice. Besides this, the digital divide between the various sections of the society ought to be taken care off ailing which, health care disparity may further worsen, which can thwart the very purpose of the Ayushman Bharat Digital mission (ABDM). Further, the differential response towards Ayushman Bharat Health Account (ABHA) across the age structure because of the differential e-compatibility calls for necessary interventions so that all the age strata are brought within the ambit of the digital mission. This can be done by creating the necessary facilitation counters at the Anganwadi centres/sub-centres/primary health centers besides the tertiary care institutions by roping in the grass-root level workers and other paramedical staff/technical staff.

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Utilizing educational institutions as Ayushman Bharat Health Account (ABHA) registration centres would be a feasible and efficient way to increase the number of Ayushman Bharat Health Account (ABHA) account holders among the students, who are the future users of health care services. A camp approach can even be used to cover a greater number of beneficiaries in a short span of time for ensuring a better coverage. Moreover, to facilitate the creation of a centralized repository of health data at one place, the necessary legislations in place ought to be made more stringent by encrypting the data between the beneficiaries and the authorities at helm, so that privacy is ensured and data breaches are checked. In order to achieve the wider goals of the Ayushman Bharat Health Account (ABHA) under Ayushman Bharat Digital mission (ABDM), integrating health related schemes with Ayushman Bharat Health Account (ABHA) would be a good option. Moreover, a robust mechanism has to be in place on the lines of Aadhaar and Pan Cards, so that Ayushman Bharat Health Account (ABHA) is made mandatory for the entire population of the country.

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