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Age Related Histo-morphological changes of Splenic connective Tissue in Goat *(Capra Hircus)*

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Abstract

The present study was conducted on 40 spleen samples of goat from prenatal to four months and above age. The small pieces from spleen were processed for histo-morphological study. The general histo-architecture of spleen showed capsule, trabeculae, red pulp and white pulp in all age groups. The splenic capsule was found to be composed of outer fibrous layer and inner muscular layer. The thickness of capsule was gradually increasing due to increase in thickness of muscular layer with the advancement of age. The components of outer fibrous layer were wavy and intermingled with collagen, reticular and elastic fibres. The elastic fibres however, became short and fragmented with advancement of age. Variation in the concentration of these fibres in capsule with advancement of age was necessary to provide firmness to the organs from outside during postnatal period and to provide elasticity of organ during prenatal and early postnatal stage. The connective tissue trabeculae radiated from capsule in to splenic pulp and supported the blood vessels. The components of trabeculae were similar to that of capsule.

Key words : Histo-morphological Changes, Spleen, Goat.

Introduction

The lymphatic organs are the specialized form of connective tissue, which contains large number of lymphocytes with reticular cells and reticular fibres. It is responsible for acquired immunity. The immune system comprised of primary and secondary lymphatic organs. Spleen is a blood-forming organ. In most of the mammals, it produces red blood cells, platelets and granulocytes during embryonic life but produces lymphoid cells throughout the life (Krause and Cutts, 1994). Spleen acts as secondary lymphoid organ, where lymphocytes respond to antigen. The lymphoid organs are present in mammals, throughout life but undergo changes with the advancement of age. The structure of the spleen and their components differ markedly in different animal species and their composition may serve a reference for the pathological conditions and may aid in diagnosis of immune deficiency diseases.

Materials and Methods

The present study was conducted on 40 samples of spleen of goat at 4 different age groups (10

spleen samples each from mid to late gestation period, kids of 0-2 months of age, kids of 2.1-4 months of age and kids of 4 months and above age). The samples were collected from healthy animals irrespective of sex from Municipal Corporation Slaughter House and carried to laboratory in ice box. To collect the sample from foeti, the gravid uteri were dissected to remove the foeti and their age was determined on the basis of crown rump length (Kadu and Kaikini, 1984). The foeti were dissected to collect the spleen. Tissue samples of 3-4 mm size were collected and fixed in 10% neutral buffered formalin for histo-morphological observations. After fixation, tissues were processed as per method suggested by Drury and Wallington (1980).

Results and Discussion

The spleen of goat was found to be composed of capsule, trabeculae, red pulp and white pulp (Fig 1). During the present study, it was observed that the spleen was ensheathed by the connective tissue capsule of uneven thickness. The capsule showed two types of layers namely, outer fibrous and inner muscular layer (Fig 2). Similar observations were recorded by Estacio *et al.* (1997) and Landsverk (1998) in domestic animals. In agreement with the present findings, Geetha *et al.* (2001) in mice, rat and guinea pig and Raju *et al.* (2003) in goat, reported that, the outer fibrous capsule

Fig.1 Photomicrograph of spleen showing
a. Capsule b. Trabeculae
c. White pulp d. Red pulp
(Haematoxylin & Eosin X 100)

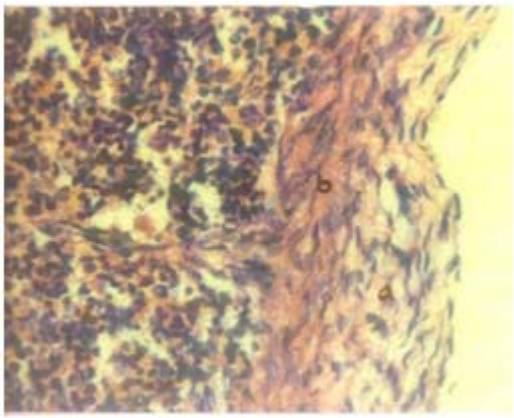

Fig. 2 Photomicrograph showing two layers of splenic capsule in group I
a. Outer fibrous layer
b. Thin inner muscular layer
(Haematoxylin & Eosin X 400)

