

# A Postmortem Study of the Number of Germinal Centers in the Vermiform Appendix of Bangladeshi People in Different Age Groups

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**Abstract** Appendicitis is the most common clinical condition of the appendix and is the most common acute abdominal surgical emergency. In 60% of patients, appendicitis is due to obstruction of the lumen of the appendix by hyperplasia of the intramural lymphoid follicles. This form of obstruction is mostly observed in children. The incidence of appendicitis coincides very well with the number of lymphoid follicles. Germinal centers are sites within lymphoid follicles in peripheral lymphoid tissues where mature B lymphocytes rapidly proliferate, differentiate and mutate their antibodies and class switches their antibodies during a normal immune response to an infection. Presence of germinal centers reflects state of activity of the immune system of the body. In Bangladesh there is no data regarding number of germinal centers of lymphoid follicles of the vermiform appendix. The present study is aimed to observe the number of germinal centers of the lymphoid follicles of the vermiform appendix in various age groups. With this aim the present study was carried out in the department of Anatomy, Sir Salimullah Medical College, Dhaka, from January 2008 to June 2009. After taking approval from the Institutional Ethics Committee sixty (60) postmortem vermiform appendices of different age groups collected by convenient sampling from unclaimed dead bodies of Bangladeshi cadavers which were under autopsy examination in the Department of Forensic Medicine of Dhaka Medical College (DMC) and Sir Salimullah Medical College (SSMC), Dhaka. The average number of germinal centers of the lymphoid follicle per histological section of vermiform appendix reduced with advancing age and showed a significant negative correlation with age ( $p < 0.001$ ). In the present study, the number of germinal centers of the lymphoid follicles of the vermiform appendix reduced significantly with advancing age.

**Keywords:** germinal center, lymphoid follicle, vermiform appendix, Bangladeshi people

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## 1. Introduction

The vermiform appendix is quite important despite its small size and obscure location because of its propensity for bacterial infection resulting in inflammation of the appendix, known as appendicitis [1]. It is the most common clinical condition of the appendix [2] and is the most common acute abdominal surgical emergency [3]. Anyone can develop appendicitis, regardless of age and sex. It occurs in all age groups but most common between the ages of 10 and 20 years [3]. In 60% of patients, appendicitis is due to obstruction of the lumen of the appendix by hyperplasia of the intramural lymphoid follicles. This form of obstruction is mostly observed in children [2]. The incidence of appendicitis coincides very well with the number of lymphoid follicles [4]. Due to the presence of extensive lymphoid tissues in the mucosa and submucosa, it has been called the 'tonsil of the abdomen'. Lymphoid tissue first appears in the appendix about 2

weeks after birth which is the time when the large bowel begins to be colonized with the necessary bacteria [5]. These follicles enlarge and increase steadily in number to a maximum of 200 at 15 -20 years of age, and then decrease and practically disappear after the age of 60 [6].

Secretory immunoglobins produced by the lymphoid follicles function as a very effective barrier that protects the milieu interior against the hostile milieu exterior [4].

Germinal centers are sites within lymphoid nodules in peripheral lymph tissues where mature B lymphocytes rapidly proliferate, differentiate and mutate their antibodies and switch the classes of their antibodies during a normal immune response to an infection. Germinal centers are an important part of the B-cell humoral immune response. They develop dynamically after the activation of B cells by T-dependent antigen. Presence of germinal centers reflects state of activity of the immune system of the body [7].

In Bangladesh there is no data regarding number of germinal centers of lymphoid follicles of the vermiform

appendix. The present study is aimed to observe the number of germinal centers of the lymphoid follicles of the vermiform appendix in various age groups. This will rectify the knowledge regarding the immunological status and the function of vermiform appendix.

## 2. Materials and Methods

The present study was descriptive with some analytical components. The study was carried out in the department of Anatomy, Sir Salimullah Medical College, Dhaka, from January 2008 to June 2009, after approval of the protocol of the research by the Institutional Ethics Committee of Sir Salimullah Medical College, Dhaka. The study was performed on sixty (60) postmortem vermiform appendices of different age groups of Bangladeshi people.

Samples of vermiform appendices with surrounding structures were collected by convenient sampling within 12 to 36 hours of death from unclaimed human dead bodies that showed no sign of putrefaction and that were autopsied on the different dates in the morgues of the Departments of Forensic Medicine of Dhaka Medical College (DMC) and Sir Salimullah Medical College (SSMC), Dhaka.

For studying the histomorphology of the vermiform appendix in relation to age, the collected samples were divided into five groups; Group-A (0-20 years), Group-B (21-30 years), Group-C (31-40 years), Group-D (41-50 years) and Group-E (>50 years) (Table 1) [10].

