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Microscopic Study Of The Age Related Changes In Vermiform Appendix.

P Venkatesh[1], Naveen R[2], and Sajey PS[3]*.

[1]Assistant Professor, Department of Anatomy, Government Medical College, Kozhikode, Kerala, India.
[2]Assistant Professor, Department of Anatomy, Government Medical College, Idukki, Kerala, India.
[3]Associate Professor, Department of Anatomy, Government T. D Medical College, Alappuzha, Kerala, India.

ABSTRACT

The objective of the present study was to establish a standardized baseline dataset for various age groups within the South Indian population regarding the histomorphological analysis of the vermiform appendix. Specifically, the study aimed to investigate parameters such as wall thickness, the quantity of lymphatic tissue, and the number of lymphatic follicles in order to provide comprehensive insights into appendix anatomy across different age demographics. The study population consists of 76 specimens including four foetal specimens were obtained from the Sree Avittom Thirunal Hospital (SATH), Thiruvananthapuram and deceased individuals undergoing autopsy at the Mortuary, Medical College Hospital, Thiruvananthapuram. Until the age of 30, our study found no evident correlation between age and muscle layer thickness. However, between 30 and 70 years, there was a noticeable increase in muscle layer thickness, followed by a decline after 70 years. In our current research, we observed a gradual rise in follicle numbers until 7 years, and then decreasing. After 30 years, only degenerating follicles were observed, with a complete absence of follicles by the age of 50. The analysis revealed a trend of negative correlation between the number of germinal centres and the age of the subjects.

Keywords: Lymphoid Follicles, Germinal Centres, Muscular tissue thickness, lymphatic tissue.

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Corresponding author

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INTRODUCTION

The vermiform appendix is a diminutive, finger-sized organ situated at the termination of the small cecum and positioned close to the commencement of the large intestine. Its length ranges from 2 to 20 cm, typically averaging at 9 cm [1, 2]. Notably, its length is greatest during childhood and progressively decreases with age. Originating as a diverticulum from the posteromedial wall of the cecum, it emerges approximately 2 cm below the junction of the ileum and cecum [3, 4].

The appendix displays variations in its microscopic characteristics. For instance, the quantity of lymphoid follicles in the mucosa or in the submucosa is believed to fluctuate depending on factors such as the level, timing, and duration of exposure to antigens [5]. Furthermore, the number of lymphoid follicles and germinal centres varies with age [6]. The appendix plays a significant role both during foetal development and in adulthood. Endocrine cells emerge in the foetal appendix around the 11th week of gestation [7]. These foetal appendix endocrine cells have been observed to produce various biogenic amines and peptide hormones, substances that contribute to various biological regulatory mechanisms. Moreover, research indicates that the appendix functions as a lymphoid organ, aiding in the maturation of B lymphocytes and the production of a specific class of antibodies known as immunoglobulin A (IgA) antibodies [8, 9].

Numerous cases of acute appendicitis arise from obstruction of the appendix lumen by factors such as lymphoid hyperplasia, fecalith, foecal stasis, foreign bodies, worms, or tumors, followed by infection [10]. Research indicates that in 60% of patients, obstruction arises from hyperplasia of the intramural lymphoid follicles [11]. These follicles undergo enlargement and progressively increase in number, peaking at around 200 during the ages of 15 to 20 years, and then gradually diminishing, practically disappearing after the age of 60 [12].

The objective of the current study was to establish a standardized baseline dataset for different age groups of South Indian population concerning the wall thickness and amount of lymphatic tissue and number of lymphatic follicles in the vermiform appendix through histomorphological analysis.

MATERIAL AND METHODS

The study aimed to examine microscopic characteristics of the human vermiform appendix. It involved 76 specimens, encompassing individuals of both genders across various age groups, ranging from one-year-old children to 87-year-old adults. Four foetal specimens were studied, one foetus was of twenty weeks of gestation, and the other three was full term. Specimens of foetuses were collected from the Sree Avittom Thirunal Hospital (SATH), Thiruvananthapuram. The other specimens were collected from the dead bodies brought for autopsy at the Mornrary, Medical College Hospital, Thiruvananthapuram.

After applying the exclusion criteria a total of 76 vermiform appendix were included and evaluated in the study. The study was approved by the Institutional Ethical Committee. Our inclusion criteria comprised specimens collected within six hours of death, appendices that were freely mobile, and apparently healthy foetuses. We excluded specimens collected after six hours of death, as well as those from patients who had died due to severe abdominal injury and had fixed appendices.

Methodology

Consisted of qualitative and quantitative study

Qualitative study

Following their removal, each specimen was swiftly and carefully transferred into the fixative solution. This step was crucial to mitigate the risk of post-mortem changes, ensuring the preservation of anatomical integrity for subsequent analysis. Bouin's fluid was the fixative used in this study. Subsequently, the fixed specimens underwent routine histological processing, including dehydration, cleaning, infiltration, and paraffin embedding. The resulting blocks were sectioned at a thickness of 5µ. Subsequently, the sections were subjected to the Haematoxylin-Eosin staining method after incubation.

Quantitative Study

The following parameters were observed and recorded.

- Amount of lymphoid tissue in the walls of appendix.
- Amount of connective tissue in the submucosa.
- Thickness of muscularis externa.
- Number of lymphatic follicles.

Statistical Analysis

The data will be recorded in an Excel spreadsheet, and all statistical analyses were carried out using SPSS (Version-16). The significance of study parameters on a continuous scale between two groups was determined using the Student "t" test. For categorical scale comparisons between two or more groups, the Chi-square test was employed. Additionally, the correlation between the age of the subjects and the length of the appendix was assessed using Pearson's correlation coefficient.

RESULTS

Table 1 shows the age distribution among the study population. We categorized the subjects into 9 groups as foetus group, 0-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years, 61-70 years and >70 years. The highest 16 (21.1%) were in the age group 41-50 years. The mean and standard deviation of age of Study population was 41.6 ± 23.45 years, ranging from 0- 79 years of age.

Table 1: Age distribution based on gender distribution among the study population

Age Distribution	Gender Distribution		Total	P value
	Female	Male		
Foetus	0 (0%)	4 (8.9%)	4(5.3%)	
0-10 Years	4 (12.9%)	4 (8.9%)	8 (10.5%)	0.824
11-20 Years	2 (6.5%)	4 (8.9%)	6 (7.9%)	
21-30 Years	2 (6.5%)	4 (8.9%)	6 (7.9%)	
31-40 Years	2 (6.5%)	4 (8.9%)	6 (7.9%)	
41-50 Years	8 (25.8%)	8 (17.8%)	16 (21.1%)	
51-60 Years	3 (9.7%)	5 (11.1%)	8 (10.5%)	
61-70 Years	6 (19.4%)	8 (17.8%)	14 (18.4%)	
>70 Years	4 (12.9%)	4 (8.9%)	8 (10.5%)	
Total	31 (100.0%)	45 (100.0%)	76 (100.0%)	

* p value <0.05 is significant. Pearson Chi-square test done.

Connective tissue in submucosa

The study revealed a rise in connective tissue within the submucosa of the vermiform appendix as individuals aged, with statistical significance noted across various age groups during ANOVA analysis. Additionally, a positive correlation between age and the percentage of connective tissue was established through regression analysis, resulting in the formulation of a simple linear model expressed by the equation $y = 15.29 + 0.744x$, where x represents age and y represents the percentage of connective tissue. With a correlation coefficient (r) of 0.94, the study demonstrates a strong positive correlation between age and the proportion of connective tissue in the vermiform appendix. The calculated F-value of 60.58 was compared against the critical value from the F-distribution table, which was 61.70 at a significance level of 0.05, indicating statistical significance. The regression graph, plotted using these data, likely illustrates the increasing trend of connective tissue proportion with age, confirming the findings of the study. (Figure 1).

