

Research Article

Microscopic study of human spleen in different age groups

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ABSTRACT

Background: The microscopic structure of spleen is variable depending on the developmental stage of the organ, and the age and immune status of the individual. The aim of the investigation was to study the microscopic structure of human spleen in different age groups, starting from a six month old foetus up to the eighth decade of life.

Methods: Seventy formalin fixed human spleens obtained postmortem, were included in the study. They were classified into different age groups, in both sexes, for a detailed study of the microscopic details.

Results: The white pulp of spleen showed peri-arteriolar lymphatic sheath (PALS) and lymphatic follicles. The corona or mantle zone and the germinal centre were discernible in many of the Malpighian bodies. The marginal zone separating the red pulp from the white pulp also could be clearly demarcated. The marginal sinus and peri-follicular zone could be seen in some sections only. The capsule thickness, trabecular network, cellularity of white pulp and red pulp, the connective tissue framework seen in the red pulp etc., showed variations in the different age groups.

Conclusion: The microscopic structure of spleen varies in different age groups, with the PALS and the white pulp showing scanty cellularity in the six month foetus, and almost uniform cellularity in all areas of spleen at full term. Thereafter the follicles showed increase in its cellularity up to the third decade, and then seemed becoming progressively atrophic. Further studies are required on age related changes in the cellular architecture of this organ correlating with its functions.

Keywords: Spleen microscopy, PALS, Malpighian body, marginal zone, mantle zone

INTRODUCTION

The spleen is a secondary lymphoid organ, with variable cellular composition. Splenic parenchyma is divided into the white pulp and the red pulp, distinguishable by their colour in fresh sections of the organ. The white pulp contains dense and highly organized accumulations of B and T lymphocytes around arterioles, while most of the red pulp contains blood-filled spaces with splenic sinuses connecting arterioles and veins, and splenic cords which are strands of loose connective tissue filled with all types of blood cells, macrophages and plasma cells. Like all other lymphoid organs in humans, the spleen also shows

variations in microscopic structure with age. Not many studies on the age changes in microscopic structure of the organ have been reported in literature.

METHODS

The study was done on formalin fixed post-mortem spleens procured in the Department of Anatomy in Government Medical College, Kottayam and Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala, India. Seventy apparently normal spleens belonging to both sexes were selected for the study. The specimens were grouped according to their age groups, and after

morphological examination the specimens were subjected to microscopic study using Haematoxylin and Eosin stains. The tissues were examined for a detailed microscopic examination with special reference to the various features in different age groups.

RESULTS

The spleen specimens obtained post-mortem were grouped according to the age of the individuals, ranging from a six month old foetus to the eighth decade of life. The observations in the Haematoxylin and Eosin stained sections were grouped under the following headings.

A. Capsule and trabeculae

The thickness of the splenic capsule in the various age groups was determined with the help of an ocular and a stage micrometer, from the outer margin of the capsule to its inner margin at three different points. The average values were taken [0.24 ± 0.20 mm] and the results were tabulated (Figure 1). The thickness in a six month old foetus was found to be 0.02 mm (Figure 2). It then increased gradually to 0.50 mm in the sixth decade and then decreased to 0.16 mm in the eighth decade. The splenic capsule was found to have an outer covering of a single layer of squamous cells, the serous [peritoneal] coat of mesothelium, deep to which lies the dense irregular connective tissue layer (Figure 3).

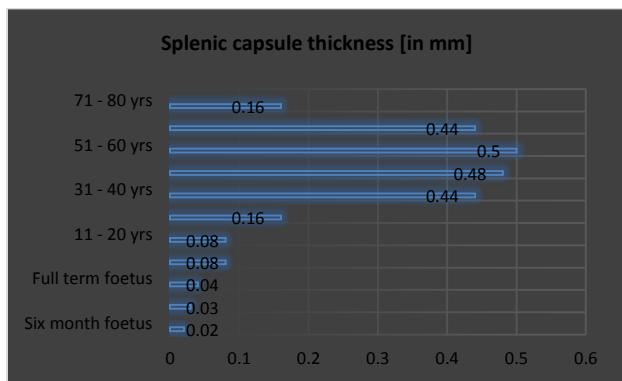


Figure 1: Thickness of spleen capsule in various age groups.