Table 1. Age grouping of the samples

Groups	Age in years	No. of samples (n=60)	Percentage frequency
A	0-20	9	15
B	21-30	19	31.67
C	31-40	12	20
D	41-50	14	23.33
E	>50	6	10

The vermiform appendix was preserved in 10% formal saline. From each age group, six (6) fresh samples were selected for histological study. Three pieces of tissues were taken from the base, middle part and near the tip of the appendix. One paraffin block was prepared with each piece of tissue. Then, several transverse sections were prepared for each region of each of the six appendices of each group. One good slide prepared as above from each tissue block was chosen for the study. As three slides were chosen from three regions of the same appendix, therefore, 3 x 6 = 18 slides were made for histological study from each group. Accordingly, 5 x 18 = 90 slides were prepared from five age groups. The sections were processed following standard histological procedures and were stained with routine hematoxylin and eosin stain. For the estimation of the number of germinal centers of the lymphoid follicles, the entire section of each slide was focused under low magnification (X10) of microscope.

**Procedure for estimation of the number of germinal centers:** Counting of the germinal centers of the lymphoid follicles was done by using a circular transparent plastic sheet on which there was a computer-generated

photographically produced line drawn at 12 o'clock position. The sheet was fixed on the top of the cover slip by adhesive tape. After that, by moving the stage of the microscope in a clockwise direction, the numbers of germinal centers of the lymphoid follicles of the particular region (base, middle or tip) of the specific vermiform appendix were counted and expressed in 'number of germinal centers of the lymphoid follicle per low power field'. The mean value ( $\pm$  SD) of the six slides of each group was then calculated for each region. Another average was calculated for each group-the average of the mean number of germinal centers of the lymphoid follicles at the three regions. From these, the mean number of germinal centers of the lymphoid follicles per low power field per sample was calculated (Figure 1).

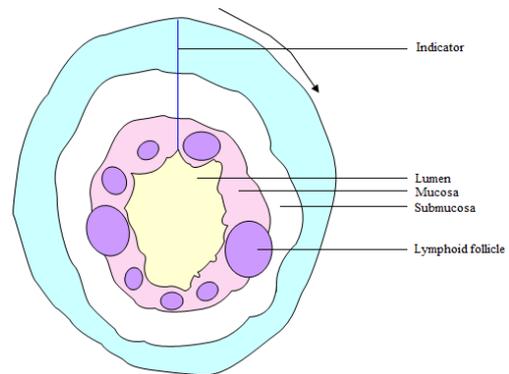


Figure 1. Procedure for counting the number of the germinal centers [11]

## 3. Results

The mean value of germinal centers per low power field ranged from 0.00 to 3.00. The mean value was highest in group A ( $2.11 \pm 0.72$ ) and lowest in group E ( $0.11 \pm 0.17$ ). The statistical differences between mean value were significant between all groups in all regions including average value, except between group A and B, A and C, at base, between group A and B, C and D, C and E at tip, between group A and B at average value. The statistical difference between mean values was not significant between group D and E in all regions including average value (Table 2 and Figure 2).

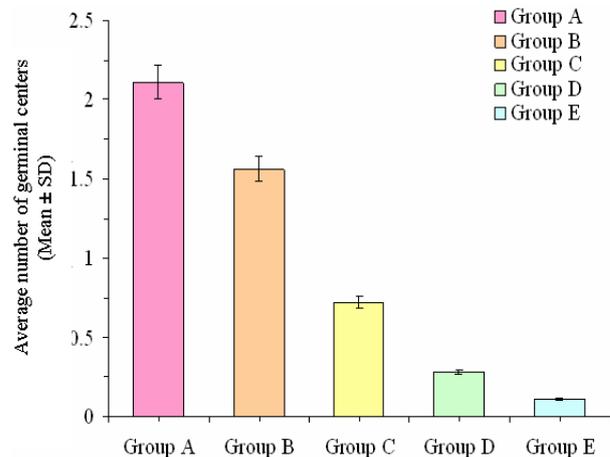


Figure 2. Average number of germinal centers per low power field in different study groups

**Table 2. Number of germinal centers at base, middle, tip and average value of the vermiform appendix in different study groups**

