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Original Research Article

Histomorphometry of human vermiform appendix

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ABSTRACT

Background: Acute Appendicitis is one of the most common acute abdominal conditions. Advances in modern radiographic imaging have improved diagnostic accuracy, however the diagnosis of appendicitis remains essentially an enigmatic challenege. Though much work has been done on morphometry but there is less work done on variation of histomorphometric features of appendix. Hence the study was carried out by keeping the following objectives in mind. Aim was to study the general histomorphology and measure the histomorphometric parameters of human vermiform appendix. And also, to study diagnostic significance of histomorphology and histomorphometric parameters in causing appendicitis.
Methods: The study was conducted in the Department of Anatomy. Specimens of appendix were obtained from embalmed cadavers. Serial sections were taken at different levels and slides were prepared. The slides were then examined using Nikon Trinocular Research microscope under magnification power of 40x for various parameters, after staining with H&E.
Results: The mean luminal diameter varied from 1.32±0.65mm at base to 1.22±0.72mm from base. Diffuse lymphatic tissue was seen in both mucosa and sub-mucosa.
Conclusions: Definitely there is a relationship between lymphoid follicle diameter and mucosal-serosal thickness on one hand and that between the luminal diameter and lymphoid follicle diameter on the other hand. Since our study was restricted to geriatric age group so studies need to be done in different age groups to highlight any further relationship.

Keywords: Histomorphology, Histomorphometric parameters, Luminal Diameter, Vermiform appendix

INTRODUCTION

Appendicitis is one of the most common acute abdominal conditions that the surgeon is called on to treat. It is one of the best recognized clinical entities and the most difficult diagnostic problems that confronts the clinician and if not promptly and correctly treated, may even have fatal outcome.[1,2]

The presence of vermiform appendix in human beings, was probably first noted as early as the Egyptian civilization (3000 B.C). It was in the year 1521 that Jacopo Beregari Da Capri, a professor of Anatomy in Bologna identified the appendix as an anatomical structure and in 1710 the term appendix vermiformis was coined by Phillipe Verheyen.[3]

The vermiform appendix is a narrow blind tube or an appendage arising from the postero-medial wall of caecum. The word vermiform, derived from Latin word "Vermiforma" means worm shaped or resembling worm.[4]

The appendix varies from 2 to 20cm in length, the average being about 9cm. It is longer in child than in adult and may atrophy and become smaller after mid adult life.[5] The external diameter varies from 3-8mm

reaching a maximum transverse diameter by the age of 4 years.[6] It is connected by a short mesoappendix to the lower part of mesentery of the ileum. The main artery to the appendix, a branch of the lower division of the ileocolic artery, runs behind the terminal part of ileum and enters the mesoappendix a short distance from the base of appendix.[5] If the mesentery is incomplete, the artery lies on the wall of the appendix in its distal part. In such cases the wall of the vessel may be eroded in suppurative appendicitis or early thrombosis of the appendicular blood-vessels may occur.[7]

The appendix is a diverticulum of the large intestine and its wall has the same general histological structure except that the longitudinal muscle coat is evenly distributed around the circumference. It is characterized by a great increase in the lymphoid tissue, the nodules occupying a large part of both mucous and sub mucous coats. Solitary and aggregated lymphoid follicles are most pronounced around the age of puberty. Thereafter they diminish in number and size, although they may persist in old age. The muscularis mucosae is rather deficient. The glands are much less closely packed than in the larger intestine. They are most numerous in early life and tend to disappear in old age.[8] The muscular coat may be deficient in parts so that the peritoneum and mucous membrane are separated only by connective tissue through which infection may readily spread from the mucous membrane to the peritoneum.[7]

In view of the rich blood supply and histological differentiation, the vermiform appendix is probably more correctly regarded as a specialized than as a degenerate, vestigial structure.[4] The appendix appears to be strategically placed and structurally composed of tissues which are vital in establishing and maintaining the various types of body defences or immunity necessary in recognition of such assaults and having a part to play in their repulsion. The appendix is thus one of the guardians of the internal environment of the body from the hostile external environment.[9] Variation in anatomical position cause different clinical presentations. Variation in blood supply and histology, have also been reported in literature. Though much work has been done on morphometry but variation of histomorphometric features of appendix do not seem to have attracted much attention as evidenced by paucity of publications. The present study is therefore aimed to analyse histomorphometric features of appendix which may add to the understanding of appendicitis.

