



# Anatomical and Histological Study of Neonatal Human Spleen

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## Abstract

The spleen is the human body's largest lymphoid organ. The appearance of the spleen is inflated by a variety of conditions, such as cell abnormalities that lodge within the spleen or storage function disorders. As a result, the spleen's size can be used as a predictor of illness severity. Place and period of study: From July 2019 to May 2020, all of these samples were taken from autopsied bodies in the mortuary room of Kirkuk forensic medicine and the institute of forensic medicine in Baghdad, with legal authority. Study design: Cross-sectional descriptive study. Materials: The current study used 20 autopsy neonate human spleens ranging in age from 1 to 28 days that were collected from available dead undergoing post-mortem examination, were studied macroscopic and microscopic after staining with Hematoxylin and Eosin. Results: The number of notches in this study ranged from zero to five, however the top score of the specimens had one or two notches. The accessory spleen, on the other hand, was not found in the area the spleen's hilum. From the first day forward, the characteristic lymphoid follicle or white pulp could be recognized. During this time, nucleated red blood cells were observed. Purpose of study: Because no data on the standard dimensions of the normal spleen exists in the Iraqi population, the findings of this study may reveal differences in spleen morphology and provide valuable data for parameter standardization, It will be useful for correct clinical diagnosis and treatment of disease by physicians, surgeons, radiologists, and anatomists.

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**Key Words:** Spleen, Splenic Capsule, Lymphoid Organ, Splenic Notch.

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## Introduction

A detailed understanding of the anatomic and histologic properties of any bodily organ or system is fundamental.<sup>(1)</sup> Spleen is the major single mass, encapsulated, secondary lymphoid organ with dual function; immunological and hematopoietic at the same time. It is an intraperitoneal organ with two surfaces: visceral and diaphragmatic. It has superior and inferior borders and anterior and posterior poles or extremities.<sup>(2)</sup> Because of the stomach's replacement, the spleen was shifted to the left side of the abdominal cavity. It's positioned between the fundus of the stomach and the

diaphragm (medial 1/3) in the upper left quadrant of the abdominal cavity.<sup>(3)</sup>

Normally, the spleen is not palpable, but splenomegaly can be caused by a variety of diseases, such as kala-azar, malaria, or a dysfunction of the spleen's storage function, such as leukemia or sarcoidosis. During deep inspiration, it magnifies more than double or triple times, and the superior border of the spleen is clinically tangible at the left costal edge.<sup>(4)</sup> The notch on the spleen's superior border helps to distinguish it from other abdominal organs.

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As a result, differences in the number and location of notches may make it difficult to diagnose an enlarged spleen clinically. The number of notches may vary from one to six. More freshly, a case where one spleen had seven notches.<sup>(5)</sup> The spleen is the human body's most vascular organ, and it is involved in the calculation of circulating blood capacity. Every day, 350 liters of blood pass through it. The spleen receives approximately 5% of the cardiac output and 40% of the blood in the portal circulation. At any given time, the spleen contains around one unit of blood, 25% of the body's total lymphocytes, 30 to 40 ml of mature red blood cells, and 1/4 of the circulating platelets. The spleen's main role is to filter undesirable particles from the blood via phagocytosis.<sup>(6)</sup> The spleen has a variety of shapes, ranging from a somewhat curved wedge to a domed tetrahedron. The size and weightiness of the spleen change with age and in different people, as well as in the same people under different circumstances. In adults, it's normally 3-4 cm thick, 7 cm wide, and 12 cm long. The spleen has a steady weight of about 150 grams, with a usual range of 80 to 300 grams depending on the amount of blood it contains.<sup>(7)</sup>

The spleen forms at the end of the fourth week of intrauterine life from mesenchymal cells termed splenunculi, which multiply and connect between the two leaves in the cephalic section of the dorsal mesogastrium to form lobulated spleen, which is then merged with the spleen at a later stage. Single or double notches emerge close the lateral termination of the superior boundary of the spleen due to the fusion of multilobular masses, indicating lobulated development of the spleen in the embryonic phase.<sup>(8)</sup> When encapsulated splenic masses fail to fuse, auxiliary spleen can form in the gastro-splenic ligament or greater omentum, and infrequently in the left spermatic cord.<sup>(2)</sup> The spleen manufactures erythrocytes while still in the fetal live and lymphocytes after birth. The spleen is the midpoint of the immune system, where both B and T cells grow and play a key role in immunological responses. The spleen is the only organ in the body that can initiate an immune response to antigens found in the bloodstream (but are not present in the tissues).<sup>(9)</sup>

