

Histological Study of Splenic Capsule in Bangladeshi Cadaver

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Abstract

Introduction: Thickness of splenic capsule shows positive correlation with age. The capsule and trabeculae of the spleen consist of dense collagenous connective tissue with some elastic fibers and smooth muscle fibers.

Objective: To find out the histomorphologic changes of splenic capsule in relation to age and sex of the Bangladeshi cadaver.

Methods: This cross-sectional descriptive study was carried out on 40 fresh spleens collected from the morgue of Department of Forensic Medicine of Mymensingh Medical College from July 2007 to April 2008. Collected tissue samples were stained with Haematoxylin and Eosin (H&E) stain, AZAN and Van-Gieson's stain. The selected fields in the slides were studied and micro photographed by a special microscopic device. Collected data were analyzed statistically by the computer generated programme SPSS using 'F' test, ANOVA, 't' test and difference among groups was determined using comparison method at 5%, 1% or 0.1% level of significance.

Results: The maximum thickness of splenic capsule were 65.30, 78.54, 92.56, 95.64, 113.05µm in male and 61.50, 80.23, 98.34, 98.62, 114.20µm in female in age group A, B, C, D, E respectively. The minimum thickness were 53.00, 67.00, 72.12, 81.23, 100.50µm in male and 56.23, 61.50, 83.50, 92.80, 102.95µm in female. With the help of special staining abundant smooth muscle cells are identified in the splenic capsule and trabeculae.

Conclusion: Most of the splenic disease can be managed with conservation of spleen by invasive and non-invasive method. Detail knowledge on splenic capsule will help the physician to manage splenic disease.

Key words: Thickness of capsule, Smooth muscle.

Introduction

Increase incident of road traffic accident in Bangladesh is a leading cause of blunt abdominal trauma. Upto 45%

of patients with blunt abdominal trauma will have a splenic injury which causes rupture of splenic capsule^{1,2}. Splenic rupture divided into traumatic and non-traumatic rupture. The most prevalent major mechanism in traumatic injury (50% to 75%) is the result of motor vehicle injury. Direct abdominal blows and falls are the remaining major causes of traumatic rupture³. Non-traumatic splenic rupture can be related to underlying pathologic conditions or may be idiopathic which may causes mortality of around 12%⁴. Spontaneous splenic rupture may occurs in only 0.1% of the patients with infectious mononucleosis⁵. Several other pathologies are associated with splenic rupture including haematological, neoplastic and inflammatory conditions⁶.

The management of splenic injuries has evolved over the past three decades with the realization of the importance of spleen in immunological defence against encapsulated organisms and a better understanding of the role of non-operative management of splenic injuries^{7,8}. So detail knowledge on splenic anatomy including splenic capsule play an important role for the management of splenic injury.

The human spleen has a thick collagenous capsule closely invested by the peritoneum which lines the abdominal cavity and covers its viscera^{9,10}. The capsule and trabeculae consist of dense collagenous connective tissue with some elastic fibers and smooth muscle fibers. Elastic fibers are present between bundles of collagen fiber and are most abundant in the deeper layers of the capsule^{11,12}. Smooth muscle elements within the capsule and trabeculae are responsible for slow rhythmical change in volume of the spleen¹³⁻¹⁵. In human the capsule is relatively thin (0.1 to 0.15mm) in periphery and thickest at the hilum^{16,17}. The trabeculae extend from the inner surface of the capsule and pass deeply into the substance of the spleen to form a rich branching and anastomosing framework. The space between tuberculae is filled by a reticular network of fibers and associated reticular cells. The substance of the spleen is called the splenic pulp in which scattered

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grey areas forms white pulp and consist of nodular lymphatic tissue. The dark red tissues are called red pulp which consists of diffused lymphatic tissue and large branching thin walled blood vessels, called sinusoids¹⁸. Detail knowledge on splenic capsule plays an important role for determination of approach to operative or non operative management of splenic injury. Understanding of splenic capsular structure may help explaining mechanical properties of normal and diseased spleen¹⁹. The present study is carried out to establish a standard data of splenic capsule and to find out the histomorphologic changes in relation to age and sex of Bangladeshi cadaver.

