GROSS ARCHITECTURAL AND HISTOMORPHOMETRICAL STUDIES ON THE SPLEEN OF POSTNATAL GOAT

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ABSTRACT

A gross and histomorphometric study was conducted on the spleen of 18 goats ($Capra\,hircus$) at various stages of postnatal development. The specimens were divided into three age groups viz., group-I (less than 6 months), group-II (6 to 12 months) and group-III (above 12 months to 3 years). The spleen of goat was roughly quadrilateral in shape. Gross morphometrical mean values of the spleen viz., weight, volume, length, width and maximum thickness were increased in dimension from the age group I to III. The histoarchitecture of splenic parenchyma showed distinct red pulp and white pulp during various stages of postnatal development. The mean thickness of capsule increased significantly (P<0.01) from the age group I to III. The mean radius, diameter and area of white pulp were increased with the advancement of age; however, increase between groups was not significant statistically. The numbers of white pulp per square mm area of spleen between age group I and II were statistically not significant, while between group II and III numbers were significant (P<0.01) statistically.

Keywords: Goats, Gross morphology, Histomorphometry, Postnatal, Spleen

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Spleen is the major secondary lymphoid organ that plays an important role in filtering the blood-borne pathogen and antigens and initiate immune responses against invading pathogens (Eurell and Frappier, 2006). The histoarchitecture of spleen is composed of capsule, trabeculae and its parenchyma organized in regions called the red pulp and a white pulp which are separated by an interface, the marginal zone. In general, spleen changes in size with advancement of age and weight. The cellular composition of spleen is also variable depending on the developmental stage of the organ, age and immune status of the individual (Alex et al., 2015). In addition, spleen can have a wide range of anomalies which may be congenital or acquired types. The gross-morphometric and histomorphometric parameters of spleen can be used for monitoring the immunological status of goats at various stages of postnatal development. Hence, the present investigation is undertaken to elucidate normal splenic biometric values and changes in histoarchitecture according to the age of postnatal goats.

MATERIALS AND METHODS

A total of 18 spleen samples were collected from apparently healthy goats irrespective of sex slaughtered at the local abattoirs of Navsari district, Gujarat. The approximate age of the goats was estimated on the basis of dentition (Noden and de Lahunta, 1985). The goats were divided equally into three age groups based on their approximate age *viz.*, group I (less than 6 months), group II (6 to 12 months) and group III (above 12 months to 3 years). Immediately after collection of specimens, gross *Corresponding author: drchaurasia77@gmail.com

and morphometrical parameters were recorded. The volume (cc) of spleen was measured by water displacement method and length, width and thickness were measured by Vernier caliper (Mitutoyo Caliper). After recording gross measurements, the tissue pieces of spleen were fixed in 10% neutral buffered formalin and processed for paraffin embedding technique. Tissue sections (5µ thick) were obtained and stained with Harri's haematoxylin and eosin method, Gomori's method, Masson's trichrome method and Hart's method (Luna, 1968 and Singh and Sulochana, 1997). The histomorphometric parameters were measured in µm under a low power objective lens (4x) by calibrating an ocular micrometer with a stage micrometer. The data were statistically analysed by Duncan's multiple range test (DMRT) and ttest (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Gross Morphology and Morphometry

The spleen was roughly quadrilateral in each age group of goats. The colour of the spleen was reddish grey in each group. These findings were similar with Gnanadevi et al. (2019) in goats. Contrary to this Mehta et al. (2016) reported a roughly triangular shaped spleen in sheep. This might be due to species differences. The spleen of goat was consisted of two surface and four borders. The parietal surface was convex and attached to the diaphragm with small part of dorsal area. The visceral surface was concave. One-half of the visceral surface was related with the rumen and devoid of the peritoneum. The anterior border was straight and thick dorsally. The dorsal border was thick

anteriorly and thin caudally. The ventral border was thinner as compared to the dorsal border and slightly rounded caudally. Both posterior and ventral borders were thin and free. The hilus was observed on the visceral surface of the spleen and close to the anterior dorsal angle (Fig. 1). These findings were in consonance with the reports of Chaurasia *et al.* (2019) in goat fetuses and Gnanadevi *et al.* (2019) in postnatal goats.

The maximum increase in the mean weight of spleen was observed between groups II to III (Table 1). The mean volume of spleen increased significantly (P<0.01) from the age group I to III (Table 1). The mean length, width and maximum thickness of spleen increased in dimension from group I to III and all the changes were highly significant (P<0.01) (Table 1). These findings were in agreement with Sivagnanam *et al.* (2017) in goats and Mehta *et al.* (2016) in sheep.