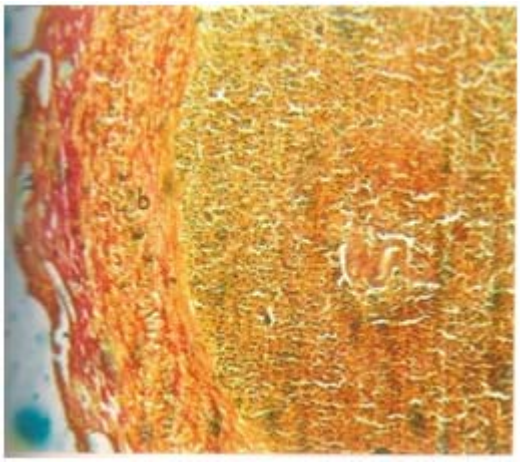

Fig.3 Photomicrograph of splenic capsule showing collagen fibers in group IV
a. Outer fibrous layer
b. Inner muscular layer
(Van Gieson's X 100)

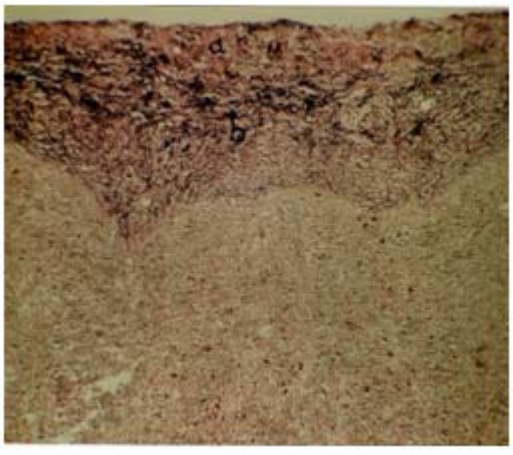

Fig.4 Photomicrograph of splenic capsule showing elasticfibers in group IV
a. Outer fibrous layer
b. Inner muscular layer
(Verhoeff's elastic stain X 100)

was composed of wavy intermingled reticular, elastic and collagen fibres with fibroblasts. It was noticed that the distribution of these fibres was unequal and varied with the advancement of age. A progressive increase in the amount of collagen fibres from prenatal to postnatal age groups (group I to group IV) was recorded. In group IV, the collagen fibres were found predominant over the other fibre types (Fig. 3). The amount of reticular fibres in the capsule, however, showed reverse trend. These fibres were predominant over other fibre type in group I. There was a progressive decrease in the size and amount of reticular fibres with the advancement of age. The elastic fibres in the outer fibrous layer of capsule showed a progressive increase in size and amount up to group III, but in group IV, these fibres appeared short and fragmented (Fig 4). These observations of the present study are in accordance with the findings reported by Geetha *et al.* (2001) in mice, rat and guinea pig. Raju et al. (2003) reported the equal amount of collagen, reticular and elastic fibres in the outer fibrous layer of capsule. Karad et al. (2002) in goat reported the presence of collagen, elastic and smooth muscle fibres in the capsule of the spleen however; they did not mention the presence of reticular fibres in the capsule of spleen.

The inner smooth muscular layer of splenic capsule was very thin in group I (Fig. 2), which later on increased in thickness with the advancement of age and became two layered during late prenatal period. These two layers of smooth muscle were oriented at right angle with each other. Similar arrangement of these two inner muscular layers was observed in group II and group III. However, in group IV, occasionally, three muscle layers were also observed (Fig. 5). These observations of the present study are on the lines of findings reported by Malik *et al.* (2001) in goat. Although, Bacha and Bacha (2000) reported similar arrangement of muscle fibres in horse and cow, they found interwoven arrangement of muscle fibres in goat. This must have appeared at the site of intersection of the muscle fibres of two layers which lie at right angle to each other.

The inner muscular layer of the capsule had collagen, elastic and reticular fibres interposed between the smooth muscle cells. A progressive increasing trend in the amount of collagen and elastic fibres, while decreasing trend in the amount of reticular fibres was noticed with the advancement of age.However, in group IV, the amount of elastic fibre was comparatively more than other fibre type.

The result of the present study pertaining to the increase in amount of elastic fibres in the capsule provide elasticity, which is necessary for enlargement of organ during its growth and its predominance in inner muscular layer in group IV may aid in function of contraction along with smooth muscle to expel the erythrocytes and to increase the number of corpuscles in general circulation. The predominance of collagen fibres in the outer layer of capsule in group IV is necessary to provide firmness to the organ after cessation of growth.

