

## MORPHOMETRIC STUDY OF HUMAN FOETAL SPLEEN: A STUDY FROM NORTH-INDIA

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### ABSTRACT

**Background:** The vertebrate spleen has important functions in immunity and haematopoiesis, many of which have been well studied. In contrast, we know very less about the mechanisms governing its early embryonic development. There is little research on morphometric analysis of spleen in human fetal cadavers.

**Aims and objectives:** The present study was carried out to determine the location and dimensions of human fetal spleen.

**Material and methods:** The study was carried out on 50 dead human fetal spleens (30 males and 20 females), aged between 9 - 32 weeks of gestation. The location of spleen and its relation with the neighboring structures, notches on borders, major ligaments and the shape of spleen and its hilum were studied. The length, width, thickness, and weight of the spleen were measured.

**Results:** The average length, width and thickness of fetal spleen of gestational age between 12 to 16 weeks were 7.65mm, 4.71mm, 2.93mm. respectively and mean weight was 0.065gm. In >16 to 20 weeks, mean length was 10.9mm, width was 7.05mm, thickness was 5mm. respectively and weight was 0.461gm. In >20 to 24 weeks, mean length was 13.45mm, width was 8.38mm, thickness was 5.72mm. and weight was 0.580gm. In >24 to 28 weeks, mean length was 19.8mm, width was 12.43mm, thickness was 8.67mm. and weight was 0.997gm. In >28 weeks gestational age, mean length was 26.5mm, width was 13.5mm, thickness was 9.2mm. and weight was 1.91gm. Spleen was found to be of three different shapes - leaf shaped (two faced), wedge shaped (three faced) and pyramid shaped (four faced). In 44 cases (88%) it was wedge shaped, in four cases (8%) it was two faced and in two cases (4%) it was pyramid shaped.

**Conclusion:** The expression of morphometric parameters of the spleen at different gestational ages can be used in determining various pathologies of the spleen and may also contribute to future studies on this subject.

**KEY WORDS:** Fetal Spleen, Morphometric Data, Splenic Development.

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### INTRODUCTION

Spleen is the largest secondary lymphoid organ [1]. The vertebrate spleen has important functions in immunity and haematopoiesis, many of

which have been studied well. In contrast, we know very less about the mechanisms governing its early embryonic development [2]. The spleen appears in the sixth week of intrauterine

life as a localized thickening of the coelomic epithelium of the dorsal mesogastrium near its cranial end. Stem cells migrate into the spleen from the yolk sac. Multiple foci coalesce to form the body of spleen. Failure of fusion of splenculi results in accessory spleens [3].

At fetal stage the cells of splenic condensation become arranged into anastomosing trabeculae. The trabecular columns produce reticular fibers, which forms the connective tissue framework of the spleen. In the 4<sup>th</sup> and 5<sup>th</sup> months of intrauterine life these cells multiply and differentiate into erythroblasts, myeloblasts and megakaryocytes as well as lymphoblasts. In later embryonic life the myeloid function ceases and are replaced by erythropoietic function. Lymphocytes are produced in the spleen throughout life [4].

The spleen displays various developmental anomalies, including complete agenesis, multiple spleens or polysplenia and persistent lobulation. Fetal splenomegaly occurs in cases of congenital transplacental infections, hematological diseases, immunologic disorders, neoplasms, metabolic diseases, congestion and congenital cysts [3]. A hypoplastic spleen is often found in cases of DiGeorge syndrome and sickle cell disease. Therefore, the measurements of fetal splenic size is a useful diagnostic tool in the detection of congenital splenic abnormalities in utero [5,6].

Most of the studies concerning the development and morphometry of spleen have been done by the use of ultrasonography [5,7]. In two studies on adults, it has been reported that there is a difference between the dimensions of spleens from alive and dead subjects at a ratio ranging from 25 to 50% [8]. Taking this ratio in account, we believe that measurements from spleen of dead fetuses may have particular importance. So we decided to study the anatomical development of the fetal spleen.

## MATERIALS AND METHODS

The present study was carried out in the department of Anatomy, Government medical college and hospital, Chandigarh. The study was carried out on 50 dead human fetal spleens (30 males and 20 females), aged between 9 and 32 weeks of gestation. Fetuses were obtained

from department of obstetrics and gynaecology (Government medical college and hospital, Chandigarh) for routine fetal autopsy and were fixed in 10% formaldehyde. Consent was taken from the parents to perform autopsy. Cases with any marked pathology or anomaly were not included. Gestational ages of the fetuses were determined based on the crown rump length (CRL) until 12<sup>th</sup> week and bi-parietal diameter, head circumference, femur length and foot length between 13 and 40 weeks of gestation.

Cases were stratified based on the gestational age as follows: (Table-1)

Group 1 comprised of fetuses with gestational age between 12 and 16 weeks.

Group 2 comprised of fetuses with gestational age between >16 and 20 weeks

Group 3 comprised of fetuses with gestational age between > 20 and 24 weeks

Group 4 comprised of fetuses with gestational age between >24 and 28 weeks

Group 5 comprised of fetuses with gestational age >28 weeks.

**Table 1:** Gestational age and gender wise distribution of fetuses.

Group no.	Gestational age(in weeks)	Number of fetuses(50)	Male (30)	Female (30)
1	12-16	9	6	3
2	>16-20	16	9	7
3	>20-24.	16	8	8
4	>24-28	8	7	1
5	>28	1	0	1

In present study, a vernier caliper, measuring tape, plastic ruler and compass were used for measurement of dimensions of spleen. Following steps were undertaken to expose the fetal spleen during autopsy:

- 1) A midline vertical incision was given on the anterior abdominal wall of the fetus. Bilateral incisions were given in the inguinal region and along the costal margin.
- 2) Two flaps of skin were obtained and reflected laterally.
- 3) Abdominal muscles were also reflected to expose the abdominal cavity and spleen was approached in the left upper quadrant of abdomen.

After exposing the spleen, its location and anatomical position were determined. The relations of the spleen with other neighbouring abdominal structures such as liver, stomach, colon, pancreas, kidney, adrenal gland and diaphragm was noted.

Existence of accessory spleen was observed. Then the spleen was removed after dissection of its ligaments. The spleen thus removed was fixed in 10% formalin solution for 1 to 2 weeks. The shape of the spleen, the number of the notches on the borders, the number of fissures on the surfaces, and the shape of the hilum were determined.

**Morphological measurements:** Linear measurements of 50 fetal spleens were taken with the help of vernier caliper, divider and compass. The following morphometric measurements were taken on spleen:

**Length (L):** the length of the spleen was determined as the distance between the two planes passing from the most protuberant points of anterior and posterior extremities.

**Width (W):** the width of the spleen was determined as the distance between the two planes passing from the most protuberant points of the superior and inferior borders.

**Thickness (T):** the thickness of the spleen was determined as the distance between the two planes passing from the most protuberant points of the visceral and the diaphragmatic surfaces. All the measurements were taken in millimeters (mm) and were taken thrice. Their mean was calculated to minimize measurement errors. The weights of the spleens were measured in grams by using electronic weighing scale.

## RESULTS

In all fetuses, the spleen was located in left hypochondrium and was intraperitoneal except at the hilum. The longitudinal axis was oblique and directed to anterior, left and inferior in all the cases. Regarding the relation of spleen with neighboring viscera, stomach and spleen were in contact with each other, spleen being left and posterior to stomach in all the cases.

Left colic flexure of large intestine was in contact with spleen in 47 cases out of 50 (94%). It was not in contact with spleen in rest of the

three cases (6%). These cases usually belonged to early period of fetal development (early 2<sup>nd</sup> trimester). Out of these three cases, one case belonged to 14 weeks, one was in 19 weeks and one in 16 weeks of intrauterine life. The spleen was located postero-superior to left colic flexure.

