A morphometric analysis of diameter of blood vessels in relation to degree of eosinophils and basophils (mast cells) infiltration for analysis of severity and prognosis of acute appendicitis

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Abstract

Background: Acute appendicitis is the most common surgical emergency that affect about 7% of the population. Obstruction of the lumen by faecoliths is the usual cause of acute appendicitis. The histopathologic stages of appendicitis are important to regarding prognosis. Study of diagnostic accuracy and pathological correlation aiming to reduce not only late complications but also the rate of negative appendicectomies had been studied worldwide.

Application of quantitative microscopic techniques for assessing the diagnosis of inflammatory condition of appendix is quite useful since it imparts objectivity and reliability to the diagnosis. This is the first study we carried out for analysis of severity of acute appendicitis by morphometric measurement of diameter of blood vessels in relation to degree of eosinophil and basophil (mast cells) infiltration.

Materials and Methods: A retrospective review of archives of the department of pathology, for the period of three years from 2010 to 2012, we identified 100, clinically and histopathologically diagnosed cases of acute appendicitis.

Slides and blocks were selected for the study. Blood vessel morphometric analysis was performed on H and E stained sections. Diameter of one largest measuring blood vessel in each layer (Mucosa, Muscularis propria, Submucosa and serosa) of the appendix, is measured by using ProGres Capture Pro software. Mean and SD of the diameters of the blood vessels in each layer of appendix in 100 specimens were calculated.

Number of eosinophils infiltration/10 hpf on H & E stained sections and number of mast cells infiltration/10 hpf on Toluidine blue stained sections in each layer of appendix in 100 specimens were counted by using light microscopy.

Results: All 100 patients, who underwent operative procedure, came with chief complaint of pain in the periumbilical region migrating to the right iliac fossa. The mean age of presentation was 25.10 years.

This study found that as there is increase in the number of eosinophils (counted as number of eosinophils/10 hpf) in each layer of appendix, there is decrease in the diameter of blood vessel in corresponding layer of the same appendicectomy specimen and as there is increase in the number of mast cells (counted as number of mast cells/10 hpf) in each layer of appendix, there is also increase in the diameter of the blood vessel in corresponding layer of the same appendicectomy specimen in acute appendicitis.

Conclusion: The study establishes the statistical correlation between morphometry and degree of cellular inflammatory infiltrate (eosinophils and mast cells counted by using light microscopy) which helps to assess severity of inflammation of the appendix.

Keywords: Acute Appendicitis, Morphometry.

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Introduction

Acute appendicitis is the most common acute surgical condition of the abdomen.[1,2] Untreated, mortality is high, mainly because of peritonitis and shock.[3] Acute appendicitis is a condition when the cause of acute abdominal pain and signs like fever, vomiting and right iliac fossa tenderness can be traced to the appendix.

There can be no doubt about the cause when the surgeon come across a red and swollen appendix during emergency laparotomy done for suspected appendicitis. But such a typical gross appearance may not be present always.

For this reason, pathologists have always grappled with the problem of how to define acute appendicitis in specimens of the vermiform appendix.

Acute appendicitis seems to be the end result of a primary obstruction of the appendix lumen.[4] The prevalence of faecoliths in patients with appendicitis is significantly higher in developed than in developing countries,[5] and an appendiceal faecolith is commonly associated with complicated appendicitis.[6]

Other causes of obstruction could be lymphoid hyperplasia, intestinal worms, tumors or other conditions.[2] To obtain accurate preoperative diagnosis may be difficult in many cases.[7]

Despite extraordinary advances in modern radiographic imaging and diagnostic laboratory investigations, the diagnosis of appendicitis remain essentially clinical requiring a mixture of observations, clinical acumen and surgical sense. On the other hand, prompt diagnosis and early operation can result in a number of negative appendectomies.[3] Many surgeons will accept a certain rate of negative laparotomy in order to avoid missing an inflammed appendix and its complications like perforation.[8]

Suppuration or the presence of neutrophils has long been the defining feature of acute appendicitis. The textbook view is that the diagnosis of classic suppurative appendicitis (phlegmonous appendicitis) should be made only in the presence of neutrophils in the muscularis propria.[9] The significance of mucosal neutrophils in the absence of muscle involvement has been contentious. Some investigators have considered more than 10 neutrophils/5 HPF found in the mucosa to be evidence of early appendicitis.[10] On the other hand, Pieper et al[11] found inflammation limited to the mucosa in appendices removed incidentally and suggested that the condition is not responsible for actual complaints.

This study was carried out to analyze the clinical presentation, diameter of blood vessels (measured by morphometry) and histopathological study (degree of eosinophils and mast cells infiltration). So this morphometric assisted study could help to correlate the variations in the diameter of the blood vessels in acute appendicitis with the use of morphometry along with histologic study.

This study also helps to correlate the severity of inflammation with the degree of eosinophils and basophils (mast cells) infiltration.

Methodology

Source of data: A data set of 100 appendicectomy specimens morphologically showing features of acute appendicitis in samples received in our pathology diagnostic laboratory between 2010 - 2012.

Method of collection of data: A retrospective review of archives of the department of pathology of AJIMS, for the period of 2010 through 2012 which were diagnosed as acute appendicitis.

Slides showing features of acute appendicitis were selected for the study. Cases in which the whole appendix was gangrenous and in which the muscle layer could not be made out were excluded because the inflammatory cell components were not countable.

Blocks were recutted and slides were stained with H&E stain and Toluidine blue stain separately.

Number of eosinophils infiltration/10 hpf on H & E stained sections and number of mast cells infiltration/10 hpf on Toluidine blue stained sections in each layer of appendix in 100 specimens were counted by using light microscopy.

Informed consent of the patient for the present study was not required as specimens were selected from routine samples received in the laboratory.

The patient details like age, clinical history and clinical diagnosis were noted from the records maintained.

Morphometric analysis: Morphometric analysis of blood vessels was performed on H and E stained sections by using photomicrographs taken at 10x magnification. The largest measuring blood vessel in each layer of the wall of the appendix was measured by using ProGres CapturePro software. Mean and SD of the diameters of the blood vessels in each layer of appendix in 100 specimens were calculated.

Inclusion criteria:
Specimens showing features of acute appendicitis.

Exclusion criteria:
1. Histologically confirmed pathologies other than acute appendicitis.
2. Appendix which are removed during other surgeries like uterus, gall bladder etc.
3. Interval appendicectomy specimens.

Results

The present study is a retrospective study comprising of microscopic grading of cellular infiltrates and morphometric analysis of archived histological specimens obtained from one hundred appendicectomies of patient diagnosed with acute appendicitis in the department of A J Institute of Medical Sciences. The mean age of the study subjects was 25.10years with the standard deviation of 13.3 years. The minimum age was 4 years and the maximum age was 72years.

Incidence of acute appendicitis is more common in males (62%) than in females (38%).

The most common clinical presentation was pain in abdomen and least common symptom was fever.

Many appendicectomy specimens were grossly inflammed and showed congestion and few showed widened submucosa.

The exudate seen in the muscle layer of grossly inflammed appendices was composed of a mixture of neutrophils and eosinophils (APA). But surface exudate was absent in all the cases. There was mucosal ulceration in few cases. Some of the eosinophils were partially degranulated. There were increased eosinophils in the mucosa and submucosa also seen in these cases.

Table 1: Descriptive Statistics

	No. of Cases	Serosa		Muscularis Propria		Sub mucosal Layer		Mucosal Layer	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
No. of Eosinophils/10hpf	100	129.92	70.2	160.99	86.11	157.60	86.36	228.07	119.10
No. of Mast cells/10hpf	100	177.03	100.9	168.24	102.03	173.05	101.53	164.30	102.89
Diameter of largest measuring blood vessels	100	623.32	225.4	588.99	250.8	609.86	235.61	571.35	268.45

This table shows number of eosinophilic count go on increasing from serosa to submucosa to muscularis propria to mucosa. So maximum eosinophils are seen in mucosa and minimum in serosa, and this exactly vice versa for the mast cells. We found maximum number of mast cells in serosa and minimum number in mucosa.

Diameter of blood vessels is also go on decreasing from serosa to mucosa.

Table 2: Correlation between No. of Eosinophils infiltration and Diameter of blood vessels in all layers

	No. of Eosinophils/10hpf in Serosal Layer	No. of Eosinophils/10hpf in Muscularis Propria Layer	No. of Eosinophils/10hpf in Sub Mucosal Layer	No. of Eosinophils/10hpf in Mucosal Layer
Diameter of Blood vessels	-0.906	-0.928	-0.924	-0.930
Significance	P<0.001 VHS	P<0.001 VHS	P<0.001 VHS	P<0.001 VHS

This table shows that there is negative (inverse) correlation between no. of eosinophils /10hpf and diameter of blood vessels in all layers of appendix.

This negative (Inverse) correlation indicates that as there is increase in the number of eosinophils, there is decrease in the diameter of blood vessels and as there is decrease in the number of eosinophils, there is increase in the diameter of the blood vessels in the same layer in acute appendicitis.

This correlation is statistically very highly significant with $P < 0.001$.

Table 3: Correlation between no. of mast cells/10hpf and diameter of blood vessels in all layers

	No. of Mast Cells/ 10hpf in Serosal Layer	No. of Mast cells/10hpf in Muscularis Propria Layer	No. of Mast cells/10hpf in Sub Mucosal Layer	No. of Mast Cells/10hpf in Mucosal Layer
Diameter of Blood vessels	0.931	0.934	0.933	0.934
Significance	P<0.001	P<0.001 VHS	P<0.001 VHS	P<0.001 VHS

This table shows that there is positive (direct) correlation between no. of mast cells/10hpf and diameter of blood vessels in all layers of appendix.

This positive (Direct) correlation indicates that as there is increase in the number of mast cells, there is also increase in the diameter of blood vessels and as there is decrease in the number of mast cells, there is also decrease in the diameter of blood vessels in the same layer in acute appendicitis.

This correlation is statistically very highly significant with $P < 0.001$.

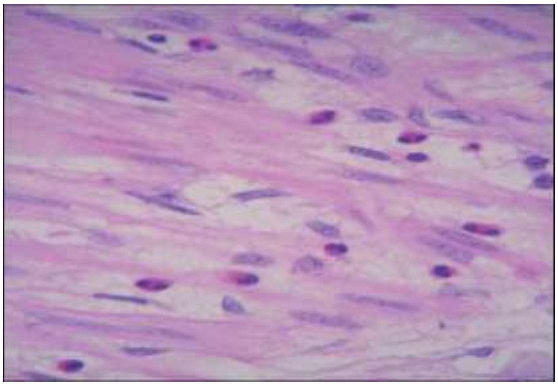

Fig. 1: Eosinophil – Edema lesion. H&E stain, 40x

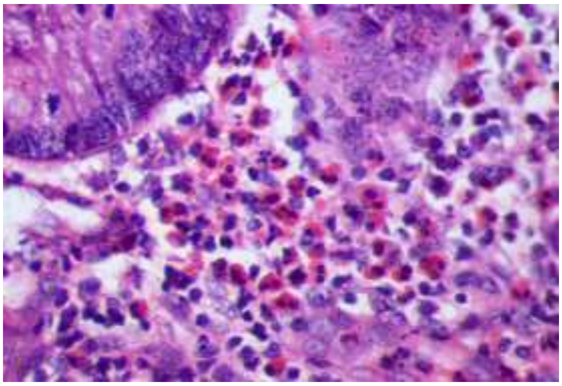

Fig. 2: Mucosa with eosinophilic infiltration. H&E stain, 40x

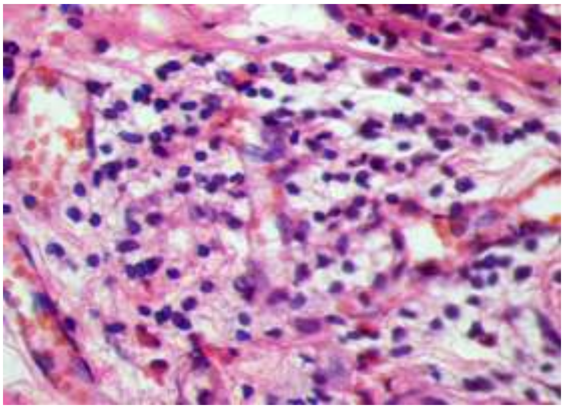

Fig. 3: Submucosa with eosinophilic infiltration. H&E stain, 40x

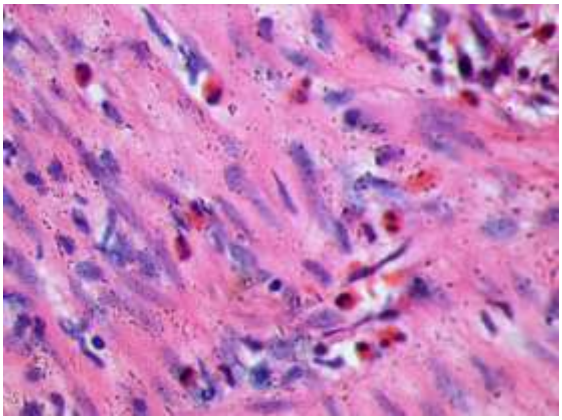

Fig. 4: Muscularis propria with eosinophilic infiltration. H&E stain, 40x

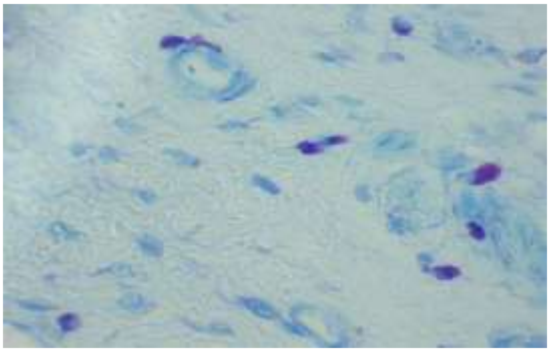

Fig. 5: Submucosa with mast cells infiltration. Toluidine blue stain, 40x

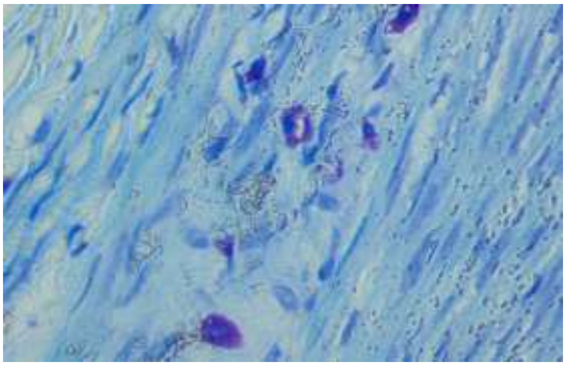

Fig. 6: Muscularis propria with mast cells infiltration. Toluidine blue stain, 40x

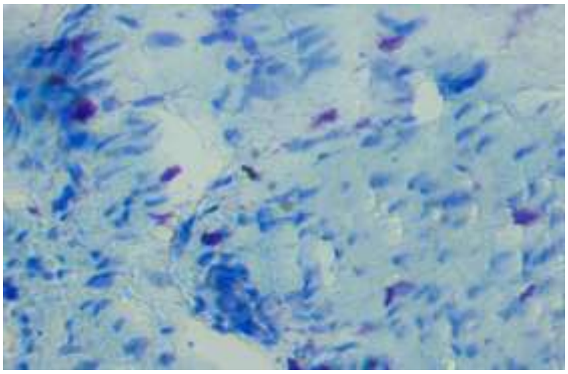

Fig. 7: Serosa with mast cells infiltration. Toluidine blue stain, 40x

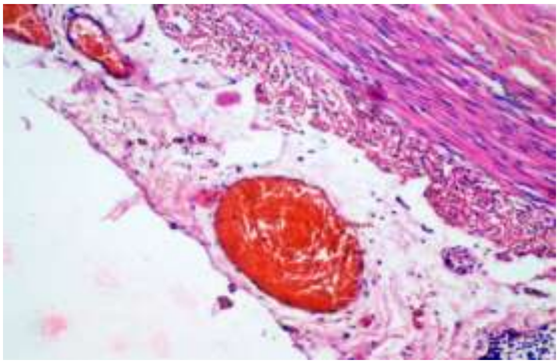

Fig. 8: Serosa with largest measuring bld vessel. H&E stain, 10x

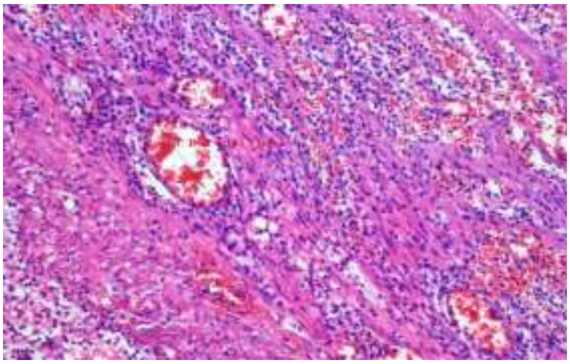

Fig. 9: Muscularis propria with largest measuring bld vessel. H&E stain, 10x

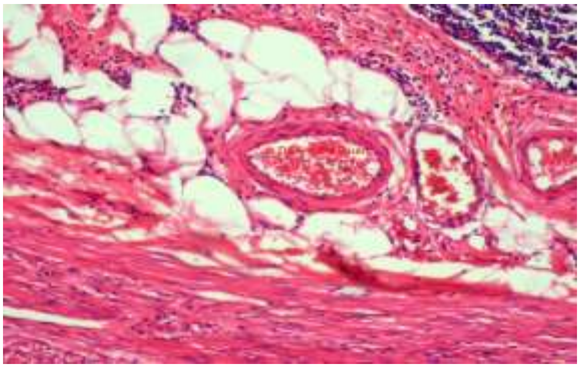

Fig. 10: Submucosa with largest measuring bld vessel. H&E stain, 10x

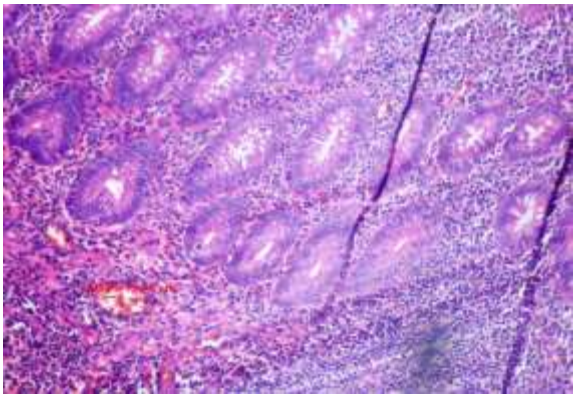

Fig. 11: Mucosa with largest measuring bld vessel. H&E stain, 10x

Discussion

Acute appendicitis is the most common acute surgical condition of the abdomen.[2,3] Appendicitis is most common between the ages of 10 and 20 years, but can occur at any age according to Humes DJ et al.[12]

But in our study subjects the mean age of presentation is 25.10 years with standard deviation of 13.3 years. Minimum age is 4 years and maximum age is 72 years. Appendicitis is more common in men according to Humes DJ et al.[12] In our study Subjects we also found more common incidence in men (62%) than in women (38%).

Acute appendicitis seems to be the end result of a primary obstruction of the appendix lumen according to Wangensteen OH and Pieper R et al.[7] The prevalence of faecoliths in patients with appendicitis is significantly higher in developed than in developing countries[9] and an appendiceal faecolith is commonly associated with complicated appendicitis.[10] In our study subjects many appendicectomy specimens (42%) showed faecoliths in their lumen.

Finding of increased number of eosinophils in the muscle layer in cases of acute appendicitis has lead to the hypothesis that it may have an allergic origin. Our study aimed to measure the degree of eosinophils infiltration in different layers of appendicectomy specimens and compare it with diameter of largest measuring blood vessel in corresponding layers which helps to find out the severity and type of inflammation.

Eosinophils as the sole infiltrate in the muscle layer in appendicitis have been described before. Stephenson and Snoddy chose to call these 'subacute appendicitis' though the clinical correlation needed to justify this term was not mentioned in their study.[13]

Aravindan[14] in a study of 120 appendicectomies found that mural eosinophil infiltration is a consistent finding in acute appendicitis. He also described cases similar to what Jona *et al*[15] described, in which eosinophil infiltrate was the sole finding. He suggested that eosinophil infiltrate seen in acute appendicitis is an early event linked possibly to type I hypersensitivity.[14]

This was in contrast to what was believed earlier - that infiltration of eosinophils is evidence of subacute or chronic inflammation.[13]

In our study we counted number of eosinophils/10hpf by using light microscopy in each layer of 100 appendicectomy specimens. We found that the eosinophils count go on increasing from serosa to submucosa to muscularis propria to mucosa. So maximum number of eosinophils seen in mucosa and minimum number is seen in serosa.

In the same way we measured the diameter of largest measuring blood vessel by using morphometry in each layer of 100 appendicectomy specimens. We found that the diameter of largest measuring blood vessel go on decreasing from serosa to submucosa to muscularis propria to mucosa. So maximum diameter is seen in serosa and minimum diameter is seen in mucosa.

Then we compared the degree of eosinophils infiltration in particular layer of the appendicectomy specimen with diameter of the largest measuring blood vessel in the same layer of the same appendicectomy specimen. Same comparison has been done in all four layers of the same appendicectomy specimen for all 100 appendicectomy specimens.

From this comparison we found that there is a negative (Inverse) correlation between the degree of eosinophils infiltration and the diameter of largest measuring blood vessel in particular layer of appendicectomy specimen which is statistically very highly significant with $P < 0.001$.

This negative (Inverse) correlation means as there is increase in the number of eosinophils, there is decrease in the diameter of the blood vessel in the same layer of the same appendicectomy specimen.

There will be few eosinophils present in normal appendix usually, but their count will be increased in acute appendicitis to limit the progression of inflammation.

Eosinophils admixed with lymphocytes and unaccompanied by edema may be seen in cases of resolving appendicitis according to Ciani S et al.[16] and also it has been mentioned that Acute eosinophilic appendicitis may merely represent those cases that do not proceed on to suppuration.

This showed that eosinophils have protective role. Eosinophils limit the progression of inflammation to suppurative inflammation.

Mast cells are known to be effector cells in various inflammatory reactions, but their role in appendicitis is unclear. In a study of total 150 appendices including normal and inflammed appendices for assessing their histological changes and density of neutrophil, lymphocyte and eosinophil infiltration by Vijaya V Mysorekar et al, mast cells were counted in 1% touidine blue-stained sections.

It was found that eosinophil counts in all the layers were significantly low in normal appendices with $P<0.01$ and in chronic appendicitis with $P<0.1$ as compared to acute appendicitis. Mast cell counts were lowest in normal appendices, significantly higher in acute appendicitis with $P<0.01$ and highest in chronic appendicitis with $P<0.001$[17].

So in our study we counted number of mast cells/10hpf in 1% touidine blue stained sections by using light microscopy in each layer of 100 appendicectomy specimens which were diagnosed as acute appendicitis. We found that the mast cell counts, significantly higher with $P<0.001$.

Also found that mast cell counts go on decreasing from serosa to submucosa to muscularis propria to mucosa. So maximum number of mast cells seen in serosa and minimum number is seen in mucosa.

In the same way we measured the diameter of largest measuring blood vessel by using morphometry in each layer of 100 appendicectomy specimens. We found that the diameter of largest measuring blood vessel go on decreasing from serosa to submucosa to muscularis propria to mucosa. So maximum diameter is seen in serosa and minimum diameter is seen in mucosa.

Then we compared the degree of mast cells infiltration in particular layer of the appendicectomy specimen with diameter of the largest measuring blood vessel in the same layer of the same appendicectomy specimen. Same comparison has been done in all four layers of the same appendicectomy specimen for all 100 appendicectomy specimens.

From this comparison we found that there is a positive (Direct) correlation between the degree of mast cells infiltration and the diameter of largest measuring blood vessel in particular layer of appendicectomy specimen which is statistically very highly significant with $P < 0.001$.

This positive (Direct) correlation means as there is increase in the number of mast cells, there is also increase in the diameter of the blood vessel in the same layer of the same appendicectomy specimen.

This positive (Direct) correlation is because of mast cells upon degranulation release certain chemical mediators like histamine etc, which causes vasodilation.

According to Vijaya V Mysorekar et al in their study of 150 appendicectomy specimens, obstruction due to faecoliths or parasites were seen in only 20.1% and 2.1% of the inflammed appendices respectively. Hence a Type I hypersensitivity reaction with release of chemical mediators by mast cells might be another triggering factor for the sequence of events leading to appendicitis[17].

But in our study we found faecoliths in 42% of specimens and no parasites seen. Increase in eosinophils and mast cells may explain the pain in histologically normal but clinically suspected acute appendicitis.

Conclusion

- Acute appendicitis is more prevalent in elderly patients with mean age of 25.10 years.
- Majority of the patients presented with pain abdomen and nausea.
- There is negative co relation between number of eosinophils and diameter of largest blood vessel in the same layer of appendix in acute appendicitis. So this showed that eosinophils have protective role in inflammation and limit the progression of inflammation to suppurative stage.

So acute appendicitis with high eosinophil count is milder form and usually in resolving stage of appendicitis.

- Vice versa number of mast cells and diameter of largest measuring blood vessel shows positive correlation. So this showed that mast cells upon degranulation release certain chemical mediators like histamine etc, which causes vasodilation.

So acute appendicitis with high mast cell count is relatively severe form.

The present study underlines the potential significance of blood vessel morphometry and degree of eosinophils and mast cells infiltration in acute appendicitis to predict the risk of progression to suppurative stage.

Summary

Application of blood vessel morphometry and degree of eosinophils and mast cells to cases of acute appendicitis will help to know type of inflammation and severity of inflammation which helps in proper management of these patients.

The pathologists report can also be quantitated and documented using morphometry. Though morphometry has a wide role in acute appendicitis, the expense of such a quantitative technique must be balanced against the utility of the information gained from its use. It is important to focus application of quantitative techniques on real diagnostic dilemmas because in practice, visual examination of a slide requires less time and effort than image analysis.

The study underlines the potential significance of blood vessel morphometry and degree of eosinophils and mast cells infiltration in acute appendicitis to predict the risk of progression to suppurative stage.

Bibliography

1. Duzgan AP, Moram M, Uzun S et al. Unusual findings in appendectomy specimens: Evaluation of 2458 cases and review of the literature. Indian J Surg 2004;66:221-6.
2. Edino ST, Mohammed AZ, Ochicha O, Anumah M. Appendicitis in Kano, Nigeria: a 5 year review of pattern, morbidity and mortality. Ann Afr Med 2004;3:38-41.
3. Hobler, K. Acute and Suppurative Appendicitis**:** Disease Duration and its implications for Quality Improvement. Permanente Medical Journal Spring 1998;2(2).
4. Wangensteen OH, Bowers WF. Significance of the obstructive factor in the genesis of acute appendicitis. Arch Surg 1937;34:496-526.
5. Jones BA, Demetriades D, Segal I, Burkitt DP et al. The prevalance of appendiceal faecoliths in patients with and without appendicitis. A comparative study from Canada and South Africa. Ann Surg 1985;202(1)**:**80-2.
6. Nitecki S, Karmeli R, Sarr MG et al. Appendiceal calculi and Faecoliths as indications for appendectomy. Surg Gynecol Obstet 1990;171(3):185-8.
7. Anderson RE, Hugander A, Thulin AJ. Diagnostic accuracy and perforation rate in appendicitis: Association with age and sex of the patient with appendectomy rate. Eur J Surg 1992;158:37-41.
8. Abu- Eshy SA, Ibn Ouf MA, Malatani TS et al. Acute appendicitis in females: a clinical study of 366 cases. Afr J med sci 1995;24:227-30.
9. Liu C, Crawford JM. The Gastrointestinal tract. In: Kumar V, Abbas AK, Fausto N, eds. Robbins and Cotran Pathologic Basis of Disease. 7th edition. Philadelphia PA: Elsevier inc;2004:797-875.
10. Barcia JJ, Reissenweber N. