

Gross Anatomical and Histomorphometrical Studies on the Spleen of Rhesus Monkey (*Macaca Mulatta*)

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ABSTRACT

The present study was carried out to investigate the anatomy and histomorphometry of spleen of monkey. Grossly, the spleen was greyish in colour and elongated in outline. Histologically, spleen was covered by capsule with thickness of $76.31 \pm 6.56 \mu$ and had two layers. Outer layer of capsule was composed of mostly collagen fibres and inner zone had predominantly smooth muscle fibers. From capsule trabeculae extended into the splenic parenchyma in form of bands dividing the parenchyma incompletely. Thickness of trabeculae ranged from 33.82μ to 45.04μ with mean value of $40.94 \pm 1.77 \mu$. Sub-capsular and peri-trabecular sinuses were distinct. Splenic parenchyma was composed of white pulp and red pulp. The white pulp of spleen was composed of splenic nodule or lymphatic nodule and peri-arterial lymphatic sheath (PALS). The clear demarcation between white and red pulp was observed. The maximum and minimum diameter of splenic nodule was $296.71 \pm 17.33 \mu$ and $234.09 \pm 19.81 \mu$, respectively. Eccentricity (E value) of splenic nodule was 0.37 ± 0.06 indicating more spherical shape. The number of splenic nodules per field at 40X was 12.67 ± 0.67 . The spaces between white pulp and trabeculae were filled by red pulp which consisted of splenic sinuses and cords.

Keywords: Capsule, Histology, Micrometry, Monkey, Spleen

INTRODUCTION

Rhesus monkey belongs to order primates, family Cercopithecidae, genus *Macaca* and species *mulatta*. Rhesus macaques are native to India, Bangladesh, Pakistan, Nepal, Myanmar, Thailand, Afghanistan, Vietnam, southern China, and some neighboring areas. It is one of the best-known species of old-world monkey. They are brown or grey in color with pink face. They show better adaptability to human presence, and generally form larger troops in human-dominated landscapes than in forests (Kumar *et al.*, 2013). They are diurnal animals and are both arboreal and terrestrial.

Spleen is the largest and major secondary lymphatic organ, serving as filter for the blood and preserves iron for use in haemoglobin synthesis (Samuelson 2007). These important functions are carried out by the peculiar stroma and parenchyma components (Onkar and Govardhan 2013). Anatomical data on morphometry of spleen is available for Iraqi sheep (Khalel 2010), cow (Alim *et al.*, 2012), pig and goats (Usende *et al.*, 2014),

Marwari goat (Devi *et al.*, 2016), goat and buffalo (Rahman *et al.*, 2016), small ruminants of Jammu region (Suri *et al.*, 2017), Ring-tailed Lemur (Makungu, 2019). However, data on the spleen of rhesus monkey is scant. Keeping this point in view, the present study was planned to generate some baseline data on gross morphological features and histo-morphometry of the spleen of rhesus monkey.

MATERIALS AND METHODS

The present study was conducted on the spleen of carcass of adult rhesus monkey donated by TVCC after animal died during accident. The abdominal cavity of the carcass was opened carefully and proper dissection was carried out to collect spleen. After collection, specimen was thoroughly washed with normal saline. Gross morphological features were recorded. Subsequently, biometry was carried out with the help of thread, meter scale and Vernier caliper.

For histological study, spleen tissues were fixed in 10% Neutral Buffered Formalin, processed using Alcohol-Benzene method, obtained paraffin sections and stained with Haematoxylin and Eosin (Luna 1968). Micrometry was done under light microscope using an ocular micrometer calibrated

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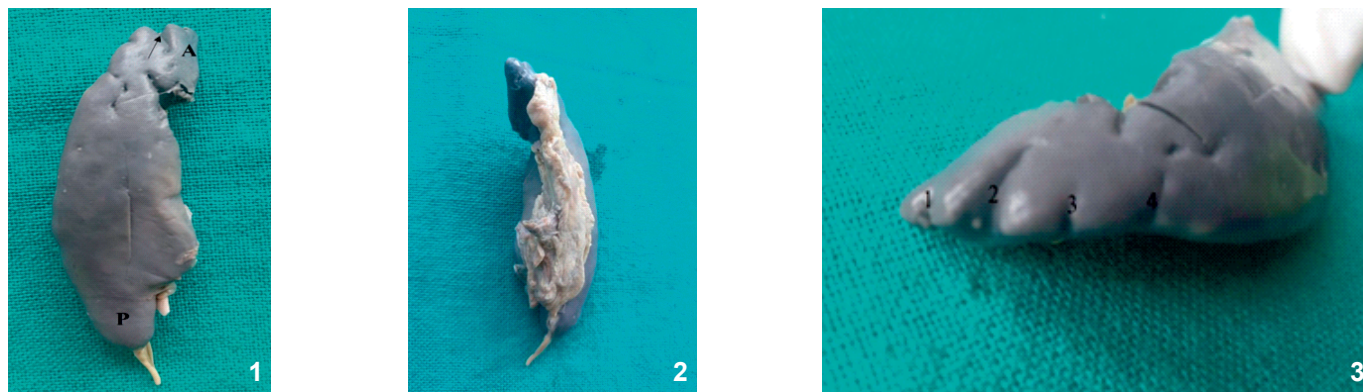


