

Study of Number of Germinal Centers of Vermiform Appendix in Bangladeshi People of Different Age and Sex

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The vermiform appendix is a tubular, blind-ended secondary lymphoid organ located near the caecum, often referred to as the “tonsil of the abdomen” due to the abundance of lymphoid follicles in its histology. The presence of germinal centers, which are indicators of lymphatic tissue response to antigens and subsequent antibody production, helps assess the immune status of individuals. By counting these germinal centers, researchers can evaluate the immune status of the Bangladeshi population across different age and sex groups. This study was conducted at Mymensingh Medical College, approximately 120 km north of Dhaka, the capital of Bangladesh. Samples were randomly selected from the local population between 2016 and 2017, including 40 vermiform appendices from various age groups: Group A (up to 20 years), Group B (21-40 years), Group C (41-60 years) and Group D (over 60 years). After obtaining ethical approval, the germinal centers, identified by their lighter staining in the center of lymphoid follicles, were counted under a microscope (X4 objective, X10 eyepiece). The average number of germinal centers per age group was then calculated. The study found that the mean±SD number of germinal centers was 3.20±1.66, 2.40±0.66, 1.50±1.11 and 0.30±0.45 across the advancing age groups. The mean differences of number of germinal center between A&B, B&C were statistically non significant at p= or >0.05 level, difference between group C&D, A&C was statistically significant at p<0.05 level and differences between group B&D, A&D were statistically highly significant at p<0.001 level. Mean±SD number of germinal center of vermiform appendix in male was higher (3.60±1.02, 2.42±0.49, 1.50±1.26 and 0.33±0.47 in Group A, B, C and D respectively) than in female (2.80±1.17, 2.33±0.94, 1.30±0.87 and 0.25±0.43 in Group A, B, C and D respectively) but mean difference between sexes in the different groups was statistically non significant at p=or >0.05 level. Overall, the study observed a gradual decline in the number of germinal centers with increasing age.

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Key words: Germinal center, Vermiform appendix, Lymphoid follicle, Bangladeshi people

Introduction

The lymphatic system can be considered a secondary circulatory system that operates alongside the cardiovascular system, reaching almost every major part of the body, except for the brain and spinal cord¹. Lymph circulates through various key lymphoid organs, where its composition is monitored and altered. These lymphoid organs are classified into two types: primary lymphoid organs, such as the red bone marrow and thymus gland, where lymphocytes are produced from immature progenitor cells; and secondary lymphoid organs, including the spleen and lymph nodes, where lymphocytes are stationed to initiate immune responses². The vermiform appendix is a secondary lymphoid organ. Although once thought to be a vestigial organ without significant function, modern histological techniques and surgical research have uncovered its important roles in the body's immune system³. Although the appendix does not play a role in digestion, it is an essential part of the Mucosa-Associated Lymphoid Tissue (MALT), containing numerous lymphatic nodules within its walls³.

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These lymphatic nodules are most abundant in early life, begin to decrease around middle age, and tend to progressively disappear thereafter⁴. The lymphoid follicles in the appendix are not limited to the mucosa but extend into the submucosa^{5,6}. This lymphoid tissue is arranged in follicles and is considered part of the mucosa-associated lymphatic tissue. The number, diameter, and location of the lymphoid follicles in the vermiform appendix vary among individuals⁷. In approximately one-third of cases, a fecolith was identified as the specific cause of appendicitis, while in the remaining two-thirds, it was believed that the blockage was due to the swelling of individual lymphoid follicles in response to inflammation^{8,9}. Due to the extensive presence of lymphoid tissues in both the mucosa and submucosa, the appendix has been referred to as the “tonsil of the abdomen”. Lymphoid tissue in the appendix typically appears about two weeks after birth^{10,11}. These follicles enlarge during early life but gradually decrease in number as a person ages¹². In old age, the lymphoid nodules diminish and are replaced by fibrous tissue^{13,14}. These lymphatic nodules are primarily composed of B lymphocytes. When activated by antigen-presenting cells that carry recognized antigens, these B lymphocytes proliferate within the central area of the nodule, resulting in the formation of a lighter staining germinal center^{15,16}. After the initial immune response, the germinal center may eventually disappear¹⁷. The presence of a germinal center indicates a series of immune events, including the activation and proliferation of B lymphocytes, the differentiation of plasma cells, and the production of antibodies, serving as a morphological marker of the lymphatic tissue's response to antigens¹⁸. Germinal centers are typically found in most lymphoid nodules of the human appendix in children up to 16 years of age. However, their numbers decrease in adults and are absent in older individuals¹⁹. By counting these germinal centers, the study aimed to assess the immune status of the Bangladeshi population across different age and sex groups. This study could add valuable data to the global understanding of the human immune system, particularly in under-researched populations. The findings could help create a more comprehensive understanding of how the immune system adapts to various environments and challenges.