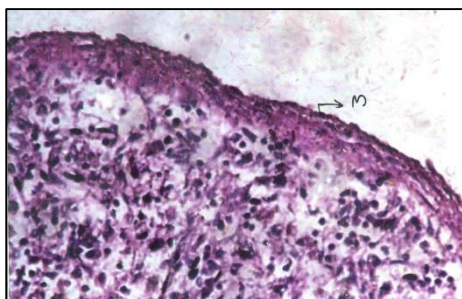


Figure 2: Capsule of spleen in the foetus – microscopy.

Trabeculae were seen scattered throughout the substance of the spleen. They contain arteries and veins which nourish the splenic tissue. Closely arranged connective tissue fibres and fibroblasts were seen in the capsule and trabeculae. Very few smooth muscle cells were identifiable in the splenic capsule, but the trabeculae contained definite, discrete smooth muscle cells interspersed between the fibres (Figure 4).

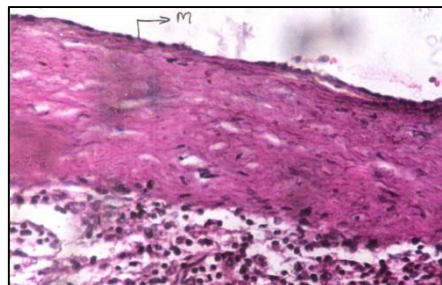


Figure 3: Capsule of spleen in an adult – microscopy.

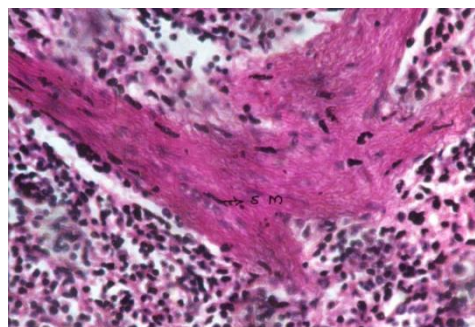


Fig. 4: Trabeculae of spleen showing smooth muscle cells and connective tissue fibres.

A more or less well developed connective tissue framework was observed in the spleen of full term foetuses. By the time the individual is about five years old, the trabecular framework appeared almost well formed. As age advanced the trabeculae become thicker and dense, occupying a major part of the section.

B. White pulp

The splenic white pulp is composed of lymphoid tissue [both T and B lymphocytes] around the splenic arterial tree. The primary arterial branches divide into arterioles within the splenic substance. These arterioles showed a *peri-arteriolar lymphatic sheath (PALS)*, composed of aggregations of lymphocytes, which are mostly T lymphocytes (Figure 5).

Dense aggregations of B lymphocytes are seen in relation to the eccentrically placed central arterioles, forming lymphatic nodules, otherwise called *Malpighian bodies*. Some follicles showed a pale staining *germinal centre*, surrounded by a cuff of closely packed lymphocytes, known as the *corona or mantle zone*. The germinal centres are areas where the larger lighter stained lymphocytes are found to divide actively. The rim of

lymphocytes in the mantle zone was composed of smaller, mature, densely stained cells.

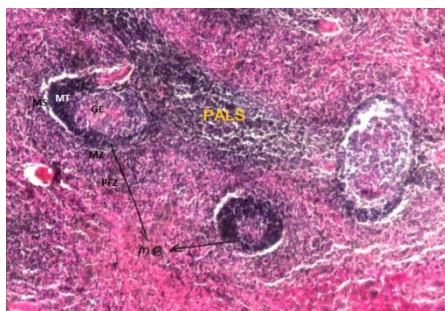


Figure 5: Elongated PALS and Malpighian bodies in relation to central arterioles.

GC: Germinal centre, MT: Mantle zone, MS: Marginal sinus, MZ: Marginal zone, PFZ: Perifollicular zone

The arterioles seen in the white pulp showed variations in their numbers and caliber. More than one arteriole was frequently present in many follicles, with the numbers going up to five or six in some of them. The *marginal zone* which marks the border between the red pulp and white pulp appeared more cellular than the red pulp, but the cellularity was less than that of white pulp. Also, in this region the connective tissue framework was denser than elsewhere. The marginal zone is separated from the follicles by an irregular capillary space, *marginal sinus*. A *peri-follicular zone*, which seemed relatively more cellular than the red pulp region, could be identified around the marginal zone of many follicles (Figure 5).