Number of germinal centers per low power field					
Groups	n	Base Mean ± SD (0.00-2.00)	Middle Mean ± SD (3.00-5.00)	Tip Mean ± SD (3.00-3.00)	Average Mean ± SD (1.33-3.00)
A	6	1.17±0.75 (0.00-2.00)	3.67±0.82 (3.00-5.00)	2.00±0.63 (3.00-3.00)	2.11±0.72 (1.33-3.00)
B	6	1.33±0.52 (1.00-2.00)	2.00±0.00 (2.00-2.00)	1.33±0.52 (1.00-2.00)	1.56±0.18 (1.33-1.67)
C	6	0.50±0.55 (0.00-1.00)	1.33±0.52 (1.00-2.00)	0.33±0.52 (0.00-1.00)	0.72±0.39 (0.33-1.33)
D	6	0.33±0.52 (0.00-1.00)	0.50±0.55 (0.00-1.00)	0.00±0.00 (0.00-0.00)	0.28±0.25 (0.00-0.67)
E	6	0.00±0.00 (0.00-0.00)	0.33±0.52 (0.00-1.00)	0.00±0.00 (0.00-0.00)	0.11±0.17 (0.00-0.33)
Groups compared		Significance of difference (p)			
		Base	Middle	Tip	Average
A vs. B		>0.05 <sup>ns</sup>	<0.001***	>0.05 <sup>ns</sup>	>0.05 <sup>ns</sup>
A vs. C		>0.05 <sup>ns</sup>	<0.001***	<0.001***	<0.01**
A vs. D		<0.05*	<0.001***	<0.001***	<0.001***
A vs. E		<0.01**	<0.001***	<0.001***	<0.001***
B vs. C		<0.05*	<0.05*	<0.01**	<0.001***
B vs. D		<0.01**	<0.001***	<0.001***	<0.001***
B vs. E		<0.001***	<0.001***	<0.001***	<0.001***
C vs. D		<0.01**	<0.05*	>0.05 <sup>ns</sup>	<0.05*
C vs. E		<0.05*	<0.01**	>0.05 <sup>ns</sup>	<0.01**
D vs. E		>0.05 <sup>ns</sup>	>0.05 <sup>ns</sup>	>0.05 <sup>ns</sup>	>0.05 <sup>ns</sup>

Group A : Age 0-20 years.

Group B: Age 21-30 years.

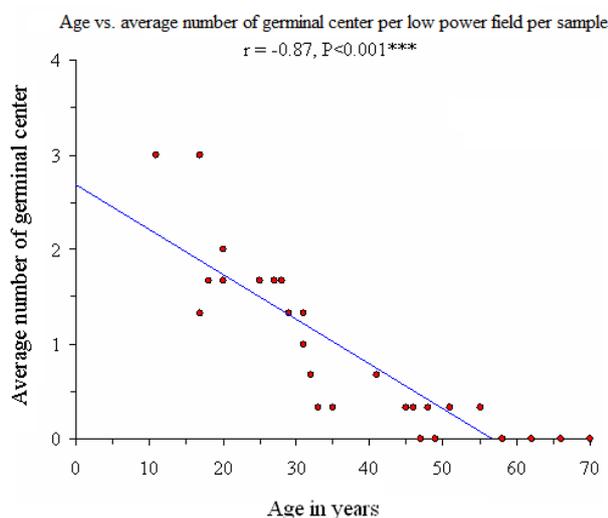
Group C: Age 31-40 years.

Group D: Age 41-50 years.

Group E: Age >50 years.

Figures in the parentheses indicate range. Statistical analysis was done by ANOVA (multiple comparison), ns = not significant, \* = significant, \*\* = very significant, \*\*\* = highly significant

Figure 3 shows the correlation between age and number of germinal centers. The regression line shows negative correlation between age and number of germinal center, which reached the level of significance ( $p < 0.001$ ).



**Figure 3.** Relationship between age and average number of germinal centers per low power field per sample

The mean value of germinal centers per low power field ranged from 0.00 to 3.00. The statistical differences between mean value were significant between all groups in all regions including average value, except between group A and B, A and C, at base, between group A and B, C and D, C and E at tip, between group A and B at average value. The statistical difference between mean values was not significant between group D and E in all regions including average value (Table 2 and Figure 2). Figure 3 shows the correlation between age and number of germinal centers. The regression line shows negative

correlation between age and number of germinal center, which reached the level of significance ( $p < 0.001$ ).

## 4. Discussion

In the present study, the mean value was highest in group A ( $2.11 \pm 0.72$ ) and lowest in group E ( $0.11 \pm 0.17$ ). It was reported by scientists that the mean number of germinal centers per low power field per sample of vermiform appendix gradually decreases with advancing age [8,9]. Again scientists reported that the numbers of germinal centre were highest in group B (21-35 years) ( $2.20 \pm 0.45$ ) and lowest in group D (51-70 years) ( $0.00 \pm 0.00$ ) [9]. It is due to the fact that immune system of our body is highly active during early period of life. Thus, the findings of the present study completely matched with the findings of the scientists mentioned above.

## 5. Conclusion

From the study it can be assumed that the number of germinal centers of the lymphoid follicles of the vermiform appendix reduced significantly with advancing age. Further studies on larger populations and different sex and ethnicity may be done to establish a complete data of Bangladeshi population.

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