The trabeculae emerge from a capsule that surrounds the spleen. The red pulp is the reticular meshwork perfused with arterial blood, whereas the clean blood is concerned with. It clears the blood of germs, antigens, platelets, and aberrant or old erythrocytes. The white pulp, which is mostly

lymphatic tissue, is the immunological component of the spleen. The White Pulp is a lymphoid organ involved in immunological protection against blood-borne antigens. The spleen's haematopoietic function is maintained throughout the fetal period and declines after delivery, however lymphocyte production continues after birth. Because splenic macrophages consume any bloodborne antigens, the spleen is known as the "graveyard of red blood cells."<sup>(10)</sup> However, due to the wide-ranging of morphological and haematological immunological roles, a study of its morphology is still required. Furthermore, for any clinical disorders of the spleen, doctors and surgeons require valuable information on morphological changes.<sup>(11)</sup>

### Summary of Materials and Methods

This study was prepared on 20 neonate cadaver with age ranging from 1-28 days, 2600-5100 grams body weight, 450-540 mm crown heel length, 310-330mm crown rump length. All these samples were collected from autopsied bodies from the mortuary room in Kirkuk forensic medicine and from institute of forensic medicine in Baghdad under legal permission from July 2019 to May

2020. Without detecting the sexual dimorphism, <sup>53</sup> spleens from both genders were combined and a statistical analysis was performed. After exposing the spleen were examined with naked eye in detail for relations shapes, sizes, borders, surfaces, hilum and anatomical position were determined. The spleen was then extracted and embedded for one to two weeks in a 10% formalin solution.

Morphological measurements: A sliding vernier caliper was used to take linear measures in cm of 20 newborn spleens. On the spleen, the following morphometric measurements were taken: We considered the maximum distance between two extremities of the spleen as its length. The depth was the greatest distances between diaphragmatic and visceral surfaces of the spleen. The breadth was recorded as the greatest distance between two points at the same level on the superior and inferior borders. Millimeters were used for all measurements. The spleens' weight was measured in grams using an automated device. Histologically the light microscope (Olympus, made in Tokyo, Japan) which was used for the microscopic description. All the variables were studied under low power objective (X 10 objective). statistical processing of data collected from the anatomical measurements were processed and statistical



analyses were done by one-way A NOVA test, using the SPSS 21 version.

### Results

In 20 formalin-fixed tissues, the morphology of the spleen was investigated. The spleen was intraperitoneal and situated in the left hypochondrium in all neonates except at the hilum. Spleen has been observed in various shapes, two poles, three boundaries, and two surfaces. Its visceral surface, on the other hand, had an uneven morphology that was generated by the pressure induced impression by surrounding structures (Figure1). The stomach and spleen were in contact in all of the cases, with the spleen being present left and posterior to the stomach. The left colic flexure of the large intestine met with the spleen, which was located postero-superior to the left colic flexure. In all cases, the left kidney and left suprarenal gland were in close proximity to the spleen, with both being located inferior, posterior, and medial to the spleen. The diaphragm was always adherent to the spleen and had an uniform morphology above and lateral to the spleen. The tail of the pancreas was in interface with the spleen's visceral surface. In all of the instances, the Phrenicocolic, gastrosplenic, and splenorenal ligaments were present. Several irregularly spaced openings for the entrance and outflow of arteries and nerves have been bored into the hilum of the spleen. Spleens were wedge-shaped in 44 percent of cases, tetrahedral in 24 percent of cases, and triangular in 32 percent of cases. The number of splenic notches found on the upper border ranges from 0 to 4, but the majority of spleens had 1 to 2 splenic notches. Splenic notches were discovered on the superior border in 76 percent of the specimens, although they were also observed on the inferior border in 24 percent (figure 2). Total length of neonate spleen was  $31.81 \pm 1.46$  mm, width was  $20.35 \pm 1.28$  mm, thickness was  $10.28$

$\pm 0.43$  mm. The average weight of the spleens was  $4.09 \pm 0.22$  gm (figure 3)

The serous [peritoneal] coat of mesothelium was discovered to have a single layer of squamous cells covering the splenic capsule. Trabeculae were found distributed throughout the spleen's material. They have arteries and veins that supply nutrients to the splenic tissue. In the capsule and trabeculae, connective tissue fibers and fibroblasts were found. The splenic capsule had very few smooth muscle cells, but the trabeculae had clear, distinguishable

smooth muscle cells interspersed between the fibers.