Materials and Methods

This cross-sectional descriptive study was carried out on relatively 40 fresh spleens out of 120 post-mortem human spleens collected from Bangladeshi cadavers of both sexes (male and female) age ranging from birth to 80 years. The specimens were collected following all medico legal procedure from dead bodies that were under post-mortem examination in the morgue of the department of forensic medicine of Mymensingh Medical College on different dates from July 2007 to April 2008. Only fresh specimens were collected from cadaver who died within preceding 12 to 24 hours. Spleen of decomposed body, injured spleens, poisoning cases, known cases of diseases affecting spleen was excluded. For histological study small pieces of tissue block were taken from peripheral region (near to the periphery) of relatively fresh spleens which were fixed in 10% formal saline solution.

Collected tissue samples were processed routinely following standard histological procedures and tissue sections were stained with Haematoxylin and Eosin (H&E) stain. To distinguish the smooth muscle cells from connective tissue components in splenic capsule, AZAN and Van-Gieson's stain were chosen for staining. After preparing the permanent slides the tissue section on the slide was divided into three parts by drawing three lines on the cover slip from the centre of the tissue. The lines were made to radiate towards the periphery through the 10 O'clock, 2 O'clock and 6 O'clock position. For measuring the thickness of splenic capsule 12 slides from each age group thus 60 slides were selected. In total, 36 fields were examined from each age group. Capsular thickness was measured in three different fields with the help of ocular micrometer which was standardized with stage micrometer. The average thickness was taken from each slide and was expressed in μm . Presence of smooth muscle in the splenic capsule and trabeculae are observed by staining the tissue with special stain AZAN and Van Gieson's. The selected fields in the slides were studied

and micro photographed by a special microscopic device. All data were recorded in a predesigned data sheet. The collected data were analyzed statistically by the computer generated programme SPSS using 'F' test. Following the analysis of variance procedure (ANOVA), 'r' test and difference among groups was determined using comparison method at 5%, 1% or 0.1% level of significance²⁰.

Results

The maximum thickness of splenic capsule were 65.30, 78.54, 92.56, 95.64, 113.05 μm of male and 61.50, 80.23, 98.34, 98.62, 114.20 μm of female in age group A, B, C, D, E respectively. The minimum thicknesses were 53.00, 67.00, 72.12, 81.23, 100.50 μm of male and 56.23, 61.50, 83.50, 92.80, 102.95 μm of female in A, B, C, D, E age group respectively. From Table-I, it was observed that the mean thickness of splenic capsule was maximum in group E (male 105.57 μm and female 108.14 μm) and minimum in group A (male 57.43 μm and female 58.86 μm). From the Figure-1, it was also observed that the thickness of splenic capsule increases with the age of individual was linear. Differences between all the groups were statistically highly significant between A-B, A-C, A-E and moderately significant between A-D, B-C.

With the help of special stain AZAN and Van Gieson's (Figure-3, 4 & 5) connective tissue and smooth muscle cells were differentiated in splenic trabeculae and splenic capsule.

Table-I: Thickness of splenic capsule in different age and sex group

Age group	Number of specimen		Thickness in μm (Mean \pm SE) Range	
	Male	Female	Male	Female
A (up to 15 years)	3	3	57.43 \pm 3.94 53.00-65.30	58.86 \pm 2.63 56.23-61.50
B (16-30 years)	7	4	73.25 \pm 1.48 67.00-78.54	72.32 \pm 3.64 61.50-80.23
C (31-45 years)	7	3	80.56 \pm 2.46 72.12-92.56	88.61 \pm 4.86 83.50-98.34
D (46-60 years)	3	3	88.43 \pm 7.20 81.23-95.64	96.55 \pm 1.88 92.80-98.62
E (Above 60 years)	4	4	105.57 \pm 2.67 100.50-113.05	108.14 \pm 2.36 102.95-114.20

Significant test by- One way ANOVA

Comparison between different age groups		P value	Comparison between different age groups		P value
A	B	0.000 HS	B	D	0.000 HS
A	C	0.000 HS	B	E	0.000 HS
A	D	0.000 HS	C	D	0.004 MS
A	E	0.000 HS	C	E	0.000 HS
B	C	0.001 MS	D	E	0.001 MS

