Original article

Histomorphometric characteristics of human vermiform appendix with special reference to lymphoid tissue

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Abstract

The luminal diameter, largest lymphoid follicular diameter, and serosal – mucosal circumference (wall thickness) of the vermiform appendix were measured in micrometers from the base, middle of appendix and near to tip and their relationship analysed and plotted. There is a strong relationship between the diameters, especially between lymphoid follicle diameter and mucosal serosal circumference (wall thickness) on the one hand and that between luminal diameter and largest lymphoid follicle diameter on the other. There is decrease in the luminal diameter, when either walls thickness, or the largest lymphoid follicle with the resulting histological and histomorphometric changes that can occur to bear in normal subjects. These profiles also explain the basis and common diameter increase in size, suggesting that obstruction can occur at these sites sites of obstruction in appendicitis.

Keywords: appendix**,** histological, histomorphometric, mucosal or luminal diameter, lymphoid follicle, serosal-mucosal thickness or circumference, segments, appendicitis.

1 Introduction

The vermiform appendix is a narrow, worm shaped tube, arising from the posteromedial caecal wall, 2 cm or less below the end of the ileum (WILLIAMS, BANNISTER, BERRY et al., 1995; ZINNER, SCHAWRTZ and ELLIS, 1997). It is located in the right lower quadrant of the abdomen (WILLIAMS, BANNISTER, BERRY et al., 1995; SABISTON and TOWNSEND, 2001). Its opening is guarded by a semicircular fold of mucus membrane known as the valve of Gerlach (SINGH, 1999).

The appendix is usually located at the junction of the taeniae, found on the surface of the caecum (SCHWARTZ, SHRIES, SPENCER et al., 1999) has much lymphoid tissue and normally communicates with the cavity of caecum (ROPER, 1987 apud SHUGABU, UMAR and SINGH, 2000).

Its length varies from 2-20 cm with an average length of 9 cm (BUSCHARD and KJAELDGUARD, 1973). The appendix contain lymphoid nodules which first appear in the appendix about 2 weeks after birth (FAWCETT and BLOOM, 1994). The lymphoid tissue is organized in the form of the follicle and has been considered as the part of MALT (STANDRING, 1989). The vermiform appendix is considered to be a vestigeal organ but the exact physiological role of the appendix is unproved, an immunologic function is suggested by its lymphoid tissue content. Nevertheless, it is a useful organ for surgeons.

Appendicitis is the most common acute abdominal condition. The surgeon is called on to treat, and acute appendicitis remains the most common indication for emergency operation.

Acute appendiceal inflammation is associated with obstruction in 50-80% of cases, with mainly obstructive causes, but a significant minority of inflamed appendix has no demonstrable luminal obstruction and the pathogenesis remain unknown (LIU and CRAWFERD, 2004).

Many times appendices of children operated on because of clinical signs of acute appendicitis were having normal histological finding i.e. epithelium and lymphatic tissue in more than 2/3rd of specimen originally classified as normal that is what the term 'negative appendicectomy' is used (MROZIK, HECKER, WIEBECISE et al., 1993).

2 Material and methods

This study was done in the Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak in the year 2007-2009 as part of my thesis for the award of DNB Anatomy by the National Board of Examination, New Delhi.

Sixty subjects were taken and separated into 2 groups:

- Group I – in which appendix removed surgically during operation but diagnosed as a negative appendicectomy or appendix removed with adjoining part of intestine for other surgery i.e. normal otherwise; and
- Group II – in which appendix removed during the operation with a preparative diagnosis of acute appendicitis and diagnosed as acute diffuse suppurative appendicitis on histopathology.

The appendicular tissue or specimen was collected from the histolopathological section of pathology department after measuring the length and diameter of appendix with the help of measuring scale and fixed by immersion in 10% formaldehyde solution for 48 hours.

Three pieces, one close to tip (labeled A), one from middle (labeled B) and one from base (labeled C) were taken. The tissues were processed for paraffin embedding and sectioning.