Fig. 1. Parietal surface of spleen of monkey showing anterior (A) pointed extremity and posterior (P) rounded extremity. Ventral border showing serrations (arrow) towards anterior extremity. **Fig. 2.** Visceral surface of spleen of monkey showing hilus. **Fig. 3.** Anterior extremity showing four numbers of serrations. Second serration is the deepest.

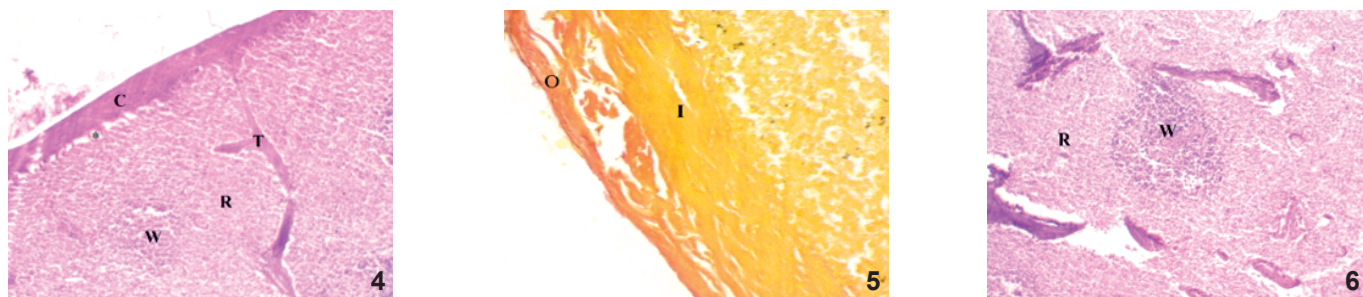


Fig. 4. Photomicrograph of spleen of monkey showing capsule (C), sub-capsular sinus (*), trabeculae (T), white pulp (W) and red pulp (R). X100 H&E Stain. **Fig. 5.** Photomicrograph of spleen of monkey showing outer layer of capsule (O) having collagen fibers and inner layer (I) having smooth muscle fibers. X400 VonGieson Stain. **Fig. 6.** Photomicrograph of spleen of monkey showing clear demarcation between white pulp (W) and red pulp (P). X400 H&E Stain.

with a stage micrometer. Following micrometrical parameters were recorded.

1. Spleen capsule thickness (μ)
2. Trabecular width (μ)
3. Longer and shorter diameter of white pulp (μ)
4. Eccentricity of white pulp was calculated as per Zedda *et al.* (2016). The degree of eccentricity was calculated according to the following mathematical formula:

$$e = \sqrt{1 - b^2/a^2}$$
 where a is longer diameter and b is shorter diameter of white pulp

The value of eccentricity lies between 0 and 1 ($0 < e < 1$). When the eccentricity is 0, the figure is round and when the value of eccentricity is towards 1, the figure becomes more elongated.

5. Number of white pulp per field (x40 magnification)

All the data generated was presented as Mean $\pm$ Standard deviation and analyzed by one-way ANOVA (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Grossly, the spleen was greyish in colour and elongated in outline. It lied on the left side of abdomen attached to stomach. Makungu (2019) reported elongated strap-like spleen in Ring-tailed Lemur. Casteleyn and Bakker (2021) reported tongue-shaped spleen in rhesus monkey attached to the left side of stomach by gastro-splenic ligament. Spleen weighed 17.70 gm and volume was 15 ml. Spleen consisted of visceral and parietal surfaces. Parietal surface was smooth and convex (Fig. 1) whereas the visceral surface was attached to the stomach and presented hilus (Fig. 2). The maximum length of spleen along parietal surface was 9.00 cm whereas the length of the hilus was 7.30 cm. The anterior extremity was more pointed whereas the posterior extremity was rounded. The maximum width of spleen was towards its posterior extremity which was 2.90 cm. The borders were dorsal and ventral. The ventral border was more convex and presented serration towards its anterior extremity (Fig. 1). Serrations were four in number out of which second was deepest (0.9 cm) (Fig. 3).