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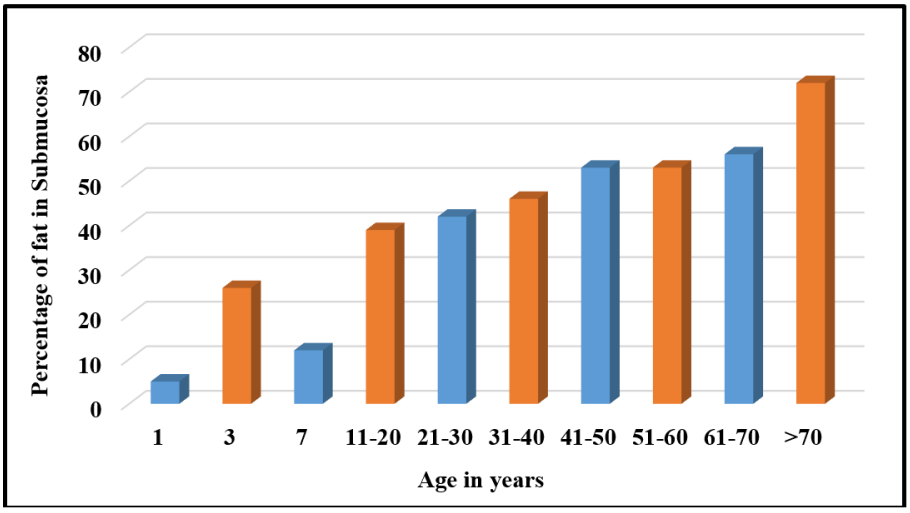

Figure 1: shows the percentage of fat in submucosa according to age

Muscular Layer

The study investigated the thickness of the muscle layer, noting that there was no apparent relationship with age up to 30 years. However, from 30 to 70 years, there was an observed increase in muscle layer thickness, followed by a decrease after 70 years.

Upon subjecting the data to ANOVA, a significant difference among different age groups was observed. However, the correlation coefficient between age and muscle thickness was low, measuring 0.2366. This suggests a weak association between age and muscle thickness.

Furthermore, regression analysis using both linear and log-linear models revealed that the p-value of the X variable (age) was found to be >0.05, indicating that the relationship between age and muscle thickness lacked statistical significance.

Table 2: Thickness of muscular layer in relation with age

Age in Years	Muscular Layer	
	Inner Circular (µ)	Outer longitudinal (µ)
20 weeks (Foetus)	20	13
Full Term	16.5	9.9
1 year	55	44
3 year	105	105
5 year	125	70
7 year	130	115
11-20 years	165	132
21-30 years	264	264
31-40 years	263	263
41-50 years	341	312
51-60 years	422	422
61-70 years	670	670
>70 year	264	264

Number of Germinal Centres present in a section

The count of germinal centres observed within each section displayed considerable variability, ranging from absence in sections located at the tip to as many as 13 germinal centres in sections situated at the base. The values gradually increased from the age of I year to a peak value at 7 years. Then on there was a decrease in the number of follicles. Only degenerating follicles were seen after 30 years. Follicles were totally absent after 50 years.

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Table 3: Arrangement and number of lymphocytic follicles according to age

Age of Specimen	Number of follicles
20 weeks (Foetus)	➢ Lymphocytes were diffusely arranged, not as nodules
Full Term	➢ Nodular Could be appreciated
1 year	➢ Lymphocytes were arranged as follicles ➢ 8-10 follicles were noted arranged in ring like fashion.
3 year	➢ Small and large lymphocyte were seen in the ratio 1:1 ➢ 10-12 follicles were observed & arranged as a ring.
5 year	➢ Lymphoid tissue organised as nodules. ➢ Nodules showed germinal centre ,10-12 nodules were seen. ➢ Nodules were arranged in a ring like fashion.
7 year	➢ Lymphocytes arranged as follicles mainly seen in a submucosa. ➢ 11-13 follicles were arranged in ring like fashion
11-20 years	➢ 5-8 nodules were arranged in the form of a ring.
21-30 years	➢ 5-6 nodules were arranged in the form of a ring. ➢ Nodules were large enough to encroach into a major portion of submucosa.
31-40, 41-50, 51-60, 61-70 years	➢ Lamina propria contained scattered lymphocytes. ➢ Lymphocytic follicles were degenerating. typical nodular pattern was not seen.
>70 year	➢ Lamina propria contained scattered lymphocytes. ➢ No follicles were seen