HS	Highly Significant (P<0.001)
MS	Moderately Significant (P<0.01)
S	Significant (P<0.05)
NS	Non Significant (P>0.05)

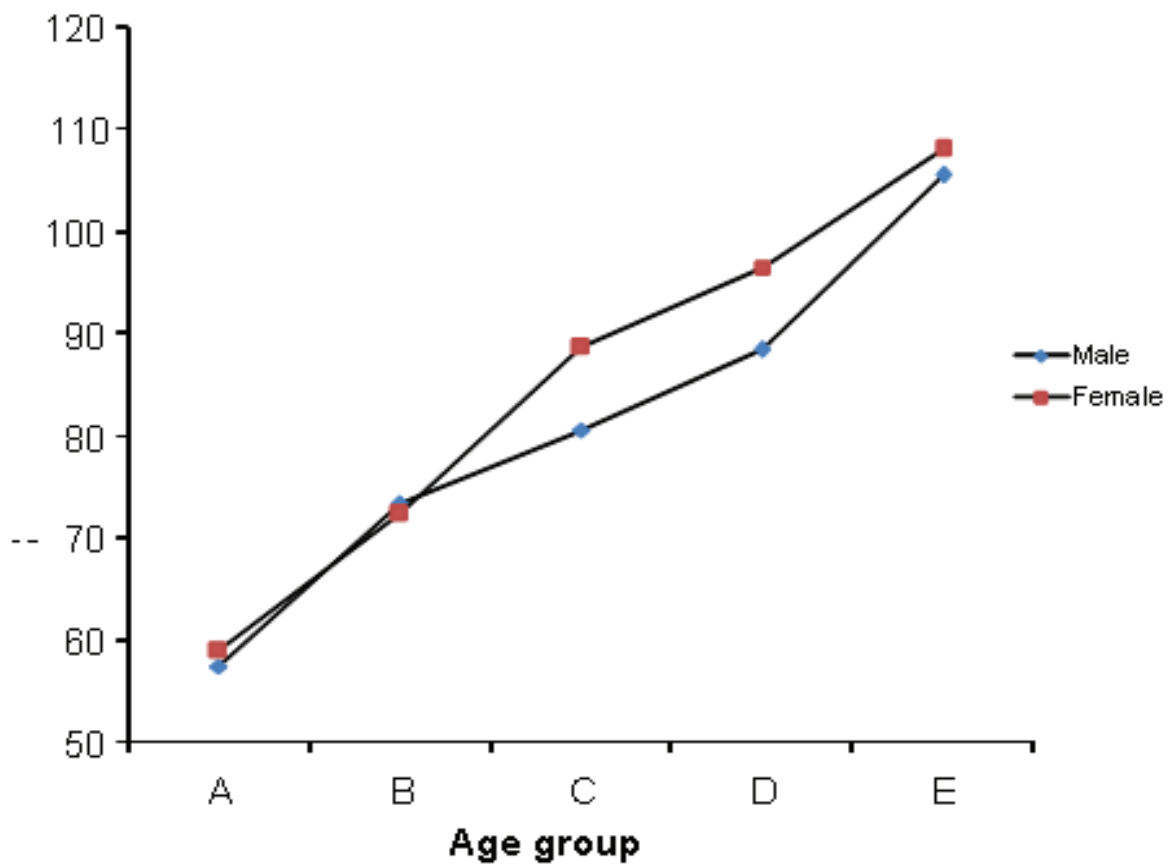


Figure-1: Line diagram showing the mean thickness of splenic capsule in different age and sex group