The histological study revealed that spleen

of rhesus monkey consisted of outermost capsule, trabeculae and inner parenchyma showing white and red pulp (Fig. 4). Spleen had a thick fibro-muscular capsule. Outer layer of capsule was composed of mostly collagen fibres and inner zone had smooth muscle fibers (Fig. 5). This corroborates with the findings of Zidan *et al.* (2000) in camel. The thickness of capsule was $76.31 \pm 6.56 \mu$ which was much less than in Awasi sheep ($140.5 \pm 13.712 \mu$) (Khalel 2010), buffalo (145.5μ) (Rahman *et al.* 2016), goat ($282.27 \pm 14.88 \mu$), sheep ($150.13 \pm 8.14 \mu$) (Suri *et al.* 2017). Aale (1994) reported that outer connective tissue layer was thicker than the inner smooth muscle layer. The capsule of the human spleen is composed of connective tissue with little smooth muscle (Weiss 1983).

From the innermost zone of capsule, trabeculae extended into the splenic parenchyma in form of bands dividing the parenchyma incompletely (Fig. 4). These trabeculae were predominantly composed of smooth muscles that serve as a rigid framework of the pulp (Usende *et al.* 2014). Sub-capsular and peri-trabecular sinus were also observed (Fig. 4). Sub-capsular sinus connected to peri-tubular sinus as earlier reported by Zidan *et al.*, (2000) in camel. Zidan *et al.*, (2000) in camel reported primary and secondary trabeculae. The primary trabeculae originated from the capsule with structure similar to that of the inner layer of the capsule. The secondary trabeculae were composed mainly of parallel smooth muscle cells with reticular fibres among them. The thickness of trabeculae was $40.94 \pm 1.77 \mu$. Khalel (2010) measured trabecular thickness in spleen of Awasi sheep and was $82.75 \pm 9.7 \mu$, whereas Usende *et al.*, (2014) recorded trabecular thickness in spleen of goat as $52.17 \pm 3.07 \mu$. Rahman *et al.*, (2016) recorded trabecular thickness in human (75.0μ), goat (76.36μ), buffalo (109.5μ) and rabbit (30.0μ). However, in goat and sheep of Jammu region, the same was $224.67 \pm 20.19 \mu$ and $104.35 \pm 8.92 \mu$, respectively (Suri *et al.* 2017).

Splenic parenchyma was composed of white pulp and red pulp (Fig. 4). The white pulp was distributed randomly throughout the parenchyma of spleen. It was composed of mainly two components namely splenic nodule or lymphatic nodule and peri-arterial lymphatic sheath (PALS) as reported by Devi *et al.* (2016) in Marwari goat and Suri *et al.* (2017) in small ruminants of Jammu region. The

central artery was eccentrically located in the nodule. There was clear demarcation between white and red pulp (Fig. 6). The average longer diameter of white pulp was $296.71 \pm 17.33 \mu$ whereas the average shorter diameter was $234.09 \pm 19.81 \mu$. E-value was 0.37 ± 0.06 indicating more spherical shape of white pulp. Diameter of white pulp in humans was 0.32 ± 0.01 mm (Alim *et al.* 2012). Khalel (2010) measured average diameter of white pulp in spleen of Awasi sheep as $505 \pm 34.245 \mu$. Alim *et al.* (2012) measured average diameter of white pulp in spleen of goat as 0.35 ± 0.03 mm and 0.35 ± 0.05 mm in cow. Rahman *et al.* (2016) recorded lymphoid follicle diameter in human (320.63μ), goat (503.18μ), buffalo (456.0μ) and rabbit (324.64μ). Average diameter of white pulp was $478.20 \pm 26.88 \mu$ in sheep and $412.22 \pm 47.85 \mu$ in goat (Suri *et al.*, 2017).

Number of white pulp per field at X40 magnification were 12.67 ± 0.67 whereas number of white pulps per field at X100 magnification were 1.30 ± 0.21 in sheep and 1.60 ± 0.23 in goat (Suri *et al.* 2017). Alim *et al.* (2012) also observed the number of white pulp per mm^2 in both goat and cow and it was recorded as 1.98 ± 0.69 in both the species.

The spaces between white pulp and trabeculae were filled by red pulp which consisted of irregular splenic cords separated by splenic sinuses. The cords composed of lymphocytes and reticular cells as reported by Khalel (2010) in domestic animals.

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