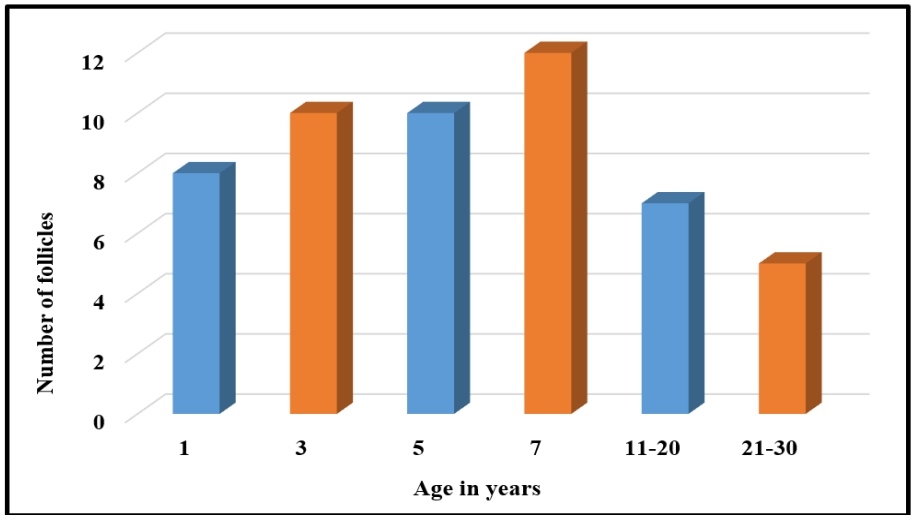

Figure 2: Average number of lymphoid follicles according to age

DISCUSSION

Indeed, there's a conspicuous scarcity of published information regarding the wall thickness of cadaveric vermiform appendices. Despite the comprehensive research efforts by Simonovský [13], Ferri et al. [14], Tamburrini et al. [15], and Huwart et al. [16], who have extensively explored the wall thickness of the vermiform appendix using diverse imaging techniques, this specific aspect remains underrepresented in the literature. The study undertaken by Bockman and Rahman delving into the functional histology of the vermiform appendix illuminated findings indicative of an average wall thickness measuring at 3.2 mm [17, 18].

Limited literature exists on the relationship between appendix thickness and age. These studies indicate a consistent pattern of decreasing thickness from the base to the tip of the appendix, coupled

with a documented decline in wall thickness as age increases [19]. Another study found that the mean wall thickness was highest in the 0-20 age group, gradually decreasing thereafter, with the lowest values observed in individuals above 50 years old. This suggests age-related variations in appendix thickness, with younger age groups generally exhibiting thicker appendices compared to older age groups [18]. The current study's findings contrast with those mentioned earlier. In our investigation, we focused on the muscle layer thickness of the appendix. Surprisingly, we found no clear relationship between age and muscle layer thickness up to 30 years. However, between 30 and 70 years of age, we observed an increase in muscle layer thickness, followed by a decrease after 70 years.

Niazi SH et al. (2014) conducted a study on the lymphoid tissues of the vermiform appendix across different age groups. The study specimens were categorized into four groups. In Group A (0-15 years), the mean number of lymphoid nodules was 9.9. In Group B (16-30 years), it was 10.3. In Group C (31-45 years), it decreased to 6.6, and in Group D (46-74 years), it further declined to 4 [20]. Their findings revealed a gradual decrease in the mean number of lymphoid nodules with increasing age.

Furthermore, Rehman et al. (2008) reported findings consistent with the present study, indicating that the diameter of lymphoid follicles was highest in the age group up to 20 years and lowest in the age group of 56-70 years [6]. This observation aligns closely with the results obtained in the current research. Similarly, Gupta et al. [21] also noted similar trends in their study, corroborating the findings of Rehman et al. (2008).

Bakar SM et al. discovered that the number of germinal centres displayed a tendency towards a negative correlation with the age of the subjects [5]. In our current study, we observed a gradual increase in follicle numbers from the age of 1 year, peaking at 7 years, followed by a subsequent decline. After reaching 30 years, only degenerating follicles were observed, with a complete absence of follicles by 50 years of age. This pattern suggests a decline in follicle presence and viability with increasing age.

Correlation with age was subsequently analysed, and regression analysis was conducted. However, it was found that simple linear or log-linear models could not be formulated. The correlation coefficient (r) was calculated to be 0.7811, indicating a correlation between age and the number of follicles. However, the p-value of the X variable was found to be >0.05, suggesting that the data lacked statistical significance.

CONCLUSION

The quantity of lymphatic tissue within the walls of the appendix diminishes with age, while the connective tissue in the submucosa increases. Both of these findings were deemed statistically significant upon analysis. The muscular tissue thickness appears to increase until middle age, after which it decreases, though this trend lacked statistical significance in the data analysis.