Histomorphology and Histomorphometry

The splenic capsule was composed of a layer of connective tissue fibres and a layer of smooth muscles which was enclosed by a serous layer of the peritoneum in all age groups of postnatal goats (Fig. 2). Present findings were similar as reported by Waghaye et al. (2017a) in goats. The outer layer of splenic capsule was predominantly made by collagen fibres intermingled with reticular and elastic fibres (Figs. 2, 3 and 4). The collagen fibres were increased in amount with advancement of age from group I to III. Elastic fibres were small and fragmented in group I goats; however, as age advances the numbers and length of these fibres increased from group I to III (Fig. 4). The inner layer of capsule was mainly composed of smooth muscle fibres. These fibres were increased in number and thickness with advancement of age. The trabeculae were branched with increase in the distribution of smooth muscle cells from group I to III (Fig. 5). Similar to present findings Devi et al. (2016) observed fibro-elastico muscular capsule in spleen of Marwari goat. Waghaye et al. (2017a) also reported two layers fibro muscular splenic capsule in goats.

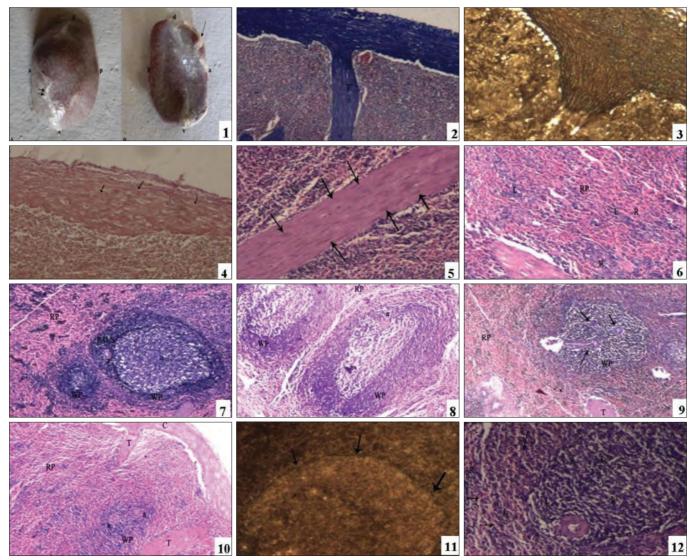
In present observations, the splenic parenchyma was composed of two major components; a red pulp and a white pulp in all age groups of postnatal goats. The red pulp showed vast amount of red blood cells held within the reticular network (Fig. 6). The blood vessels were observed in the parenchyma of spleen in all age groups. The lymphocytes were also observed in the three-dimensional networks formed by reticular fibres and reticular cells in each group (Fig. 6). During postnatal development, the goat spleen showed well differentiated and distinct red pulp in all age groups, however, in group

III with advancement of age, relatively the diffuse area of the red pulp increased on account of the lymphoid tissue as compared to group I and group II. Udoumoh and Abiaezute (2017) reported poorly defined red pulp and white pulp areas in the spleen of 1 day old African Dwarf goats, whereas the outlines of both pulp were more obvious at 2 weeks and became expansive with increased concentration of red blood cells from 1 to 7 months old.

The white pulp was observed among the red pulp distributed throughout the spleen in all age groups of goats (Figs. 7 and 8). It was composed of lymphatic nodules and periarterial lymphatic sheath (PALS). The shape of splenic lymphatic nodules was ovoid to round of different size and has germinal centres in most of the splenic lymphatic nodules in each group (Figs. 7, 8, 9 and 10). These findings were in agreement with the report of Eurell and Frappier (2006) in ruminants, Devi et al. (2016) and Waghaye et al. (2017b) in goats. The periarterial lymphatic sheath was formed along the arteries that leave the trabeculae and enter the parenchyma which were infiltrated with lymphocytes to form diffuse and nodular lymphatic tissue (Figs. 7 and 8). The position of artery was eccentric in white pulp. Two or three sections of arteries were observed in some of the white pulp in each group (Fig. 9). The stroma of white pulp was composed of reticular cells and associated reticular fibres forming three-dimensional networks (Fig. 11). At the periphery of periarterial lymphatic sheath, circumferentially arranged reticular fibres with flattened reticular cells were noticed (Fig. 11). The meshes of reticular framework were occupied by small to medium-sized lymphocytes with few plasma cells (Figs. 11 and 12). The present finding was in accordance with Devi et al. (2016) in Marwari goat. The mean thickness of splenic capsule was increased significantly (P<0.01) from the age groups I to III (Table 2). A similar trend of increasing thickness of the capsule of spleen with advancement of age was observed by Alim et al. (2012) in human spleen and Waghaye et al. (2017b) in goats.

The mean radius, diameter and area of white pulp per square mm were increased from age group I to III; however, increase in radius, diameter and area of white pulp was not significant statistically (Table 2). Waghaye *et al.* (2017b) recorded increase in the diameter of splenic nodule with the advancement of age in goats. Alim *et al.* (2012) reported that the mean diameter of the white pulp of the human spleen showed no difference among different age groups of 15-29, 30-49 and 50-69 years, respectively.