Each section was processed for microscopic demonstration in the straight, intact form by dehydration in graded series of alcohol 50, 70, 80, 90, 95%, absolute), cleaning in xylene for 3 hours, embedding in paraffin wax and sectioning at 5 nm on rotary microtome.

Serial sections near the tip, middle and base of the appendix were taken and three best out of these sections were taken and measured out of which again one was then stained in haematoxylin and Eosin.

Each section representing a segment was then examined carefully under microscope for the general histomorphology and following histomorphometric parameters, outer circumference (serosal) of appendix, luminal (mucosal) diameter of appendix, circumference of largest lymphoid follicle at the maximum thickness were taken and drawn on a paper. The image was projected on a graph paper with 15 times magnification and length of the image was measured.

The length of these outline were measured with the help of a planimeter. For each section 3 reading were taken from 3 different sites and mean of three reading for each appendix was calculated as average measurement. With the help of formula: $D = C/\pi$, $A = rD^2/4$ and wall thickness = serosal – mucosal circumference/2, following parameter per section were calculated for each appendix in micrometer, mean luminal diameter, mean lymphoid follicular diameter and mean serosal – mucosal circumference (wall thickness).

3 Observations

The present study was based on observations made in 60 cases of human appendix of 2 groups comprising 30 cases of normal appendices and 30 cases of appendicitis in different age groups. Section were taken from all the 30 subjects from each group divided into age groups of 0-15 years, 16-30 years and >30 years and observations were made.

The average length and diameter was 6.8 cm and 8.7 mm in children (<15 years) and 5.25 cm and 7.2 mm in adults (>15 years) in normal group, whereas it was 6.12 cm, 8.6 cm and 5.8 cm, 8.3 cm in children and adults respectively in appendicitis group.

In group 1 the luminal diameter was seen in the various segments and was found to be almost equal in the middle and base and lowest at the tip, whereas the lymphoid follicle diameter was largest at middle and smallest at the base but difference were not statistically significant.

The organ thickness was greatest in middle and almost equal in base and tip i.e. there was an initial decrease at the base, then increase in the middle segment before decreasing in the tip.

In group II the mean of luminal diameter and lymphoid follicle diameter decreased from base to tip following the normal trend of appendicitis.

The serosal mucosal diameter (wall thickness) increased from base to tip again following the normal trend of appendicitis but differences were not statistically significant (Table 1).

The average luminal diameter, lymphoid follicle diameter and wall thickness was compared in two groups and results are shown in Table 2-4.

The luminal diameter in group I varied from 53 µm to 322 µm (mean value 168.4 ± 69.58 D) and 74.3 µm to 311.4 µm (mean 147.14 ± 49.64 SD) in group 11, so mean value of luminal diameter decreased from 168.4 to 147.14 µm but difference was not statistically significant. Average lymphoid follicle diameter in group 1 varied from 53 µm to 130.9 µm (mean value of 91.21 ± 17.43 SD) and 60.1 µm to 137.9 µm (mean value 91.82 ± 18.40 SD) in group 11 so mean value showed slight increase in value from 91.21 to 91.82 µm but again difference was not statistically significant.

The wall thickness varied from 138.0 µm to 422.8 µm (mean value 214.48 ± 63.35) in group I and 187.1 µm to 461.7 µm (mean value 281.3 ± 56.70 SD) in group 11 so mean wall thickness increased from 214.48 to 281.3 µm in group II and difference was statistically highly significant ($p < 0.001$).

4 Discussion

The present study provides a general anatomical morphology, histomorphological and histomorphometric

Table 1. The mean of luminal, lymphoid follicle, serosal-mucosal diameter (thickness of wall) of the normal appendix (group I) in micrometers from base (C) middle (B) and Tip (A) and appendicitis (group II).

