

**Original article**

**Histomorphometric study of the human spleen**

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**Abstract:**

**Context:** The spleen is the largest single mass of lymphoid tissue in all vertebrates. Histological changes are evident in advancing age along with functional capability of the human spleen. A cross-sectional, descriptive type of study was done in the Department of Anatomy, Dhaka Medical College, Dhaka, from January to December 2008, to see the age related microscopic changes in the human spleen. **Methods:** 30 human spleens were collected from the unclaimed dead bodies that were under examination in the Department of Forensic Medicine of Dhaka Medical College, Dhaka. The samples were divided into three age-groups including group A (15-29 years), group B (30-49 years) & group C (50-69 years). Then 5 best prepared histological slides from each group were taken and examined under the light compound microscope to determine the thickness of capsule, diameter, number and percent amount of white pulp in the human spleen. **Results:** The thickness of the splenic capsule were found  $84.60 \pm 7.97$   $\mu$ m,  $117.92 \pm 4.16$   $\mu$ m, and  $132.17 \pm 6.37$   $\mu$ m in group A, B and C respectively. The diameter of the white pulp were found  $0.32 \pm 0.01$  mm,  $0.32 \pm 0.01$  mm and  $0.31 \pm 0.01$  mm, while the number of white pulp per sq. mm were  $2.28 \pm 1.04$ ,  $2.38 \pm 0.93$  and  $2.04 \pm 0.76$  in group A, B and C respectively. Moreover, the amounts of the white pulp were determined  $23.09 \pm 1.38$ ,  $24.45 \pm 1.84$  and  $22.54 \pm 1.08$  in group A, B and C respectively. The difference in thickness of the splenic capsule was statistically significant among the study groups ( $p < 0.001$ ). However, no difference was found in diameter, number and percentage of the white pulp of the spleen among those age groups. **Conclusion:** The thickness of the capsule of the spleen was found to increase with advancing age in humans. However, no age change was evident in diameter, number or amount of the white pulp.

**Key words:** Histomorphometry, spleen, capsule of spleen, white pulp of spleen.

**Introduction:**

The spleen is the largest single mass of lymphoid tissue. It is almost entirely covered by peritoneum, which is adherent to its capsule<sup>1</sup>. It is present in all the vertebrates<sup>2</sup>. The capsule of the spleen is a continuous layer, rich in collagen but also contain some elastic fibers<sup>3</sup>. The capsule is thickened at the hilum of the spleen, where it is attached to the folds of peritoneum<sup>4</sup>. From the capsule numerous trabeculae extend into the substance of the spleen, branching within it to form a supportive framework<sup>3,5</sup>. Microscopically, the parenchyma of the spleen consists of two major components, known as white pulp and red pulp<sup>3</sup>. The white pulp forms periarterial lymphoid sheaths by aggregation of T- lymphocytes around the arteries where these leave the trabecule to enter the parenchyma. The periarterial lymphoid

sheaths continue the vessels nearly to the point where they break up into capillaries<sup>4,6</sup>. Some equivocal results were evident in microscopic structure of the spleen on age related changes in earlier studies<sup>7</sup>. Besides, it is notable that only few histological studies were done in our country. Much more attention is needed to find out age related microscopic changes and functional capability of spleen in humans. Therefore, the aim of the present study was to reinvestigate age related changes in splenic capsule and white pulp in a Bangladeshi population.

**Methods:**

A cross-sectional descriptive study was carried out in the Department of Anatomy, Dhaka Medical College, Dhaka, from January to December 2008, based on the collection of postmortem human spleen from the unclaimed dead bodies that were under examination in the Department of Forensic

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Medicine of Dhaka Medical College, Dhaka, from February to October 2008. After legal formalities the samples were collected within 24-36 hours of death without any sign of putrefaction. All the human samples were collected from medicolegal cases. During collection appropriate age, sex and cause of death were noted from morgue's record book. The samples were tagged immediately, which was bearing a code number for subsequent identification. Soon after collection each sample was gently washed in tap water on a dissection tray. Blood and blood clots were removed as far as possible. Then the samples were fixed in 10% formol saline solution. The samples were divided into three age-groups including group A (15-29 years), group B (30-49 years) & group C (50-69 years), according to Rayhan et al. (2008)<sup>8</sup> (Table-I).