METHODS

The study was done on ten specimens of human adult appendix obtained from embalmed cadavers, made available from the department of Anatomy.

The abdominal wall was dissected, and peritoneal cavity opened to expose the viscera. Ascending colon was identified and the three tainae were traced to the base of appendix and then it was dissected out from the base.

All the appendix obtained were then stretched and measured in centimetres, labelled and kept in separate containers containing 10% formalin. Each appendix was processed for microscopic examination by dehydration in graded series of alcohol, cleared by acetone and xylene and then tissue was impregnated with paraffin wax and sectioning was done at 5nm on a rotatory microscope. Serial sections of appendix were taken at different levels at a distance of 3cm, 6cm, 9cm (if applicable) to include entire thickness of wall.

The slides were examined using Nikon Trinocular Research Microscope Model-E-200 under magnification power of 40x with NIS element BR software after staining with Haematoxylin and Eosin (H/E) for the following parameters.

Figure 1: The calculation of luminal diameter of appendix.

Luminal diameter (mm): Since the lumen was irregular so the luminal diameter was calculated using the formula for equivalent circular area (Figure 1).

$A = \pi r^2$

$r = \sqrt{A/\pi}$

$d = 2\sqrt{A/\pi}$

Where, A = Area, d = diameter, r = radius.

Depth of crypts extending up to:

- Mucosa,
- Sub-mucosa.

Lymphoid nodules:

- Present/Absent,
- Position and extent,
- Scattered/well defined.

Thickness of wall (mm)

- Total wall,
- Mucosa,
- Sub mucosa,
- Muscularis externa.

Observations were taken at four different points where layers were well defined and then mean was calculated.

RESULTS

The present study was conducted in the department of Anatomy to study the morphometry and light microscopic study of human vermiform appendix.

Histology

The sections of appendix were taken at different levels, 3cm apart i.e. at base, 3cm from base and 6cm from base where applicable. These were studied and analysed after staining with haematoxylin and eosin staining for the following parameters (Table 1). The mean luminal diameter was 1.32±0.65mm at base, 1.28±0.47mm at 3cm from base and 1.22±0.72mm at 6cm from base. No statistically significant difference was seen between cross sections taken at various levels. Crypts of variable sizes were seen. Short crypts extended from epithelial lining to lamina propria. Long crypts extended from epithelial lining to muscularis mucosa. Diffuse lymphatic tissue was seen in both mucosa and sub-mucosa. Very few well defined lymphatic nodules were observed as our subjects were of geriatric group so this is in accordance with other studies. The mean total wall thickness was 1.46±0.21mm at base, 1.45±0.20mm at 3cm from base and 1.44±0.24mm at 6cm from base. The mean thickness of mucosa was 0.47±0.10mm at base, 0.47±0.06mm at 3cm from base and 0.60±0.23mm at 6cm from base. The mean thickness of sub-mucosa was 0.57±0.15mm at base, 0.61±0.19mm at 3cm from base and 0.52±0.01mm at 6cm from base. The mean thickness of muscularis externa was 0.38±0.09mm at base, 0.34±0.08mm at 3cm from base and 0.32±0.10mm at 6cm from base.

Table 1: The mean histomorphometric measurements of appendix at base, 3cm and 6cm from the base.