Left kidney was in relation with spleen in 27 cases (54%), while there was no contact of spleen with left kidney in 23 (46%) cases. In all cases, left kidney was located posterior, inferior and medial to spleen. The percentage value of contact increased with increase in the fetal age. The left suprarenal gland was in contact with the spleen in all cases and was located posterior, inferior and medial to the spleen. In all cases, diaphragm was in contact with spleen, located superior and lateral to spleen. In six cases (12%), the liver did not have any contact with spleen while it was in contact with spleen in 44 cases (88%). Tail of pancreas was in contact with visceral surface of spleen in 43 of cases (86%) while it was not in contact with spleen in seven cases (14%). The gastrosplenic and splenorenal ligaments were present in all the cases. Phrenicocolic ligament was absent in eight cases and in remaining all it was present. All these eight cases were from 1<sup>st</sup> trimester. We did not observe any case of accessory spleen in our study.

Concerning the shape of spleens, it was of three different shapes - leaf shaped (two faced), wedge shaped (three faced) and pyramid shaped (four faced). In 44 cases (88%) it was wedge shaped, in four cases (8%) it was two faced and in two cases (4%) it was pyramid shaped. (Table-2) In one case, spleen was abnormally enlarged. One spleen had hook like curved anterior end.

**Table 2:** Distribution according to shape of spleen.

Group no.	Two faced (leaf shaped)	Three faced (wedge shaped)	Four faced (pyramid shaped)
1	2	7	0
2	0	16	0
3	0	15	1
4	2	5	1
5	0	1	0
total	4	44	2

Concerning the notches on the borders, notches were present in 38 cases (76%) out of 50. Number of notches varied from one to three, and three

**Table 3:** Mean parametric values of fetal spleens according to gestational age.

Group no.	Gestational age (in weeks)	Mean length (in mm.)	Mean width (in mm.)	Mean thickness (in mm.)	Mean weight (in grams)
1	Dec-16	7.65	4.71	2.93	0.065
2	>16-20	10.9	7.05	5	0.461
3	>20-24.	13.45	8.38	5.72	0.58
4	>24-28	19.8	12.43	8.67	0.997
5	>28	20.2	13	9	1.91

and a half. In 25 out of 38 cases, only one notch could be appreciated. In seven cases, 2 notches were seen. In two cases, two and a half (2.5) notches were seen. In three cases, 3 notches were visible. In only one case three and a half notches were visible. In all these cases, notches were present mostly on superior border, except in one case in which more than 3 notches were present on inferior border.

The shape of the splenic hilum was found to be of four different types. In 72% cases, it was longitudinal in shape, in 20% cases it was triangular, in 5% cases it was V shaped and in 3% cases it was irregular in shape. The data on various morphometric variables viz. length, width, thickness and weight of spleen was measured and tabulated in present study (table-3). In group1, mean length was 7.65mm, width observed was 4.71mm, thickness was 2.93mm. and mean weight was .065gm. In group 2, mean length was 10.9mm, width was 7.05mm, thickness was 5mm. and weight was .461gm. In group 3, mean length was 13.45mm,width was 8.38m , thickness was 5.72mm. and weight was.580gm. In group 4, mean length was 19.8mm, width was 12.43mm, thickness was 8.67mm.and weight was .997gm. In group 5, mean length was 26.5mm, width was 13.5mm, thickness was 9.2mm.and weight was 1.91gm.

## DISCUSSION

Review of the literature suggests that there are very few studies regarding position and visceral relations of fetal spleen. According to Ungor et al [9] and studies done by Saheb et al [10] and Ramanujan et al [11], all the spleens were located in left hypochondrium of abdomen. In our study also, all the spleens were located in left hypochondrium and were intraperitoneal which is consistent with general textbook data [1,3].

In the present study, the relation of spleen with neighboring viscera was different as compared to adult spleen except its relation with the stomach and diaphragm which followed adult pattern. The relation of spleen with large intestine varied with gestational age. Left colic flexure was in contact with spleen in 94 % cases which belong to group 3&4 of our study. Similar to our finding in a study done by Ungor et al [9] large intestine was in relation with spleen in late second and third trimester and it was not in contact in first and early second trimester cases. Regarding the relation of spleen with tail of pancreas, Skandalakis et al [12] and Soyluoglu et al [13] reported that pancreatic tail was in contact with spleen in all the cases they studied, though the position of tail varied with age of foetus. In another study, pancreatic tail was in contact with spleen in 93.6% of cases [9]. In our study tail of pancreas was in contact with spleen in 86% of cases.