**Dissection of the spleen:**

The 10% formol saline fixed samples were washed with the free flowing tap water to wash away the formol saline so as to avoid irritation of eyes and nasal mucosa by the formalin vapour and also for somewhat softening of the fixed tissue. Then samples were taken in a metallic tray and the surrounding fats and other unwanted tissues were removed carefully with the help of sharp scissors, fine dissecting forceps and BP blade to expose spleen and its related structure.

**Preparation of histological slides:**

The spleens were fixed in 10% formol saline in a plastic container. The spleen was placed on a metallic tray in such a way that the upper border lies at the top and the inferior border at the base. A sharp knife was placed on the hilum of the spleen, and it was cut sharply obtain a slice of tissue having 3mm in thickness. Each of the samples was fixed with 10% formol saline for 24 hours. Then the splenic capsule was dissected from both superior and inferior surfaces of the spleen. The tissues were washed in running tap water, dehydration was done with ascending grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin. Paraffin blocks were cut at 5mm thickness and were stained with routine Harris' Haematoxylin and Eosin (H & E) stain.<sup>5</sup> best prepared slides from each of the groups were chosen for the study.

**Microscopic measurement:**

The light compound microscope which was used for the microscopic measurement was OLYMPUS CHB, made in Tokyo, Japan. All the variables were studied under low power objective ( $\times 10$  objective,

10 eyepiece).

**Measurement of thickness of the splenic capsule:**

The thickness of the splenic capsule was determined with the help of an ocular and a stage micrometer. The thickness of the splenic capsule of each species was measured from the outer margin of the capsule to the inner margin at three different points and the average value was taken. The stage micrometer calibration was focused under the objective to be used and the ocular micrometer calibration was superimposed in such a way that starting mark on the ocular micrometer matches exactly with a starting mark on the stage micrometer. Then the markers on the stage and the ocular micrometers corresponding to each other most closely were noted. In this way how many smallest division of the ocular micrometer corresponds to how many smallest division of the stage micrometer was determined. Measurement was done by how many ocular micrometer divisions correspond to the thickness of the splenic capsule. Then the average thickness was calculated in mm by conversion measurement of an ocular micrometer and a stage micrometer<sup>8</sup>.

**Measurement of diameter of white pulp of the spleen:**

The white pulp of the spleen was spherical rather than rounded, which was somewhat difficult in measuring the actual diameter. To overcome such type of drawback, the numbers of ocular micrometer divisions were read out from near to remote margins of the white pulp and measurements were taken twice for each pulp i.e. one was maximum transverse diameter and another perpendicular to the first one. Then the numbers of micrometer divisions were multiplied by the correlation factor derived earlier keeping the magnification constant ( $\times 10$  objective,  $\times 10$  eyepiece). Therefore, the diameter of white pulps was calculated as follows:

$$\text{Diameter of white pulp} = (\text{Maximum transverse diameter} + \text{Maximum perpendicular diameter}) \div 2$$

Then the average diameter was calculated in mm by conversion measurement of an ocular micrometer and a stage micrometer<sup>8</sup>.

**Estimation of number of white pulp of the spleen per sq. mm area of the microscopic field:**

According to Rayhan et al.<sup>8</sup>, the stained tissue section on the slide was divided into three equal parts by a computer generated, photographically produced equal sized room over a transparent plastic sheet by