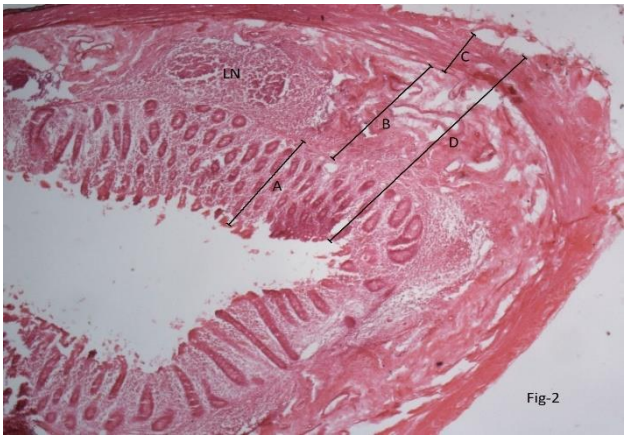
	Mean			SD		
Dimensions (mm)	**Base**	**3cm from the base**	**6cm from the base**	**Base**	**3cm from the base**	**6cm from the base**
Luminal diameter	1.3266	1.2844	1.2266	0.6524	0.4764	0.7246
Total wall thickness	1.4680	1.4500	1.4433	0.2144	0.2088	0.2438
Thickness of mucosa	0.4790	0.4710	0.6000	0.1067	0.0684	0.2330
Thickness of sub mucosa	0.5750	0.6150	0.5233	0.1545	0.1926	0.0153
Thickness of muscularis externa	0.3880	0.3400	0.3267	0.0948	0.0859	0.1021

DISCUSSION

The human vermiform appendix is a complex and organized structure both in its development and maturation. 20% of the general population develops appendicitis during their life time. When appendicitis manifests in its classic form, it is easily diagnosed and treated. Unfortunately, few patients with acute appendicitis develop classic symptoms, therefore an accurate and timely diagnosis of atypical appendicitis remains clinically challenging and one of the most commonly missed problems.

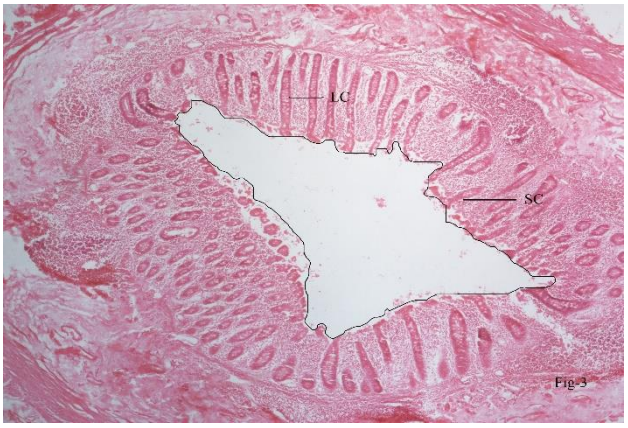
The basic pathophysiology of appendicitis is obstruction of appendical lumen, resulting in disruption and interference of circulation, which in turn causes ischemia and necrosis of the wall. Obstruction of appendiceal lumen may be due to lymphoid hyperplasia (60%), fecaliths (35%), foreign bodies (4%), tumours (1%) and worms. In children the cause of obstruction is mainly due to hyperplasia of sub mucosal follicles, whereas in adults the fecaliths are the most common cause of obstruction.

In the study, we observed four layers of appendix going from the luminal to superficial aspect i.e. mucosa, sub-mucosa, muscularis externa and serosa (Figure 2). The mucosa showed crypts of variable sizes (Figure 3). Shorter crypts did not extend up to the muscularis mucosae, while the longer ones extended from epithelial lining to muscularis mucosae. In most of the slides, lymphatic tissue was scattered in mucosa and sub-mucosa (Figure 2). Very few well defined lymphatic nodules were seen and was comparable to a study done by Berry and Lack on 103 human appendices of both sexes and all ages who observed that lymphoid follicles diminish progressively with age.[10] Our study was also in accordance with the study of Rahman et al who studied100 normal appendices of both sexes and all ages. He observed that number of lymphoid follicles gradually decreased with advancing age and were replaced by fibrous connective tissue in mucosa and partly in sub-mucosa.[11] In 60% cases appendicitis is due to lymphoid hyperplasia leading to occlusion of the interior of appendix, indicating that the appendix is usually prone to abnormal proliferation of its lymphoid tissue.