In agreement with the observations recorded in the present study, Copenhaver et al. (1975) stated that the collagen fibres passes through a developmental stage in which they are argyrophilic and identical with reticular fibres. These reticular fibres are merely an immature stage of collagen fibres and get converted into matured collagen fibres. Possibly this could be the reason for progressive increase in the amount of collagen fibres and a decrease in amount of reticular fibres in capsule with the advancement of age observed during present study.

During the present work, the average thickness of splenic capsule was recorded as 86.03±0.77 μm, 101.63±4.89 μm, 137.80±6.61 μm and 162.52±8.06 μm in group I, II, III and IV respectively. This progressive increase in the thickness of capsule showed highly significant statistical difference at 1% level with the advancement of age. Similar increasing trend in thickness of capsule was reported by Vyas and Singh (1991) in goat, Gadre *et al.* (1985) in cross bred calves, Malik *et al.* (2001) in goat, Baishya *et al.* (2002) in pig foetus and Hecser *et al.* (1992) in cattle, sheep and pig.

During the present work, it was observed that the connective tissue trabeculae radiated from capsule into the splenic pulp and supported the blood vessels. The trabeculae were few in early prenatal

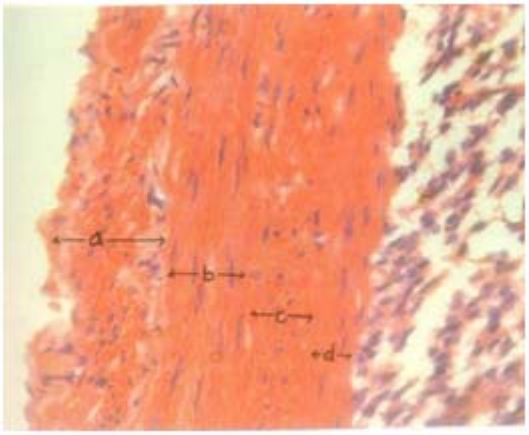

Fig.5 Photomicrograph of splenic capsule in late prenatal stage of group IV
a. Outer fibrous layer
b, c and d. Three layers of inner smooth muscular layer.
(Haematoxylin & Eosin X 400)

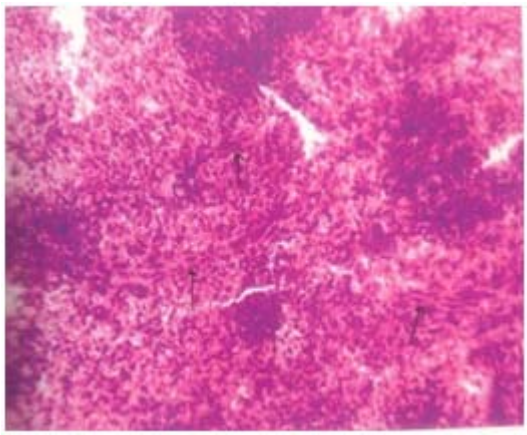

Fig.6 Photomicrograph of spleen showing few trabeculae in early prenatal age of group I (Arrow)
(Haematoxylin and Eosin X 200)

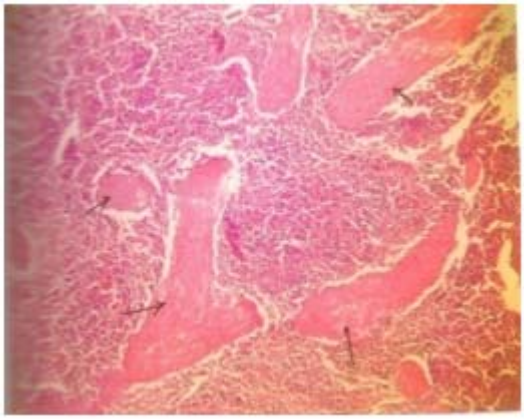

Fig.7 Photomicrograph of spleen showing well developed branched trabeculae in group III (Arrow)
(Haematoxylin and Eosin X 100)

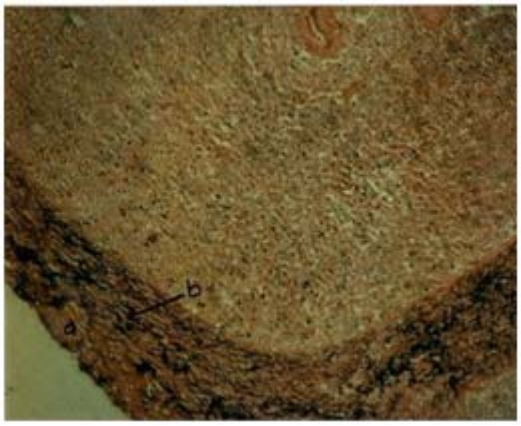

Fig.8 Photomicrograph of splenic capsule showing elasticfibers in group IV
a. Outer fibrous layer
b. Inner muscular layer
(Verhoeff's elastic stain X 100)