The developing lymphoid tissue in the foetus, and the changes in various age groups of postnatal life were studied in detail. The earliest feature observed during development was the appearance of localized areas of few scattered lymphocytes in the mesenchymal tissue, in relation to eccentrically placed central arterioles, clearly seen in the spleen of a six month old foetus (Figure 6).

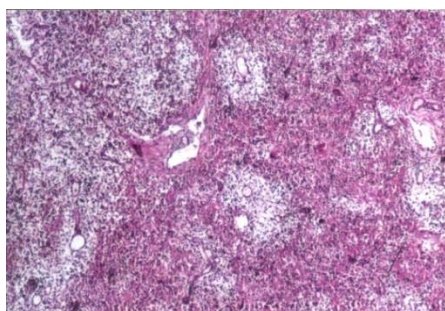


Figure 6: Primordia of lymphoid follicles at six months. Eccentrically placed central arterioles seen clearly

At this stage the developing white pulp seemed to be less cellular than the red pulp. The follicles appeared smaller in size, and more than one arteriole was seen in most of the follicles. The shape of the follicles varied from near round to oval in the foetal group.

As growth progressed the follicles enlarged and became more cellular. In the eight month old foetus the cellularity of the red pulp and white pulp appear almost similar. The two could be distinguished only by the eosinophilic dense connective tissue framework of the red pulp. In the full term foetus the white pulp cellularity seemed further increased. But a germinal centre was not discernible (Figure 7).

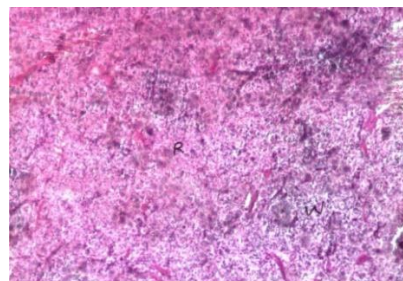


Figure 7: Lymphoid follicles in white pulp clearly made out in a full term foetus.

In the first and second decades of life the white pulp showed typical *primary* and *secondary lymphoid follicles* [primary follicles do not have clear germinal centre, but secondary follicles have] (Figure 5). From the third decade onwards the germinal centres became less distinct. Also the follicles seemed to be larger than in the younger age group, but fewer. From the fourth decade onwards the cellularity of the follicles in the white pulp gradually decreased. By the seventh decade the cellularity seemed very much decreased in both red pulp and white pulp. The follicles seemed totally atrophied by the eighth decade (Figure 8). The shapes of many of the follicles also was seen to become irregular by aging.

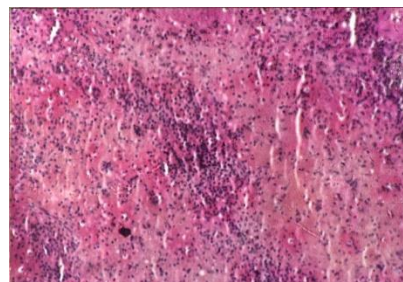


Figure 8: Atrophic splenic tissue in 8th decade; reduced cellularity and vascularity

The total number of follicles in ten low power fields put together were counted in the various age groups, and studied (Table 1). The numbers were highest in the eight month old foetus, and steadily declined with advancing age, the average being 24 ± 14.6 . So also the maximum diameter of the follicles was measured as an average of the diameters of five largest rounded follicles in each age group, and correlated with the number of follicles. The average maximum diameter of the follicles was $117.5 \mu\text{m} \pm 32.5$ considering all age groups.

A correlation of the maximum diameter of the follicles with the number of follicles in ten high power fields was done [$p < 0.05$ was significant], and it was seen that the more their diameter, the lesser the number of follicles (Figure 9). There is a linear correlation between the two variables.

Table 1: No. of follicles in 10 HPF and their maximum diameter.

Age group	No. of follicles in 10 HPF	Max. follicle diameter [μm]
8 month IUL	54	82
Full term foetus	47	96
0-10 years	28	126
11-20 years	18	153
21-30 years	16	188
31-40 years	16	128
41-50 years	15	112
51-60 years	18	101
61-70 years	16	97
71-80 years	13	92

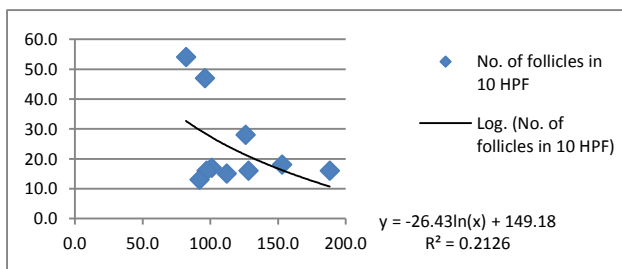


Figure 9: Correlation of the number of follicles to their number in 10 HPF – Logarithmic.

