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Microscopic study of age-related changes in germinal centre in the human appendix by using immunohistochemistry technique

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Abstract---Background: This study was conducted to observe the age-related changes in germinal centre in human appendix Subject and Methods: The study was conducted on 50 male and female appendix. All the specimens were preserved in 10% formalin solution, after paraffin processing sections were routinely stained with mouse antihuman CD 35 antibody using polymer HRP detection system using IHC technique. Results: The maximum number of lymph nodes and germinal centres found in group A (0-20yrs) and minimum found in Age group C (40-60yrs). Conclusion: Appendix play an important role in immune system.

Keywords---Vermiform Appendix, Lymphatic Follicles, Germinal Centre, Immunohistochemistry.

Introduction

The Appendix is GALT (Gut associated Lymphoid Tissue) It is a blind-ended muscular tube attached to the posteromedial wall of caecum, about 2cm below

ileocaecal junction. The mesoappendix (appendix mesentery) is short and triangular, variable. It extends the whole length of appendix. The breadth of meso-appendix usually falls short of length of appendix. The body of appendix is kinked on itself where the free border of meso-appendix ends, hence it is coiled like worm and is named the vermiform. Appendicular vessels pass in free margin of mesoappendix. The appendix is 9 cm (7 to 11cm) in length but can range from 2 to 20 cm. The diameter of the appendix is 1 to 7 mm. It is relatively longer in children and decreases after 40 years of age. The appendix shows variations in the microscopic features according to age. The Number of Lymphoid Follicles and Germinal centres varies with age.¹ The appendix serves major role in the foetus and in adults. Endocrine cells appear in the appendix of the human foetus at around the 11th week of development.² These endocrine cells of the foetal appendix have been shown to produce various biogenic amines and peptide hormones, compounds that assist with various biological control (homeostatic) mechanism. The appendix has been shown to function as a lymphoid organ, assisting with the maturation of B lymphocytes and in the production of the class of antibodies known as immunoglobulin A (IgA) antibodies.³ The appendix is involved in the production of molecules that help to direct the movement of lymphocytes to various other locations in the body Molecular Immunogenetics In this context, the function of the appendix appears to be to expose white blood cells (WBC) to the wide variety of antigens, or foreign substances, present in the gastrointestinal tract. Thus, the appendix probably helps to suppress potentially destructive humoral (blood and lymph-borne) antibody responses while promoting local immunity. The appendix-like the tiny structures called Peyer's patches in other areas of the gastrointestinal tract-takes up antigens from the contents of the intestine and reacts to these contents. The appendix wall consists of Mucosa submucosa, Muscularis externa and serosa.

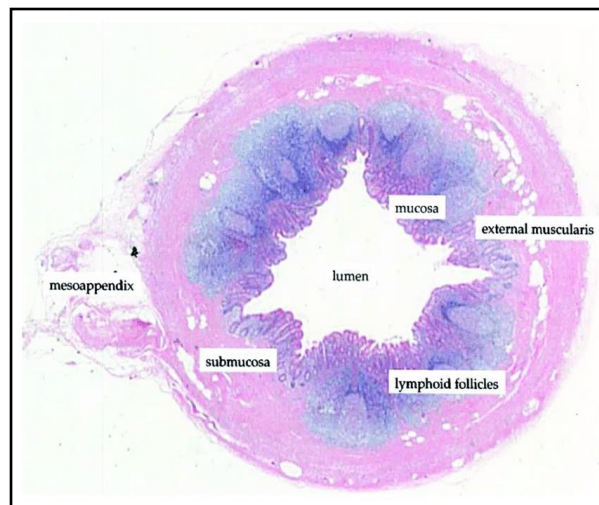


Fig 1: Normal histological features of Appendix

The Number and Size of lymph nodes decrease with age.⁴ The germinal centre (GC) is the histological structure dedicated to the generation and selection of B lymphocytes that produce high-affinity antibodies. It also represents the site from

which most B cell lymphomas originate. CD35 anti body used for detecting the follicular dendritic cells. The FDCs are source of B-Cell attraction in germinal centre, B-cell closely associated with their dendritic process. That germinal centres are not related to the formation of lymphoid cells, but rather develop in response to certain factors. Therefore, he proposed the term reaction centre.⁵

Subjects and Methods

This study was conducted to find out the lymphoid follicles and germinal centres in appendix according to age.

50 appendix (50 Male And 50 Female) Divided into four groups according to age –

Group 1- 0 to 20 yrs

Group 2- 21 to 40 yrs

Group 3- 41 to 60 yrs

The specimens of human appendix used for the study were obtained from the Dept. of Gen. Pathology of Mahatma Gandhi hospital, Jaipur and tissue sectioned at the base of appendix. subjected to processing and staining in the pathology lab.

All the specimens were preserved in 10% formalin solution. After paraffin processing sections were routinely stained with mouse antihuman CD 35 antibody using polymer HRP detection system using IHC technique. Detailed light microscopic features were studied and recorded.