The average numbers of white pulp per square mm area were recorded as 4.996±0.735, 5.553±0.221 and 3.940±0.326 in group I, II and III, respectively. The numbers



Figs. 1 to 12. (1) Photograph of spleen of goat showing parietal surface (A), visceral surface (B), dorsal border (d), ventral border (v), anterior border (a), posterior border (p), hilus (arrow) and peritoneal reflection (double arrows); (2) Photomicrograph of spleen in group I postnatal goats showing blue collagen fibres in capsule (C) and trabeculae (T). (Masson's trichrome method X 4); (3) Photomicrograph of spleen in group II postnatal goats showing elastic fibres in capsule (arrows). (Harts method X 10); (5) Photomicrograph of spleen in group III postnatal goats showing well developed smooth muscle fibres in trabeculae (arrows). (H&E X 10); (6) Photomicrograph of spleen in group II postnatal goats showing well developed red pulp (RP), lymphocytes (L) and reticular cells (R). (H&E X 10); (7) Photomicrograph of spleen in group I postnatal goats showing well differentiated red pulp (RP), white pulp (WP), artery (a) periarterial lymphatic sheath (PALS) and nodule (n). (H&E X 4); (8) Photomicrograph of spleen in group II postnatal goats showing red pulp (RP), white pulp (WP) and arteriole (a). (H&E X 4); (9) Photomicrograph of spleen in group II postnatal goats showing two to three sections of artery (arrows) in white pulp (WP), red pulp (RP) and sections of trabeculae (T). (H&E X 4); (10) Photomicrograph of spleen in group III postnatal goats showing capsule (C), trabeculae (T), red pulp (RP), white pulp (WP) and artery (a). (H&E X 4); (11) Photomicrograph of spleen in group II postnatal goats showing lymphocytes (L), reticular cells (R), group of erythrocytes in red pulp (arrows). (H&E X 10)

Table 1. Gross biometrical values (Mean ± SE) of spleen in different age groups of postnatal goats

Parameters	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)	Fvalue	P Value
Spleen weight (gram)	$22.976^a \pm 2.272$	$37.926^{b} \pm 2.499$	$65.520^{\circ} \pm 1.996$	90.745	0.00**
Spleen volume (cc)	$21.50^{a} \pm 2.778$	$37.83^{\text{b}} \pm 2.455$	$62.50^{\circ} \pm 2.141$	69.736	0.00**
Spleen length (mm)	$55.820^a \pm 2.847$	$70.147^{\text{b}} \pm 1.121$	$89.553^{\circ} \pm 3.289$	42.597	0.00**
Spleen width (mm)	$45.743^a \pm 1.977$	$53.860^{\text{b}} \pm 1.935$	$65.063^{\circ} \pm 1.754$	26.304	0.00**
Spleen maximum thickness (mm)	$17.226^a\pm 2.021$	$25.036^{b} \pm 1.226$	$30.503^{\text{b}}\ \pm2.306$	12.244	0.001**

Means bearing different superscript (a, b, c) in a row vary significantly (P<0.01).

Table 2. Histomorphometric values (Mean ± SE) of spleen in different age groups of postnatal goats

Parameters	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)	F value	P Value
Thickness of capsule (µm)	$143.168^a\!\pm\!7.009$	$211.801^{b} \pm 11.426$	$239.683^{\text{b}}\!\pm\!10.529$	25.47	0.00**
Radius of white pulp (µm)	$247.74^a\ \pm 14.576$	$270.000^{\text{ab}}\!\pm\!19.451$	$297.758^{\scriptscriptstyle b}\ \pm 11.881$	2.57	0.10
Diameter of white pulp (µm)	$495.501^{\text{a}} \pm 29.153$	$557.548^a \pm 42.620$	$595.608^a \pm 23.719$	2.37	0.12
Area of white pulp per square mm (μ m)	196066.760° ± 22231.015	234846.414 ^a ± 35641.182	$280608.982^{a} \pm 23424.627$	2.32	0.13

Means bearing different superscript (a, b, c) in a row vary significantly (P<0.01).

Table 3. Numbers of white pulp (Mean \pm SE) per square mm area of spleen in different age groups of postnatal goats

Groups	Numbers of white pulp per square mm	t-value	P-value
Group-I & II	4.996±0.735	-0.712	0.508
	5.553 ± 0.221		
Group I & III	4.996 ± 0.735	1.114	0.315
	3.940 ± 0.326		
Group-II & III	5.553 ± 0.221	4.771**	0.005
	3.940 ± 0.326		

of white pulp per square mm between age group I and II were statistically not significant, while between group II and III numbers were significant (P<0.01) statistically (Table 3). A decrease in the number of white pulp per square mm area might be due to an increase in the size of white pulp with advancing age. Similarly, Waghaye *et al.* (2017b) reported decreases the average number of splenic nodule per square mm area with advancement of age in goat spleen. In contrast with these findings, Alim *et al.* (2012) observed no age-related evident changes in number or amount of the white pulp in human spleen.

In the present study, the capsule and trabeculae of spleen showed fibro-muscular and their composition varied during postnatal development. The splenic parenchyma showed distinct red pulp and white pulp during various stages of development; however, the average number of white pulp per square mm area of spleen was changed with advancement of age. Further more studies are required to correlate age-related changes in cellular histoarchitecture of goat spleen with its functional capability.

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