LN=Lymph node, A=Thickness of mucosa, B=Thickness of sub mucosa, C=Thickness of muscularis externa, D=Total wall thickness

Figure 2: Lymph node and thickness of various layers of appendix.



LC=long Crypts, SC=Short Crypts

Figure 3: Crypts of appendix.

The luminal diameter did not show any uniform pattern. The mean luminal diameter at base was found to be 1.32mm, whereas at 3cm from base the maximum number of specimens were observed in the range of 1.00-1.5mm. Only three specimens were longer than 6cm, in them at the level of 6 cm from base, the value of luminal diameter observed was in the range of 0.50-1.0mm in first specimen, 1.0-1.5mm in second specimen and more than 2mm in third specimen. This difference in luminal diameter was not statistically significant.

In the present study the total wall thickness was studied at three different levels. The maximum thickness in the range of 1.50-1.75mm was seen in 50% of the specimens at base, in 30% at 3 cm from base and in 20% specimens it was at 6cm from base. The total wall thickness in one specimen at 3cm from base was 1.87mm. This was in accordance with the studies of Shugaba et al who observed that thickness does not decrease uniformly as we go from base to tip.[12] Our values are comparable to study of Simonovsky (2.1 mm±0.5). The normal range as quoted by him is 1.2-3.0mm.[13] The luminal diameter is inversely proportional to the thickness of wall. Wherever the wall is thicker, the luminal diameter becomes smaller and vice-versa. Since the wall is thicker at base the luminal diameter is smaller. Our specimens were taken mostly from geriatric age group, factors like the amount of lymphoid follicle in the sub-mucosa, age of the subject and the thickness must be playing a role in determining the luminal diameter and thickness of wall.

When analyzing thickness of mucosa, the range varied from 0.24-2.75mm 50% of the cases had a maximal thickness at base ranging from 0.50-0.75mm. As we proceed down the length of appendix, the thickness of mucosa decreased.

When studying the width of sub-mucosa, it was observed that majority of specimens (70%) were having thickness in the range of 0.50-0.75mm at 3 cm from base. The thickness of muscularis externa did not change much in majority of cases along the length of appendix.

A similar study was done by Alimalas et al who observed development of vermiform appendix during the fatal period. They reported not much difference between the cross sections taken from the proximal, median and distal parts in terms of wall thickness, presence of lymphoid aggregation, number of lymphatic nodules and the luminal diameter.[14]

Definitely a relationship exists between the thickness of wall and size of lumen. If due to any cause there is hypertrophy of the wall, commonly from lymphoid follicle aggregations the luminal diameter would be markedly reduced thereby increasing chances of stasis and obstruction. This is one of the important causes of high incidence of appendicitis in children. As age increases thickness of wall decreases and there are decreased chances of obliteration of lumen. So, incidence of acute appendicitis decreases. If it occurs the chances of perforation are more, leading to complications. Our study was restricted to geriatric age group so studies need to be done in different age groups to highlight any further relationship.

CONCLUSION

The vascular patterns, various positions of appendix have been studied extensively than the histomorphology and histmorphometric parameters which have been grossly understudied. The present study noted the various characteristic features and measured the thickness of various layers, luminal diameter microscopically. Though our study was restricted to geriatric group but definitely relationship exists between lymphoid follicle diameter and thickness of wall on one hand and that between luminal diameter and largest lymphoid follicle diameter on the other hand. This correlation is capable of explaining the basis and the predisposed sites of obstruction in appendicitis.

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