Results

According to microscopic study the number of germinal centre varies in different - different age group the maximum number of germinal centres present in age group 0-20yrs (8.84±3.83) and in age group 21-40 (8.74±3.42) yrs and minimum number of germinal centres present into age group 41 to 60. (5.17±1.47)

Table 1
Distribution of study subjects according to age groups

Age Groups	Number
0-20 Years	17
21-40 Years	17
41 to 60 Years	16
Total	50

Table 2
Average number of germinal centres according to age

Average number of Germinal centres	Mean \pm SD
Age	
0-20 Years	8.84 \pm 3.83
21-40 Years	8.74 \pm 3.42
41 to 60Years	5.17 \pm 1.47
Total	7.94 \pm 3.49

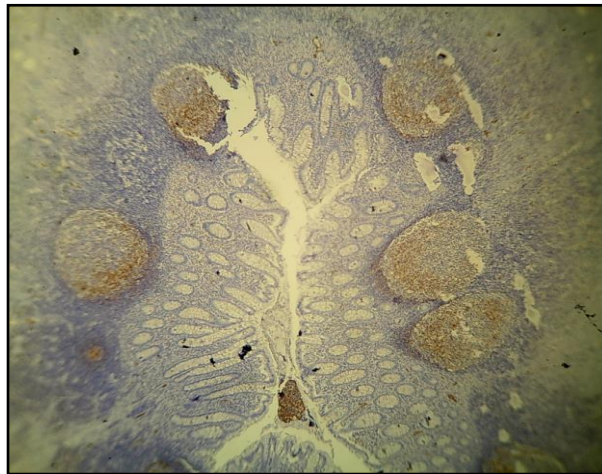


Fig 2: The section was focused under 10x of a light microscope and showing maximum lymph nodes and germinal centres

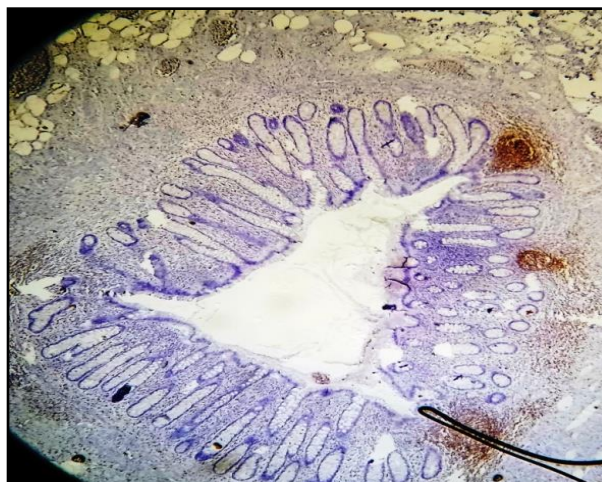


Fig 3: The section was focused under 10x of a light microscope and showing minimum lymph nodes and germinal centres.

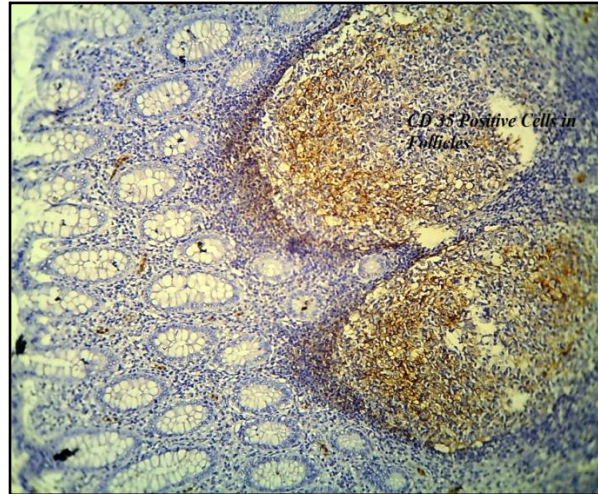


Fig 4: Showing the CD35 positive Follicular dendritic cells present into germinal centre.

Discussion

Sikander hayatniazi et al (2014)⁴ study the lymphoid tissues of vermiform appendix at various age group, there was a gradual decrease of mean numbers of lymphoid nodules with advancing age but even at the age of 74 few numbers of lymphoid nodules were observed there was great variations of diameter of lymphoid nodule at different age groups but in advance age it was much decreased In Group A(0-15yrs)-mean number of lymphoid nodule was 9.9, in group B (16-30yrs)-10.3, in group C (31-45yrs)-6.6, in group D (46-74 yrs)-4.⁴

S.M.A bakar et al (2015)⁶, The Anatomy of vermiform appendix shows variations in microscopic dimensions and microscopic features, some of which have potentials of influencing the clinical aspect of appendix, the use of 30 adults (age 18 to 67yrs) post-mortem appendix, the number of germinal centres varied between 2.33 and 10 the overall luminal diameter range between 1764.58 and 3208 micrometer.

Rahman MM, Begum J et al (2008)¹ find out the number of lymphoid follicles of vermiform appendix, findings were classified in four groups Group A 0 to 20yrs Mean were highest 5.40 +/-1.30, Group D 21 to 35 yrs mean were 1.05+/-0.35.¹

Suganthyrabi, Inbamindra Singh (2017)⁷, In the normal appendix, CD35 positive cells were present in a reticular pattern in the germinal centre of the follicle. In acute appendicitis, FDCs were scattered in the mucosa of the appendix. The lymphatic follicles were not intact and few discrete CD35 positive cells were seen in the lamina propria surrounding the intestinal glands. CD35 positive FDCs, along with lymphocytes extruding into the lumen through the disrupted epithelium, were noted. CD20 positive B lymphocytes were present in the

lymphatic follicle, interfollicular areas, around the crypts and in the lamina propria. Apposition of CD35 and CD20 cells was noted.

Trapping of antigen by FDCs and immune complex play an important role in the generation of memory B cells during germinal centre reactions (Klaus et al 1980)⁸. The major constituents of germinal centres of lymphoid follicles are activated B-Cells, tinged body Macrophages, T lymphocytes and FDCs (Butcher et al 1982, Stein et al 1982)⁹

Conclusion

The appendix is not a rudimentary organ but it plays an important role in immunity. According to this study the average number of lymphoid follicles and germinal centres of appendix were highest in group A (0-20 yrs) and minimum in Group C (41-60yrs).

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