Methods

This cross-sectional descriptive study was conducted at the Department of Anatomy, Mymensingh Medical College, Dhaka, Bangladesh from October 2016 to March 2017 after permission from ethical review committee. About 40 specimen of human vermiform appendix from Bangladeshi cadaver of both sexes and from different age groups were collected from autopsy laboratory of the department of Forensic Medicine of Mymensingh Medical College. All the collected specimen was from medico-legal cases (suicidal, homicidal and accidental death). The samples were collected from dead bodies as early as possible. The collected specimen was categorized into four groups: Group A (upto 20 years), Group B (21-40 years), Group C (41-60) years and Group D (above 60 years). Each group was again subdivided in to male and female groups. For this study 10 specimen from each group were collected. So, total 40 vermiform appendices were collected after obtaining permission from ethical review committee of Mymensingh Medical College. We employed a purposive sampling technique to select participants from various age groups. For statistical analysis, number of germinal centers between different age and sex groups were analyzed by using students unpaired ‘t’ test. For this purpose, about 3mm long of whole thickness transverse section was taken from the middle of the vermiform appendix and kept in a 10.0% normal-saline solution, which was about 15-20 times of the volume of the tissue, for about 18 hours. After fixation, each segment of tissue was washed in tap water and was processed for dehydration; infiltration and embedding in liquid paraffin separately. Six micron thick sections were prepared from the blocks. The sections were stained with haematoxylin and eosin stain (H&E) and covered with cover slips and mounted by DPX (Dibutylphthalate plasticizer xylene). Thus the permanent slides were made for microscopic examination. To count the germinal center lighter staining centrally placed area of lymphoid follicle were observe in whole microscopic field (X4 objective X10 eye piece) and their number was counted and noted (figure 1). After that the mean number of germinal center per age group was calculated.

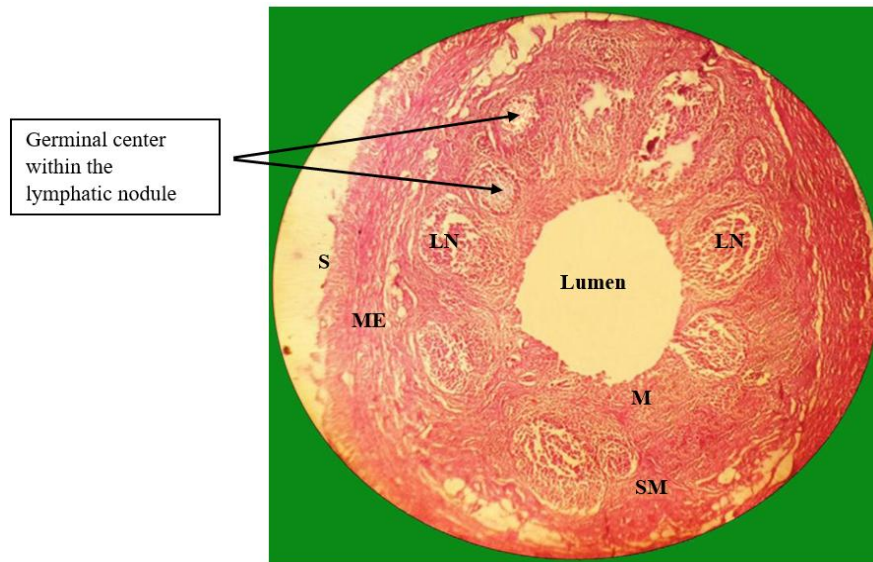


Figure 1: Photomicrograph of vermiform appendix showing lymphatic nodule with germinal center (M=mucosa, SM= submucosa, ME= muscularis externa, S= serosa, LF= lymphatic nodule. (H&E Stain. X4 objective X10 eye piece)

All data were recorded in the pre-designed data sheet, analyzed by SPSS program and compared with the findings of other national and international studies and standard text books.