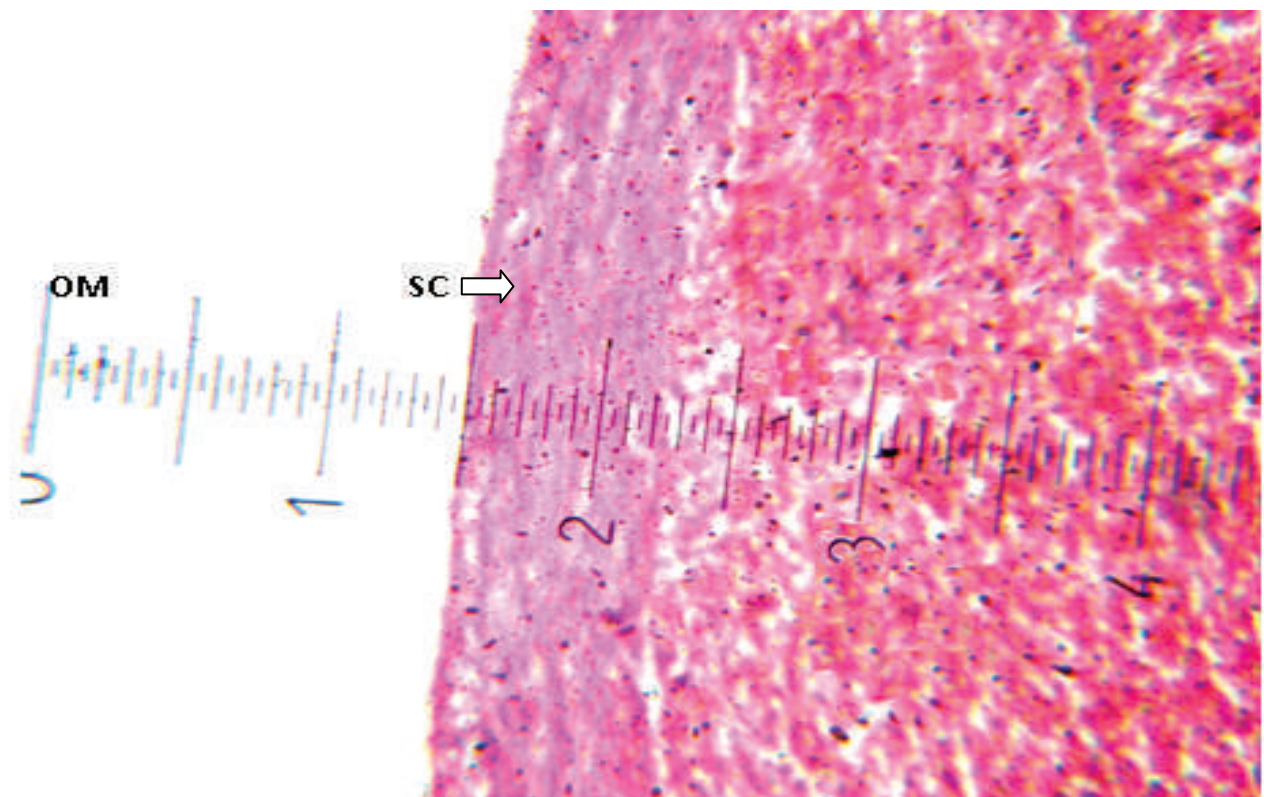


Figure-2: Photomicrograph of spleen of age group E (above 60years) showing measurement of splenic capsule (SC) by Ocular Micrometer (OM). H&E stain X10