Group I

Diameter	Base			Middle			Tip		
	Luminal	Lymphoid follicle	Serosal-mucosal	Luminal	Lymphoid follicle	Serosal-mucosal	Luminal	Lymphoid follicle	Serosal-mucosal
Mean	171.2	86.3	212.4	171.94	93.73	214.60	160.61	90.19	212.82
SD	103.9	22.7	67.68	68.81	18.09	66.98	69.06	22.42	73.41

Group II

Diameter	Base			Middle			Tip		
	Luminal	Lymphoid follicle	Serosal-mucosal	Luminal	Lymphoid follicle	Serosal-mucosal	Luminal	Lymphoid Follicle	Serosal-mucosal
Mean	156.98	94.40	274.9	145.2	92.6	284.13	142.0	90.9	284.79
SD	69.74	31.24	66.34	64.0	26.5	63.04	82.6	29.8	68.12

Table 2. Average Luminal Diameter in total sample (all age groups) of normal (group I) and appendicitis (group II).

Sr. no.	Case no.	Group I average luminal diameter (μm)	Case no.	Group II average luminal diameter (μm)
1	GNA 1	183.9	GAA 1	141.5
2	GNA 2	201.6	GAA 2	212.3
3	GNA 3	74.2	GAA 3	148.6
4	GNA 4	206.1	GAA 4	120.3
5	GNA 5	134.4	GAA 5	154.2
6	GNA 6	162.7	GAA 6	139.4
7	GNA 7	322.0	GAA 7	166.2
8	GNA 8	92.0	GAA 8	113.2
9	GNA 9	258.3	GAA 9	192.6
10	GNA10	293.6	GAA10	92.0
11	GNA11	120.3	GAA11	159.2
12	GNA12	116.7	GAA12	311.4
13	GNA13	173.4	GAA13	141.5
14	GNA14	162.7	GAA14	198.1
15	GNA15	53.0	GAA15	173.4
16	GNA16	176.9	GAA16	201.7
17	GNA17	247.7	GAA17	102.6
18	GNA18	318.4	GAA18	123.8
19	GNA19	127.3	GAA19	159.2
20	GNA20	230.0	GAA20	99.1
21	GNA21	201.7	GAA21	99.1
22	GNA22	109.6	GAA22	208.7
23	GNA23	212.3	GAA23	74.3
24	GNA24	113.2	GAA24	127.3
25	GNA25	120.3	GAA25	230.0
26	GNA26	116.8	GAA26	134.4
27	GNA27	120.3	GAA27	99.1
28	GNA28	134.4	GAA28	106.1
29	GNA29	159.2	GAA29	169.8
30	GNA30	109.6	GAA30	115.0

Table 3. Average Lymphoid follicle diameter in total sample (all age groups) of normal (group I) and appendicitis (group II).

Sr. no.	Case no.	Group I average lymphoid follicle diameter (μm)	Case no.	Group II average lymphoid follicle diameter (μm)
1	GNA 1	70.7	GAA 1	67.2
2	GNA 2	102.5	GAA 2	63.6
3	GNA 3	53.03	GAA 3	88.4
4	GNA 4	130.9	GAA 4	91.9
5	GNA 5	95.5	GAA 5	109.6
6	GNA 6	88.4	GAA 6	109.6
7	GNA 7	116.7	GAA 7	74.3
8	GNA 8	81.3	GAA 8	77.8
9	GNA 9	109.6	GAA 9	106.1
10	GNA10	116.7	GAA10	109.6
11	GNA11	91.6	GAA11	77.8
12	GNA12	99.0	GAA12	106.1
13	GNA13	99.0	GAA13	88.4
14	GNA14	88.4	GAA14	60.1
15	GNA15	74.2	GAA15	77.8
16	GNA16	91.9	GAA16	70.7
17	GNA17	95.5	GAA17	74.2
18	GNA18	106.1	GAA18	102.6
19	GNA19	99.0	GAA19	99.0
20	GNA20	84.8	GAA20	137.9
21	GNA21	95.5	GAA21	91.9
22	GNA22	63.6	GAA22	95.5
23	GNA23	109.6	GAA23	77.8
24	GNA24	88.4	GAA24	99.0
25	GNA25	81.3	GAA25	123.8
26	GNA26	63.6	GAA26	88.5
27	GNA27	81.3	GAA27	99.0
28	GNA28	95.5	GAA28	74.3
29	GNA29	67.1	GAA29	109.7
30	GNA30	95.5	GAA30	102.6

profile and variations of the observed parameters that are being considered in normal and inflamed appendix. It was aimed at evaluating the diagnostic significance of lymphoid follicle and wall thickness in causation of appendicitis.