The red pulp was made up of thin collagen fibers that were continue with trabeculae and capsule collagen fibers. Fibroblasts, fibrocytes, and blood cells were found in the red pulp's interstices. At this stage, the spleen has a structure that is nearly identical to that of an adult spleen. The lymphoid follicle's periphery revealed a central arteriole. In the red pulp, there were many sinusoids. There are a lot of primary lymphoid follicles that don't have germinal centers.

The splenic tissue of neonates looked to be homogeneous, with a distinct multiple white pulp component. The lymphoid tissue that surrounds the splenic arterial tree makes up the splenic white pulp. Within the splenic material, the principal arterial branches split into arterioles. These arterioles have a peri-arteriolar lymphatic sheath (PALS), which was made up of lymphocyte aggregations. Lymphatic nodules, also known as Malpighian bodies, are formed by dense aggregations of lymphocytes in relation to eccentrically situated central arterioles. A pale staining germinal core encircled by a cuff of closely packed lymphocytes, known as the corona or 54 mantle zone, was seen in certain follicles. The germinal centers are clusters of bigger, lighter-stained lymphocytes that are actively dividing.

Smaller, mature, heavily stained lymphocytes made up the rim of lymphocytes in the mantle zone. The red pulp's peripheral zone appeared more cellular than the white pulp, although it had less cellularity than the white pulp. Furthermore, the connective tissue framework in this location was denser than in other parts of the body. The marginal sinus, an irregular capillary gap, separates the marginal zone from the follicles. A peri-follicular zone could be identified around the marginal zone of several follicles, which looked to be more cellular than the red pulp region.(Figure 4)

**Table 1.** Caparison the variation of notches in superior and inferior border

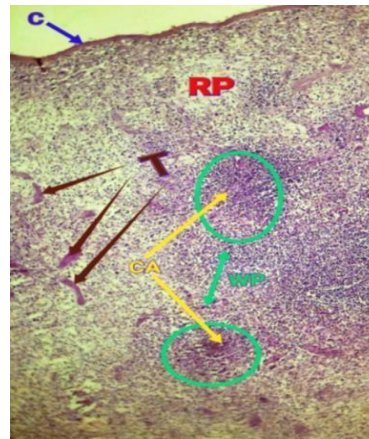
	<b>Girish V.et al.(20)</b>	<b>R. Siva Chidambaram et al.(5)</b>	<b>Ray SK. et al.(19)</b>	<b>Present study (2021)</b>
Superior border	95%	63.35%	70%	76%
Inferior border	3.3%	10.8%	8%	24%





**Table 2.** Comparison the shape of spleen with present and previous studies

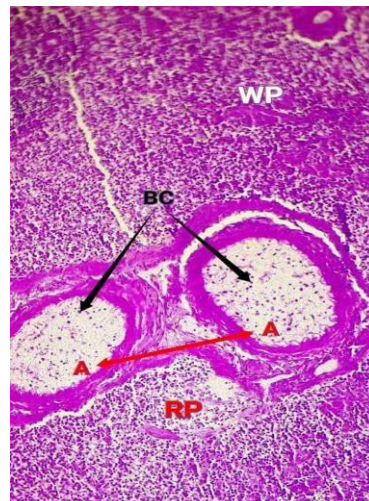
Studies	Shape of spleen		
	Wedge	Tetrahedral	Triangular
Hollinshed W. et al. (21)	44%	14%	42%
Ray SK. Et al. 2016 (19)	46%	38%	16%
Present study 2021	44%	24%	32%



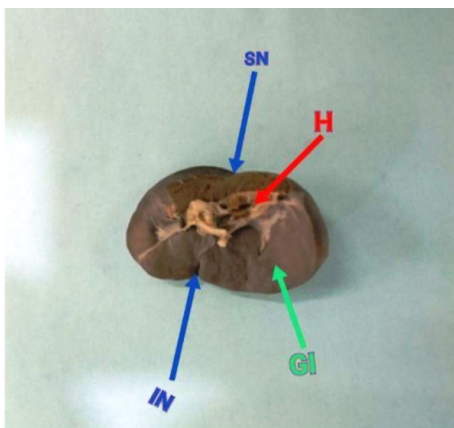
**Figure 4.** Histological section of neonatal spleen section show C Capsule, T Trabeculae of spleen with smooth muscle cells and connective tissue fibers. RP Red Pulp, WP White Pulp, CA Central Artery. Hematoxylin and Eosin 10X