Results

It is evident from Table I that maximum number of germinal was 5.00, 3.00, 3.00 and 1.00 in group A, B, C and D respectively and the minimum number of germinal center was 2.00, 1.00, 0.00 and 0.00 in group A, B, C and D respectively. Mean±SD number of germinal center was 3.20±1.66, 2.40±0.66, 1.50±1.11 and 0.30±0.45 in Group A, B, C and D respectively. It was also observed that the number of germinal center gradually decreased as age advanced. The maximum number of germinal center was 3.20±1.66 in group A and 0.30±0.45 in group D. Table II shows that the mean differences of number of germinal center between A&B, B&C were statistically non significant at $p=$ or >0.05 level, difference between group C&D, A&C was statistically significant at $p<0.05$ level and differences between group B&D, A&D were statistically highly significant at $p<0.001$ level. Table III depicts that the mean±SD number of germinal center of vermiform appendix in male was higher (3.60±1.02, 2.42±0.49, 1.50±1.26 and 0.33±0.47 in Group A, B, C and D respectively) than in female (2.80±1.17, 2.33±0.94, 1.30±0.87 and 0.25±0.43 in Group A, B, C and D respectively) but mean difference between sexes in the different groups was statistically non significant at $p=$ or >0.05 level (Table IV).

Table I: Number of germinal center of lymphoid follicle in different age groups

Age Group	Number of specimen (n=40)	Mean±SD (Minimum - Maximum)
A(Up to 20 years)	10	3.20±1.66 (2.00-5.00)
B(21 to 40 years)	10	2.40±0.66 (1.00-3.00)
C (41 to 60 years)	10	1.50±1.11 (0.00-3.00)
D (Above 60 years)	10	0.30±0.45 (0.00-1.00)

Table II: Comparison of number of germinal center between age groups

Comparison between age groups	Mean difference	Std. error difference	t	p
A & B	0.80	0.392	1.7888	0.09048
B & C	1.250	0.419	2.0769	0.05240
C & D	1.150	0.395	4.0249	0.0007
A & D	3.20	0.367	8.23193	0.0000
A & C	2.05	0.539	3.15682	0.00545
B & D	2.40	0.139	10.8544	0.0001

Table III: Number of germinal center of lymphoid follicle in different sex groups

Age groups in years	Sex	Number of specimen (n=40)	Mean±SD
A (Up to 20)	Male	5	3.60±1.02
	Female	5	2.80±1.17
B (21 to 40)	Male	7	2.42±0.49
	Female	3	2.33±0.94
C (41 to 60)	Male	6	1.50±1.26
	Female	4	1.30±0.87
D (Above 60)	Male	6	0.33±0.47
	Female	4	0.25±0.43

Table IV: Comparison of number of germinal center of lymphoid follicle between sexes

Comparison between sex in age group	Mean difference	Std error difference	t	p
A	0.800	0.694	1.152	0.282
B	0.090	0.437	0.206	0.842
C	0.070	0.729	0.251	1.000
D	0.080	0.294	0.272	0.792

Discussion

Rahman made a study of 100 vermiform appendices to find out the number of germinal centers of vermiform appendix in Bangladeshi people. He reported that the number of germinal center of lymphoid follicle in male vermiform appendix was 1.80±0.45 in Group A (0-20 years), 2.20±0.45 in Group B (21-35 years), 0.20±0.45 in Group C (36-50 years), 0.00±0.00 in Group D (51-70 years) and in female vermiform appendix was 1.80±0.45 in Group A, 2.20±0.45 in Group B, 0.20±0.45 in Group C, 0.20±0.00 in Group D and also observed that germinal center gradually decreased as age advances which is similar to our present study²⁰. Rahman studied 60 human postmortem vermiform appendices of different age groups of Bangladeshi people at department of Anatomy, Sir Salimullah Medical College,

Bangladesh. According to his observation the mean value of germinal centers was 2.11±0.72 in 0-20 year's age group, 1.56±0.18 in 21-30 years age group, 0.72±0.39 in 31-40 years age group, 0.28±0.25 in 41-50 years age group, and 0.11±0.17 in >50 years age group¹². The results of this study were more or less similar to the value mentioned by above authors.

Conclusion

The results of the present study are expected to provide an idea about the germinal center of the vermiform appendix and their changes in relation to age and sex of Bangladeshi people. This finding is consistent with previous studies and gives us knowledge that the role of the vermiform appendix in human immune system and how this

immune system gradually decreased as age advances.

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