drawing three lines which radiated from the centre towards the periphery at 10 o'clock, 2 o'clock and 6 o'clock position. Then, this sheet was fixed on the top of the cover slip by adhesive tape. The centre of this sheet corresponded with the centre of the tissue section. From each triangular area, one microscopic field was selected near the centre for study. Thus from each slide, three different fields were chosen for counting the number of white pulp of spleen. Therefore, from human spleen, a total of 45 fields and from both the cow and the goat 18 fields were taken for the study. A counting circle of 5 mm diameter was printed on a transparent plastic sheet, which was cut to fit into the eyepiece of the light microscope. Thus a black circular outline was superimposed over the actual microscopic field. The circle encircled some total of the white pulp, while some other white pulps were partly included inside that circle. However, the rest of the white pulps were also seen. Considering this circle as the field to be studied (rather than the whole microscopic field), the portions of the white pulps inside this field were taken in consideration by an eye estimate e.g. 1, 0.75, 0.5, 0.25 etc. From the three counts of three different fields of each slide, an average count was calculated for each slide. Thus, the average counts of the slides for each species were available. The count was then converted into number per square mm by conversion measurement of an ocular micrometer and a stage micrometer<sup>8</sup>.

**Amount of white pulp of the spleen in percentage:**

To measure the area of white pulp, the well-resolved area of the slide was chosen and several photomicrographs were taken by using a SONY Cyber-shot (DSC-W130) digital camera, made in Japan. Then those were scanned by using CANON scanner (CanoScan LiDE 20), made in Japan and taken into a computer. The scanned photomicrographs were transferred to the AutoCAD software. At first, the measurement of full photomicrograph was taken, and then the area occupied by the white pulp was measured and expressed in percentage<sup>8</sup>.

**Statistical processing of data:**

The data collected from the histological studies were processed and statistical analyses were done by one-way ANOVA test, using the SPSS 11.0 version.

**Ethical clearance:**

This research work was approved by the Ethical

Review Committee of Dhaka Medical College, Dhaka.

**Results:**

The thickness of the splenic capsule were found  $84.60 \pm 7.97 \mu\text{m}$ ,  $117.92 \pm 4.16 \mu\text{m}$ , and  $132.17 \pm 6.37 \mu\text{m}$  in group A, B and C respectively. The diameter of the white pulp were found  $0.32 \pm 0.01 \text{ mm}$ ,  $0.32 \pm 0.01 \text{ mm}$  and  $0.31 \pm 0.01 \text{ mm}$ , while the number of white pulp per sq. mm were  $2.28 \pm 1.04$ ,  $2.38 \pm 0.93$  and  $2.04 \pm 0.76$  in group A, B and C respectively. Moreover, the amounts of the white pulp were determined  $23.09 \pm 1.38\%$ ,  $24.45 \pm 1.84\%$  and  $22.54 \pm 1.08\%$  in group A, B and C respectively. The difference in thickness of the splenic capsule was statistically significant among the study groups ( $p < 0.001$ ). However, no difference was found in diameter, number and percentage of the white pulp of the spleen among those age groups. The results of the present study are shown in table-II & III. Figures

**Table-I: Grouping of the samples**

Group	Age limit (in years)	No. of samples
A	15-29 years	14
B	30-49 years	11
C	50-69 years	05

**Table-II: Thickness of the capsule of the spleen in different age group**

Group	Thickness of capsule in $\mu\text{m}$ (Mean $\pm$ SD)
A (n=5)	$84.60 \pm 7.97$ (74.97-84.61)
B (n=5)	$117.92 \pm 4.16$ (112.46-123.17)
C (n=5)	$132.17 \pm 6.37$ (123.17-139.26)
P value	
A vs B	$< 0.001$ ***
A vs C	$< 0.001$ ***
B vs C	$< 0.01$ **

in parentheses indicate range. Comparison between age group of human done by One-way ANOVA (PostHoc), ns = not significant, \*\*/\*\* = significant. Group A (15-29 years) Group B (30-49 years) Group C (50-69 years)

**Table-III: Diameter, number and amount of white pulp in different age group**

Group	Diameter of white pulp in mm (Mean ±SD)	Number of white pulp in mm (Mean ±SD)	Amount of white pulp in percentage (Mean ±SD)
A (n=5)	0.32±0.01 (0.31-0.34)	2.28±1.04 (1.20-3.40)	23.09±1.38 (20.76-24.17)
B (n=5)	0.32±0.01 (0.31-0.33)	2.38±0.93 (1.70-3.40)	24.45±1.84 (22.35-27.21)
C (n=5)	0.31±0.01 (0.30-0.32)	2.04±0.76 (1.70-3.40)	22.54±1.08 (21.23 - 24.02)
	P value	P value	P value
A vs B	>0.10 <sup>ns</sup>	>0.50 <sup>ns</sup>	>0.10 <sup>ns</sup>
A vs C	>0.10 <sup>ns</sup>	>0.50 <sup>ns</sup>	>0.10 <sup>ns</sup>