The observations from this study suggest that age-related changes indeed occur in the vermiform appendix. This research delineates the typical histological variations that accompany aging. There is considerable potential for future immunohistochemical investigations in this area, particularly regarding the details of changes in Paneth cells and Argentaffin cells with age and disease. Consequently, this study may facilitate the correlation of clinical conditions such as appendicitis and peritonitis with lymphoid pathology.

REFERENCES

[1] Buschard K, Kjaeldguard A. Investigation and analysis of the Positron Length and Embryology of the vermiform appendix. Acta Chirugica Scandinavica 1973;139:293–298.
[2] Venkatesh P, Jayachandran G, Umesan KG. Association between the age of subjects and length of the vermiform appendix in south Indian population. Res J Pharm Biol Chem Sci 2024; 15(1): 435-440.
[3] Williams PL, Bannister LH, Berry MM. Alimentary system. In: Williams PL, Bannister LH, Berry MM, Collins P,Dyson M, Dussek JE and Ferguson MWJ (eds) Gray's anatomy, 38th edn. Churchill Livingstone.1995. New York, pp 1775–1776
[4] Zinner MJ, Schawrtz SI, Ellis H. Appendix and appendectomy. Maingot's Abdominal Operations 1997; 2:1191-227.

[5] Bakar SM, Shamim M, Salam A, Sultana SA. Microscopic studies on postmortem vermiform appendix of the adult males of Bangladesh. Irish Journal of Medical Science 2016;185: 249-57.
[6] Rehman MM, Begum J, Khalid M, Latif SA, Nessa A, Jahan MK, Shafiquzzaman M, Parvin B, Akhunda AK. Histomorphological study of lymphoid follicle of vermiform appendix. Mymensingh Med J 2008; 17(2): 134-40.
[7] E L Rabotnikova. Endocrinocytes of the appendix in the human fetus Arkh Anat Gistol Embriol 1988;95(8):78-81
[8] IA Kooji, S Sahami, SL Meijer. The immunology of the vermiform appendix: a review of the literature. Clin Exp Immunol 2016; 186(1): 1–9.
[9] Bichhwaliya, A. K., Kumar, S., Choudhary, R., & Sharma, D. Microscopic study of age-related changes in germinal centre in the human appendix by using immunohistochemistry technique. International Journal of Health Sciences 2022; 6(S7):2002–2007.
[10] Berry RJ. The true caecal apex, or the vermiform appendix: its minute and comparative anatomy. Journal Of Anatomy And Physiology 1900;35(Pt 1):83.
[11] Ndoye JM, Ndiaye A, Dia A, Fall B, Diop M, Sow ML. Cadaveric topography and morphometry of the vermiform appendix. Morphologie: Bulletin de L'association des Anatomistes 2005;89(285):59-63.
[12] Rosai, J, editor, Rosai and Ackerman's surgical pathology, 9th rev ed, St. Louis, Missouri, Mosby, Inc., 2004 (1), p. 711-715.
[13] Šimonovský V. Sonographic detection of normal and abnormal appendix. Clinical Radiology 1999;54(8):533-9.
[14] Ferri E, Bonvicini U, Pisani M. Ultrasonography of normal vermiform appendix. Chirurgia Italiana 2001;53(2):231-8.
[15] Tamburrini S, Brunetti A, Brown M, Sirlin CB, Casola G. CT appearance of the normal appendix in adults. European Radiology 2005; 15:2096-103.
[16] Huwart L, El Khoury M, Lesavre A, Phan C, Rangheard AS, Bessoud B, Menu Y. What is the thickness of the normal appendix on MDCT?, Journal De Radiologie 2007;88(3 Pt 1):385-9.
[17] Bockman DE, Functional histology of appendix, Arch Histol Jpn 1983; 46 (3): 271-92.
[18] Rahman MA, Karim F. Histomorphological Study of the Wall Thickness and Luminal Diameter of Cadaveric Vermiform Appendix in Bangladeshi People. American Journal of Medical Sciences and Medicine 2019;7(3):89-93.
[19] Salih M. J, Mehdi A, Abdullah S. Age related changes of vermiform appendix (Histomorphometric study). Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry 2020;12(1):31-8.
[20] Niazi SH. Histomorphometric study of vermiform appendix. The Professional Medical Journal 2019;26(10):1635-9.
[21] Gupta G, Srivastava SK, Mathur SK, Gupta V. Histomorphometric characteristics of human vermiform appendix with special reference to lymphoid tissue. Journal of Morphological Sciences 2017;29(3): 135-139.