**Figure 1.** Show spleen related to viscera in abdominal cavity



**Figure 5.** Histological section of Neonatal spleen section show Artery A, contain large amount of blood cells BC, in addition to white pulp WP, red pulp RP, Hematoxylin and Eosin 10X



**Figure 2.** Visceral surface of spleen show superior notch (SN) hilum (H), gastric impression (GI) inferior notch



**Figure 3.** Measurement width of spleen, using digital vernier caliper

### Discussion

The function of the spleen in connection to immunological and cytological activity, particularly in terms of RBC storage and blood filtration potential, makes it a vital organ. (12) According to a review of the literature, there are few research on the location and visceral relationships of the neonatal spleen. All spleens were found in the left hypochondrium of the abdomen, according to research by (13,14). Wholly of the spleens in our investigation were intraperitoneal and placed in the left hypochondrium. The adult trend was followed in this study when it came to the relationship of the spleen with nearby viscera. The spleen was in direct contact with the left colic flexure. Similar to what we discovered in a study conducted by (15). large intestine was in relation

with spleen. When it came to the relationship between the spleen and the pancreatic tail, Vashishtha<sup>(16)</sup> found that the pancreatic tail was in contact with the spleen in all of the cases they studied. The tail of the pancreas came into contact with the spleen in our research.

Shaik Hussain and his associates<sup>(13)</sup> for gestational age 12 to 24 weeks, the average length, thickness and width of the fetal spleen were 1.7cm, 0.8cm, and 1.1cm, respectively; for gestational age 25 to 36 weeks, 2.5cm, 1.0cm and 1.6cm; and for gestational age greater than 36 weeks, 2.7cm, 1.0cm, and 1.7cm respectively. All of our previous measurements reliable backup our conclusion. In 25 to 36 week fetuses<sup>(14)</sup> observed length, breadth, and thickness of 2.4 mm, 1.5 mm, and 1.0 mm, respectively, and weight of 1.91gm >28. When the weights of the spleen recorded in our investigation were compared to published studies, we discovered almost acceptable values of 3.8 gm when taking into account the age of our study and the studies closest to it.<sup>(17)</sup> We discovered that spleens come in a variety of shapes, sizes, and weights in recent investigations.<sup>(18)</sup> This could be due to a number of factors, including genetics, geographical conditions, food habits, financial status, and physical characteristics.

The splenic notches were discovered on both the superior and inferior borders in this study. The number of notches ranged from zero to four, although most usually only one or two were present. The occurrence of splenic notches in the superior and inferior borders has been previously described by several authors (see table 1).<sup>(5,19,20)</sup> This variation in the existence and number of notches may be explained by the spleen's developmental basis. In mature spleens, lobulation is indicated by notches on the borders, which are typically visible on the superior and inferior borders.

The morphology of the spleen was observed in the current investigation. Wedge, triangular, and tetrahedral are the three different shapes. The proportion of various spleen forms was found to be similar in our human spleen investigation (see table 2).<sup>(19,21)</sup> The indentations of the organs that are adjacent to the spleen, such as the stomach, colon, pancreas, and kidney, cause variations in the spleen's morphology.<sup>(5)</sup>

The structure of the spleen is similar to that of an adult. In this investigation, the splenic capsule appeared to be made up of sparsely distributed collagen fibers with an exterior layer of flattened

cells with round to oval nuclei. The presence of trabeculae is confirmed by.<sup>(22)</sup> The RBCs and sinusoids were clearly visible in the red pulp, and sinusoids, as well as lymphocyte aggregations in the white pulp, were observed to be denser and more distinct from the surrounding structure (Figure 5). Germinal centers began to form in different numbers from 15 days after birth onwards, as explained previously<sup>(23,24)</sup>. As well as the study by Mrinmoy Pal and team.<sup>(22)</sup> According to their findings, from the 31st gestational week onwards, well-defined lymphoid follicles or the white pulp could be seen. Similarly, germinal centers were found in all of the newborn splenic sections in the current investigation. The spleen plays an important role in immune responses because it may produce both innate and adaptive immune responses.<sup>(25)</sup> The marginal zone is involved in both innate and adaptive immunity, whereas the white pulp is restricted to being involved in adaptive immunity, through its specific macrophage populations and marginal-zone B cells.<sup>(26)</sup>

## Conclusion

Physicians, surgeons, pathologists, and radiologists <sup>56</sup> will all benefit greatly from the outcomes of this study. It also provides a lot of information about the spleen's clinical importance.

## Recommendations

Further research with a bigger sample size in various age groups, including sexes, height, body weight, body surface area, and basal metabolic index beyond a lengthy period of time is needed.

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