In the present study the average length of appendix in children (<15 years) was more than in adult groups as observed by other authors and contradicted by authors (GALALIPOIR, ARYA, AZARHOOSH et al., 2003; COLLINS, 1932; BAKHEIT and WARILLE, 1999). The observed difference is unexplained and more investigations are recommended.

The average luminal diameter of appendix follows the normal trend in which there is decrease in luminal diameter from normal to appendicitis. In the present study, there was decrease in luminal diameter from normal to appendicitis but the difference was not statistically significant. This is supported by the fact that, other factors like the thickness of wall, amount of lymphoid follicles in the submucosa, the age of the subject or indeed the normality of the processed organ must be playing role in determining the luminal diameter. The present observation in our study does not match with the study reported by Shugabu, Umar and Singh (2000) in which luminal diameter does not shows uniform pattern.

Average lymphoid follicle diameter in the present study did not show any significant difference though it slightly increased from normal to appendicitis cases. This finding is supported by study done by Mrozik, Hecker, Wiebecise et al. (1993) who concluded by planimetric study of lymphatic tissue that lymphoid follicles and germinal centers become longer with appendicitis as compared to normal although the difference was again not found to be statistically significant (GALALIPOIR, ARYA, AZARHOOSH et al., 2003). Herein lies the area for further research both in human appendix and those in lower animals, especially now when the disease of appendix are beginning to be understood in the light of nutrition and immunology. These two together with both the age and other concurrent disease condition of the subjects go a long way in determining the amount of lymphoid follicle and there by the diameter. Of course this diameter will together interplay with either the luminal diameter or the thickness of the organ to determine the probable site or sites of obstruction of this organ (SHUGABU, UMAR and SINGH, 2000).

The most interesting observation of the present study was that of organ thickness. It would have been naturally assumed that this thickness should decrease from the base to the tip (since the base is directly from the thick walled caecum) but this is not the case with group I in the present study though the thickness showed the expected trend from base to tip in group II. In the present study, most striking finding was that there was increase in wall thickness from normal to appendicitis cases and this difference was statistically highly significant which explain the occurrence of inflammatory pattern that occurs in cases of appendicitis (Figures 1 and 2).

Present study and other experimental and clinical observation led us to hypothesis about the possible cascade of event occurring in appendicitis cases. The infection which is reaching the appendix from gut causes the inflammatory agents to stimulate the growth of lymphoid follicle.

Table 4. Average Wall thickness in total sample (all age group) of normal (group I) and appendicitis (group II).

Sr. no.	Case no.	Group I average wall thickness (μm)	Case no.	Group II average wall thickness (μm)
1	GNA 1	106.1	GAA 1	240.6
2	GNA 2	189.2	GAA 2	244.1
3	GNA 3	422.8	GAA 3	350.3
4	GNA 4	222.9	GAA 4	325.1
5	GNA 5	240.6	GAA 5	259.7
6	GNA 6	238.8	GAA 6	263.6
7	GNA 7	253.0	GAA 7	231.5
8	GNA 8	237.0	GAA 8	346.8
9	GNA 9	219.3	GAA 9	187.1
10	GNA10	157.4	GAA10	461.7
11	GNA11	215.2	GAA11	313.1
12	GNA12	290.1	GAA12	240.6
13	GNA13	194.6	GAA13	291.9
14	GNA14	226.4	GAA14	300.8
15	GNA15	226.4	GAA15	295.4
16	GNA16	184.2	GAA16	254.7
17	GNA17	145.0	GAA17	194.6
18	GNA18	148.6	GAA18	251.2
19	GNA19	219.3	GAA19	268.9
20	GNA20	156.8	GAA20	291.9
21	GNA21	194.5	GAA21	258.3
22	GNA22	217.7	GAA22	231.2
23	GNA23	138.0	GAA23	260.0
24	GNA24	222.9	GAA24	300.7
25	GNA25	224.6	GAA25	304.3
26	GNA26	371.5	GAA26	288.3
27	GNA27	166.3	GAA27	376.8
28	GNA28	192.8	GAA28	212.3
29	GNA29	201.7	GAA29	258.3
30	GNA30	210.7	GAA30	339.7