The gastrosplenic and splenorenal ligaments were present in all the cases. Phrenicocolic ligament was not present in eight cases and in the rest it was present. All these eight cases were from 1<sup>st</sup> trimester. In the study by Ungor et al [9] phrenicocolic ligament was not seen in 18 out of 21 cases in first trimester. This could be due to the fact that left colic flexure reaches its normal position in late fetal life.

In our study spleen was wedge shaped in 44 cases (88%), in four cases it was two faced and in two cases it was pyramid shaped. Ungor et al [9] also reported three faced wedge shaped fetal spleen in 60% cases, four faced in 36.5% and two faced in 3.5% cases. The differences observed could be due to different fetal age groups in the study. According to Herald Ellis the spleen has cupped-hand shape [14].

Notches were present in 38 cases (76%) out of 50. Number of notches varied from one to three, and

three and a half. In most of cases (25 out of 38), only one notch could be appreciated in our study. Ungor et al [9] reported notches on superior border in 95% cases with mean notch number of 3.03. In our study also all the notches were observed on superior border except in one where it was on inferior border.

To the best of our knowledge, there is only one study regarding the shape of fetal splenic hilum by Ungor et al [9] who reported longitudinal hilum in 69.5% cases, triangular in 20.6% cases, 2.8% had irregular shape and 2.8 % having V shaped hilum. In our study we found 72% cases having longitudinal hilum, 20% cases it was triangular, in 5% cases it was V shaped and in 3% cases it was irregular in shape. We did not find T or bow shaped hilum in our study as was reported by Ungor et al<sup>9</sup> in 4.2 percent cases.

Saheb et al [10] reported morphometric data of fetal spleen and found average length, width and thickness to be 1.70 cm, 1.08 cm and 0.8 cm in 12 to 24 weeks fetuses, and 2.53 cm, 1.64 cm and 1.0 cm in 25 to 36 week fetuses. Ramanujam et al [11] reported length, width and thickness as 1.4 cm, 1.1 cm and 0.8 cm in 12 to 24 week fetuses and 2.4 cm, 1.5 cm and 1.0 cm in 25 to 36 week fetuses. We observed almost similar findings in our study. Marecki [12-15] studied unpreserved fetuses and reported higher values of dimensions of fetal spleen as compared to our study probably because of fresh fetuses. On comparing our results with ultrasonographic studies on live fetuses and newborns<sup>5,7</sup> just after delivery, our values in terms of length were smaller probably because of relatively smaller fetal age in our study. Jephtha et al reported the length of spleen at 6<sup>th</sup> week of gestation to be 10 mm [16]. We did not include less than 12 week fetuses in our study.

**Table 4:** Comparison of mean weight of fetal spleen (in grams).

Gestational age (in weeks)	Present study	Ungor et al [9]	Hansen et al [16]	Singer et al [17]
12-16	0.065	0.074 (14 wks)	0.09 (14 wks)	NA
>16-20	0.461	0.26 (18 wks)	0.36 (18 wks)	0.38 (18 wks)
>20-24.	0.58	0.91 (24 wks)	1.24 (24 wks)	1.3 (24 wks)
>24-28	0.99	1.67 (26 wks)	NA	2 (26 wks)
>28	1.91	1.87 (29 wks)	NA	3.8 (29 wks)

When weights of the spleen observed in our study were compared to published studies we observed almost similar values as found in other studies [17,18] (Table-4). Slightly lower weight observed in our study could be due to longer periods of preservation.

## CONCLUSION

There is limited research, specially from Indian subcontinent, on spleen of dead human fetuses. That makes our study all the more relevant and important. The knowledge of human foetal spleen is very helpful to paediatric clinicians because of its several erythropoietic and lymphopoietic functions in children. The importance of such studies is that it not only enhances the fundamental knowledge of fetal spleen but also furnishes many insights on splenic disorders. Previous studies<sup>9</sup> suggested that the data about the dimensions of fetal spleen is valuable for the detection of splenic pathologies in utero. We hope that the morphometric data acquired in our study will be beneficial for the evaluations of fetal spleen.

**Conflicts of Interests: None**

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