Figures in parentheses indicate range. Comparison between age group of human done by One-

Group A (15-29 years)

Group B (30-49 years)

Group C (50-69 years)

**Discussion:**

Rodrigues et al. (1999)<sup>9</sup> found a correlation between age and the splenic capsule thickness; there was no difference of thickness among the three topographic aspects of splenic capsule. The average thickness of capsule at inferior capsular surface was found 54.58±1.586 μm, in the superior capsular surface 49.95±1.115 μm and in the middle portion of capsular surface 49.77±1.880 μm. According to Borley (2008)<sup>3</sup>, the thickness of the splenic capsule is about 1.5 mm. Rayhan et al. (2008)<sup>8</sup> found that the thickness of the splenic capsule increases with age; the lowest was 60.90±5.48 μm (0-19 years) and the highest was 120.73±4.53 μm (>60 years). The findings of the present study are higher than that of Rayhan et al.<sup>8</sup> and Rodrigues et al.<sup>9</sup> and lower than that of described by Borley<sup>3</sup>. However, it was found in the present study that the thickness of the capsule of spleen increases with advancing age.

According to Jacobsen and Shurin (2003)<sup>1</sup>, the white pulp is grossly visible as white nodules 0.1 to 0.2 mm in diameter. The size of the nodules of the white pulp increases in childhood, especially with immunization and infection, peaks at puberty, and begins to involute in adulthood. According to Borley<sup>3</sup>, the diameter of white pulp ranges from 0.25 to 1mm. Follicles undergo atrophy with increasing age and may be absent in the very old age<sup>3</sup>. Rayhan et al.<sup>8</sup> found that average diameter of the white pulp increased up to 4<sup>th</sup> decade of life, then started to

decrease. The highest was found 0.34±0.01 mm (30-39 years) and the lowest was 0.25±0.02 mm (>60 years). The diameter of the white pulp found in the present study is slightly larger than that of described by Jacobson and Shurin<sup>1</sup>, but similar to Rayhan et al.<sup>8</sup> and within the range of described by Borley<sup>3</sup>. However, no age related changes were found in this study.

Rayhan et al.<sup>8</sup> found the number of white pulp per sq. mm ranging from 1.83±0.77 to 1.90±1.22, with no age related changes. The findings of the present study were higher than that of Rayhan et al.<sup>8</sup>. However, the present study also did not show any age related difference.

Rayhan et al.<sup>8</sup> found the amount of the white pulp ranging from 18.13±0.60% to 19.39±1.43% and there was no age related differences. However, van Krieken et al. (1983)<sup>10</sup> found that 75% of the volume of the spleen were made up of red pulp, 8% perifollicular zone and the remaining part (17%) was white pulp. Borley<sup>3</sup> stated that the spleen contain about 25% white pulp. These findings are more than that of van Krieken et al.<sup>10</sup> and Rayhan et al.<sup>8</sup> and slightly lower than that of described by Borley<sup>3</sup>. Results correlates with some other studies on different organ on same population<sup>11-13</sup>. However, no age related difference was also observed here.

**Conclusion:**

The thickness of the capsule of the spleen was found to increase with advancing age in humans. However, no age change was evident in diameter, number or amount of the white pulp. Further studies with larger samples and more sophisticated stereological techniques are recommended.

**Acknowledgement:**

We would like to express our gratitude to the authority of the Health, Nutrition & Population Sector Programme (HNPS) of Directorate General Health

Services (DGHS) of the Government of the People's Republic of Bangladesh, and Dhaka Medical College, Dhaka for providing us with the grant for research.

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