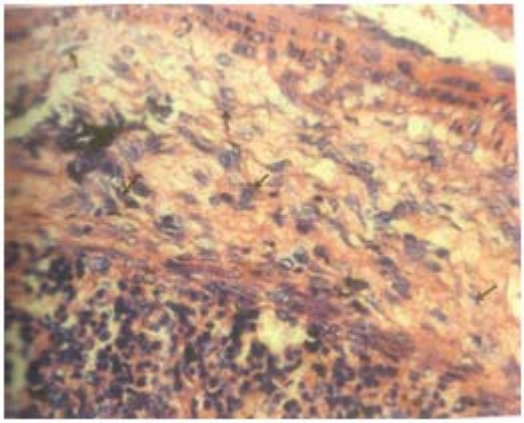

Fig.9 Photomicrograph of spleen showing numerous fibroblasts around the blood vessels in larger trabeculae (Arrow)
(Haematoxylin and Eosin X400)

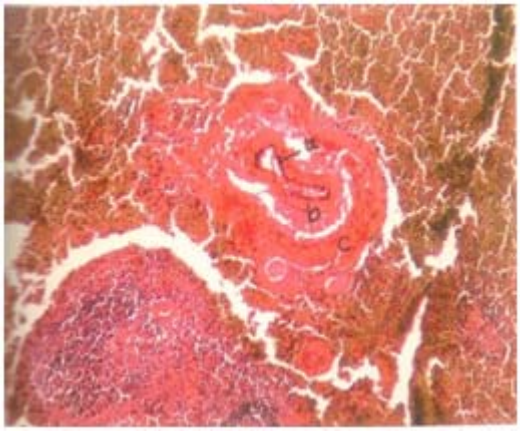

Fig.10 Photomicrograph of spleen showing distinct internal elastic lamina with few elastic fibers in the wall of trabecular vessel in group III
a. Internal elastic lamina
b. Trabecular vessel
c. Trabecula
(Verhoeff's elastic stain X100)

age (Fig 6), but their amount and size increased and became branched with the advancement of age (Fig 7).

The reticular fibres in the trabeculae were wavy, intermingled and predominant than the collagen and elastic fibres in the group I, which later on decreased progressively with the advancement of age. However, the collagen fibres and elastic fibres in the trabeculae showed progressive increase in their amount with the advancement of age (Fig. 8).

It was observed that the smooth muscle cells were the major component of splenic trabecule, but their amount was more especially in the group III and group IV. These observations of the present study are in agreement with Baishya *et al.* (2002) in cross bred pig foetuses and Malik *et al.* (2001) in prenatal goat. They mentioned that the muscular component and trabecular thickness and network increased with the advancement of age.

Copenhaver *et al.* (1975) stated that predominancy of elastic fibres and smooth muscle cells in the trabeculae enable spleen to make rapid change in volume and in contraction to expel erythrocytes and increase the number of these corpuscles in the general circulation.

In all age groups of animals, it was observed that, the vessels entered in the splenic pulp and were found supported by the trabeculae. The largest vessels were observed near the hilus. This finding is similar to those recorded by Landsverk (1998), Trautmann and Fiebiger (2002) in domestic animals, Karad *et al.* (2002) in goat, Faroon and Henry (1988) in sheep.

The larger trabeculae showed numerous fibroblasts around the blood vessels (Fig. 9). The trabecular vessels showed general histological plan of their wall having distinct internal elastic lamina with few elastic fibers in their wall (Fig. 10).

The sections stained with Gomori's reticulin stain, showed the network of reticular cells and reticular fibres throughout the splenic pulp in all age groups of animals. This finding is in agreement with that of Landsverk (1998), Trautmann and Fiebiger (2002) in domestic animals, Faroon and Henry (1988) in sheep, Tetsuo *et al.* (1992) in doe and Gadre *et al.* (1985) in calf.

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Conflict of Interest: All authors declare no conflict of interest.

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