C. Red pulp

The framework of the red pulp (Figure 10) was found to consist of a mesh of connective tissue fibres which were continuous with the collagen fibres of the capsule and the trabeculae. The red pulp was permeated by narrow passages which drain into *venous sinusoids/ sinuses*. These were lined by long, narrow, endothelial cells called *stave cells* which run longitudinally along the sinusoidal wall. The red pulp tissue between the adjacent sinusoids resembled cords of cells, termed *splenic cords/ pulp cords of Billroth*. The cords contained blood cells and lymphocytes. In a random section, the sinusoids and pulp cords get cut in various planes.

DISCUSSION

Developmentally spleen has a dual origin, i.e., from a thickening in the coelomic epithelium, and from the underlying angiogenic mesenchyme of dorsal mesogastrium.¹ The organ has a variable cellular composition with a large proportion of migrating lymphocytes from the blood settling down here for some time, and leaving again into the circulatory system, in a

process of lymphocyte recirculation. This supports the detection of antigens in spleen and the consequent spread of immune responses in the body.² The lymphoid tissue of the white pulp has B and T lymphocytes which proliferate under antigenic stimulation. The red pulp has a filtration mechanism which enables the spleen to clear particulate matter as blood traverses it. The complex system of interconnected spaces of red pulp, with a large number of phagocytic macrophages, remove damaged RBC, micro-organisms, cellular debris and other particulate matter.³

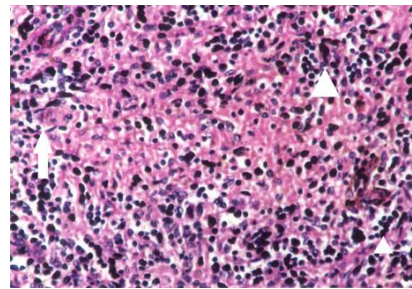


Figure 10: Red pulp with sinusoids lined by stave cells [arrow], splenic cords [arrow head].

Foetal spleen

The splenic capsule in a six month foetal spleen in the present study seemed to be composed of sparsely arranged collagen fibres with an outer lining of flattened cells with round to oval nuclei. The capsule in the earlier stages, i.e., in tenth week has reportedly shown a single layer of columnar cells lining the outer surface of the fibrous capsule.⁴ The primary reticular framework of spleen with vascular loops is reportedly laid down by about eight to nine weeks of development.⁵ The development of white pulp begin with colonization of the organ with lymphocytes, which begins as their accumulation around the central arterioles by the end of fifth month. In the present study loosely arranged lymphocytes, both T and B cells, were clearly seen around the central arterioles by the sixth month. The number of arterioles in each of the developing follicles varied from 3-5. Thereafter the cellularity of both red pulp and white pulp seemed increasing, with definite Malpighian follicles at full term. In the red pulp, spaces with discontinuous epithelium, probably sinusoids, were identifiable by six months in the present study. By eight months plenty of sinusoids and splenic cords were clearly seen, in concurrence with study by Mrinmoy Pal and team.⁴ As per their conclusions, well defined lymphoid follicles, or the white pulp could be observed from the 31st gestational week onwards. So also, the splenic corpuscle has been reported to form nodular structures around the arteries, not until the 6th month of foetal life.⁶ But in the present study the Malpighian follicles of white pulp were seen to be clearly demarcated by the 24th gestational week.

Post natal spleen

Thickness of the human splenic capsule in μm (Mean \pm SD) (n=15) was found to be 111.56 ± 21.45 (74.97-139.26), according to the study by Alim A and team.⁷ But this seems to be lower compared to the present study, (0.24 ± 0.20) which studied the thickness in different age groups.

At the very same time the connective tissue capsule as described in Gray's Anatomy is a continuous layer approximately 1.5 mm thick,³ containing abundant collagen fibres and some elastin fibres. This capsule has an outer and inner lamina, in which the direction of collagen fibres differ, thereby increasing its strength. In the splenic capsule stained by special stains in the present study, in addition to these two laminae with difference in direction of fibres, it was seen that the inner lamina contains more elastic fibres compared to the outer lamina, which was comprised mostly of collagen fibres.