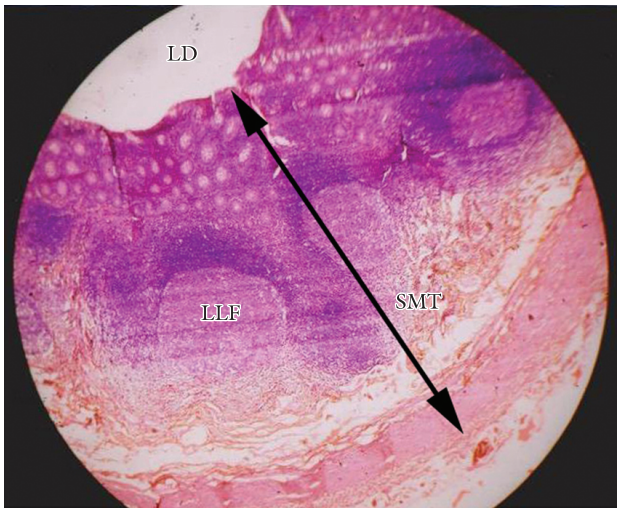

Figure 1. Normal Human Appendix shows Normal Wall Thickness at the site of Largest Lymphoid Follicle (H&E × 100). LLF-Largest Lymphoid Follicle, SMT-Serosal Mucosal Thickness, LD-Luminal Diameter.

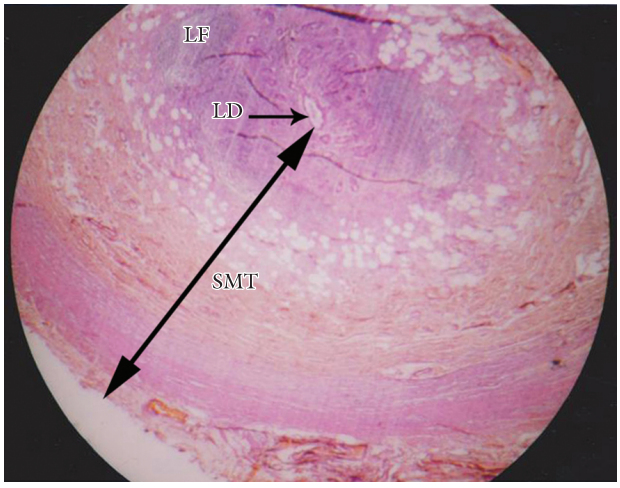

Figure 2. Abnormal Human Appendix shows Narrowed lumen and an Increased Wall Thickness (H&E × 100). LF-Lymphoid Follicle, SMT-Serosal Mucosal Thickness, LD-Luminal Diameter.

The finding observed in present study is both convincing and scientific in the sense that these two parameters, the thickness of the organ and lymphatic follicle diameter ultimately determine the caliber of the luminal diameter and hence its affectation.

5 Conclusion

The analysis of various parameters of vermiform appendix of normal and inflamed appendix has provided insight of histomorphometric changes in its structure capable of answering the age long question of whether disease associated with it are caused only by vascular phenomenon and luminal obstruction of extra appendiceal tissues or as a result of the interplay of its structural configuration with relation to different age. The lumen decreases where either the thickness or more especially the lymphoid follicle diameter increase in size suggesting that obstruction can occur because of the resulting microscopic and macroscopic changes that came to beam on normal subjects. This study gives a basis for occurrence of appendicitis and its related sequelae in clinical medicine.

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