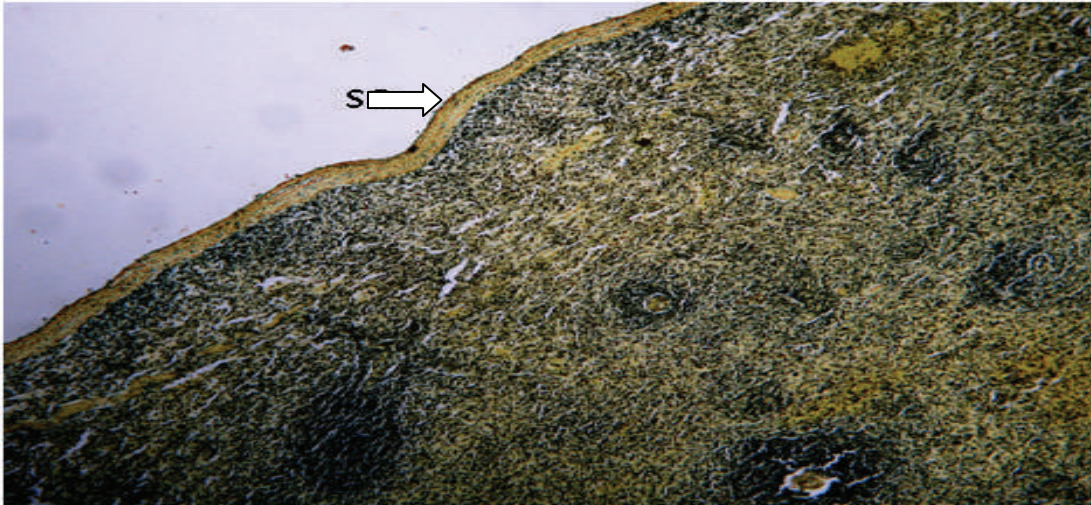


Figure-3: Photomicrograph (up to 15 years) showing Splenic Capsule (SC), White Pulp (WP) and Red Pulp (RP). Van Gieson's stain X4

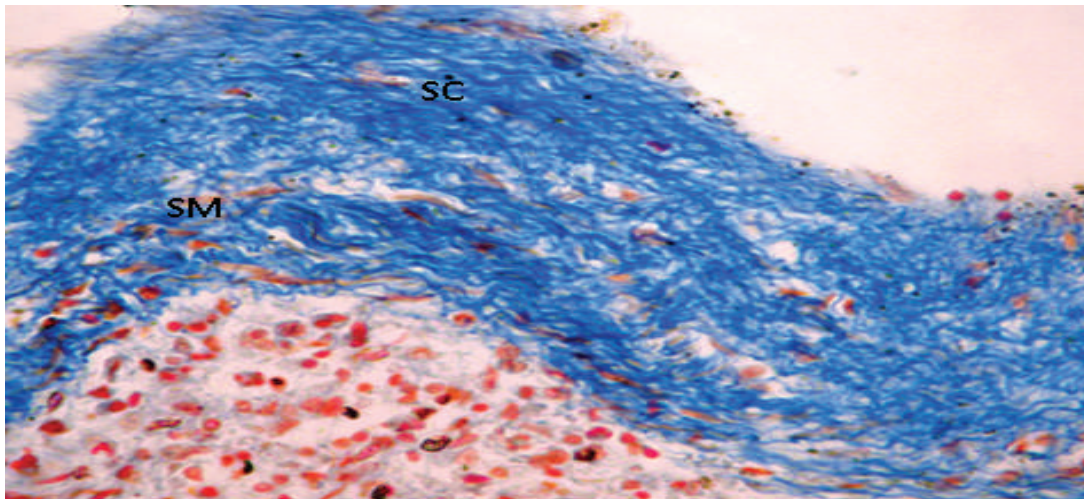


Figure-4: Photomicrograph of spleen of age group C (31-45 years) showing Smooth Muscle (SM) in Splenic Capsule (SC). AZAN stain X 40

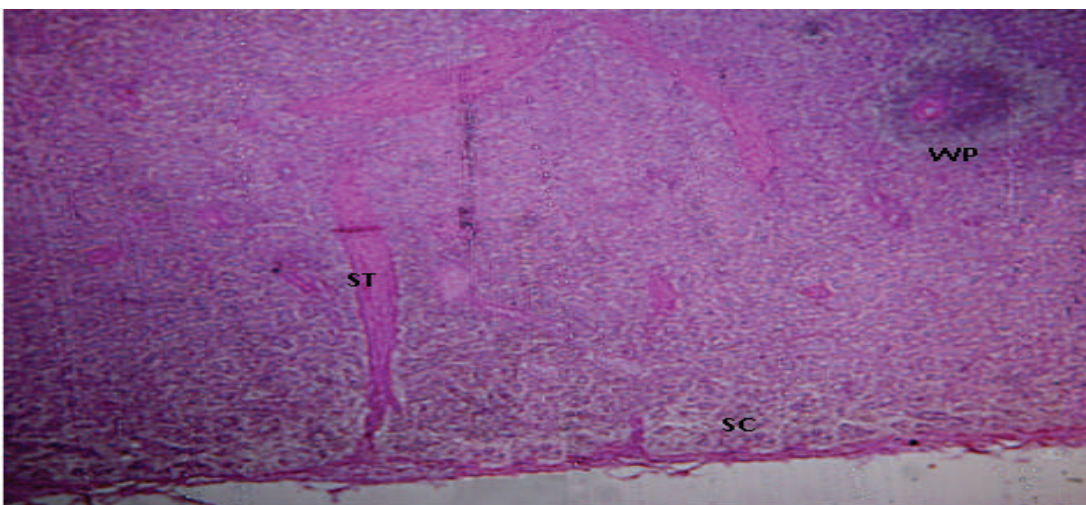


Figure-5: Photomicrograph of spleen of age group A (up to 15 years) showing Splenic Capsule (SC), White Pulp (WP) and Splenic Trabeculae (ST). PAS stain X10

Discussion

The mean thickness of splenic capsule in male was maximum 105.57 μ m in group E (above 60 years) and minimum 57.43 μ m in group A (up to 15 years). The mean thickness of capsule in female was maximum 108.14 μ m in group E and minimum 58.86 μ m in group A. The present study shows that thickness of splenic capsule increases with age and shows positive correlation with age. The differences of thickness in between groups were statistically significant.

The findings of present study is supported by Shumi who found that the mean capsular thickness of spleen was 107.71 \pm 8.70, 125.71 \pm 31.29 and 136.39 \pm 6.49 μ m in age group A, B and C respectively from June 2013 to July 2014. The mean difference of capsular thickness between group A & B, group B & C was statistically non-significant but the difference between C & A was highly significant ($p < 0.001$). According to her mean \pm SD thickness of splenic capsule was higher in male than that of female among the age groups but there was no significant difference between sexes¹⁹. In 2006, Rayhan found that mean (\pm SE) thickness of splenic capsule was 60.90 \pm 5.48 μ m in 0–19 years age group, 70.69 \pm 5.31 μ m in 20–29 years age group, 85.36 \pm 5.11 μ m in 30–39 years age group, 95.19 \pm 8.07 μ m in 40–49 years age group, 110.96 \pm 11.50 μ m in 50–59 years age group and 120.73 \pm 4.25 μ m in above 60 years age²¹. According to Bevelander, capsule of the spleen is a continuous layer about 150 μ m thick²² which was higher than the maximum thickness 113.05 μ m in male and 114.20 μ m in female of present study. In a study on 94 splenic specimen, Higginson found that the splenic capsule increasing during childhood but remain constant in adulthood²³ which support the findings of the present study. According to Rayhan and Rodrigues et al, there is a positive linear correlation between age and the thickness of splenic capsule^{21,24} which supports the findings of the present study.

In 1974, Arey LB states that in human the capsule is relatively thin 100 μ m to 150 μ m and it is thickest at the hilum¹⁶. The maximum mean thickness of both male and female in group E of present study is within the range describe by Arey. Borley et al (in 2005) described that the capsule and trabeculae of the spleen were consist of dense collagenous connective tissue with some elastic and some smooth muscle fibers. Elastic fibers are present between bundles of collagen fibers and are most abundant in the deeper layers of the capsule¹¹. Leeson et al (in 1985) states that smooth muscle element within the capsule and trabeculae are responsible for the slow and rhythmical changes in volume of the spleen¹³. Moore and Dalley (in 1999) stated that spleen normally contains a large number of

bloods that is expelled periodically into the circulation by the action of the smooth muscle in its capsule and trabeculae²⁵. Arey LB (in 1974) state that splenic capsule consist of dense fibro-elastic tissue and scanty smooth muscle fibers¹⁶. In 1968, Cruickshank state that the capsule of the spleen containing numerous elastic fibers and few smooth muscle cells¹⁷. Fawcett (in 2003) found smooth muscle cells in the splenic capsule and trabeculae⁹. Findings of the above mentioned authors are consistent with the findings of the present study where smooth muscles are observed in the capsule and trabeculae with the help of AZAN and Van Gieson's stain.

Conclusion

Most of the splenic disease can be managed with conservation of spleen by invasive and non-invasive method. Detail knowledge of splenic anatomy, advanced methods and operative technique plays an important role. Findings of the present study on splenic capsule will help the future research work on elaborated study of hemodynamic system of Bangladeshi population which will help the physician to take accurate decision during treating a patient with spleen related disease.

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