Diameter of white pulp in mm (Mean \pm SD) as per the same study⁷ was 0.32 ± 0.01 (0.30-0.34). This seems grossly different from the mean diameter of the follicles in the present study, probably because the follicles in younger and older age groups also were included. Human splenic follicles have a globular egg-shaped form.⁸ The arterial tree in human spleens is highly branched. Larger arterioles are associated with a broad PALS, while the smaller vessels have a thinner layer of T cells. The most conspicuous compartment of the white pulp are the follicles, which may totally interrupt the PALS. The arterioles may lose their T-cell sheath, run across germinal centres or follicular mantle zones and then regain the sheath.⁹

A continuous peri-arterial lymph sheath has been demonstrated too infrequently in the microscopic studies on spleen. In the present study, though the long arterial branch is not seen clearly in the section, a continuous elongated lymphatic aggregation is very well demonstrated [Fig. 5]. The Malpighian follicles are seen a globular or oval extensions of lymphoid aggregations of B lymphocytes, from the longitudinal T lymphocyte accumulation. The B lymphocyte aggregations lie around a branch of the central arteriole, which thus becomes eccentric.

The lymphoid follicles in the white pulp have also been reported as to be 0.25 to 0.01 mm in diameter.³ This is in variance to the observations of the present study, where the maximum diameter of a follicle was found to be 0.188 mm. Most of the T cells in the PALS are CD4+, while CD8+ T cells form a smaller population.¹⁰ When antigenically stimulated, the white pulp increases in size by B cell proliferation, and develop germinal centres similar to lymph nodes. The germinal centres regress when infection subsides³ and their existence is always of limited duration.

The closely packed smaller lymphocytes which lie around the germinal centre constitute the corona or mantle zone. Marginal zone lies at interphase between red pulp and white pulp.³ From here blood is delivered into the red pulp, where many lymphocytes leave the circulation to migrate into the T or B lymphocyte areas. In humans, there is a perifollicular zone surrounding the surface of primary or secondary follicles. It is composed of small blood-filled spaces without endothelia, similar to the red pulp. However, it contains much more conspicuous accumulations of erythrocytes, granulocytes and monocytes than the remainder of the red pulp.¹¹

The PALS and the follicles are surrounded by the marginal zone, which separates both compartments from the splenic red pulp. It is a broad region primarily occupied by relatively large memory B cells. It gives a lighter staining, because memory B cells have paler nuclei and more cytoplasm than the T cells of the PALS or the B cells of primary follicles.¹² The marginal zone is delimited from the PALS and the follicles by a very irregular capillary blood vessel called the 'marginal sinus'.¹³ According to Kraal G, in humans, there is no clear cut border between the mantle zone and the MZ, because a marginal sinus is absent.⁸

The splenic cords represent the 'open' part of the splenic circulation.¹⁴ They contain large stellate fibroblasts [reticular cells], lying around the sinusoids, lymphocytes, plasma cells, macrophages, granulocytes, red cells and thrombocytes. The splenic sinuses are regarded as the 'closed' part of the splenic vasculature.¹⁴ Blood percolates through these reticular spaces between the splenic cords, where the phagocytic function is partly carried out.

Role of spleen in immune responses: In the spleen, both innate and adaptive immune responses can be efficiently mounted.¹⁵ Whereas the white pulp is restricted to being involved in adaptive immunity, the marginal zone is involved in both innate and adaptive immunity, through its specific macrophage populations and marginal-zone B cells.^{16,17}

Any number of molecular studies regarding the functional aspects of the cellular components of spleen are available in literature. At the same, the accelerated growth in the younger age groups and the atrophy of splenic tissue after the individual reaches adult status has not been studied extensively.

CONCLUSIONS

The splenic white pulp showed PALS and lymphatic follicles, with the mantle zone and the germinal centre distinguishable in many of them. The marginal zone, marginal sinus and peri-follicular zone could be seen in some sections only. The capsule thickness, framework of trabeculae, and the cellularity of white pulp and red pulp

showed variations in the different age groups. The histology varies in depends on the age group studied. Further microscopic and physiological studies are required on the age related changes in the cellular architecture of this organ.

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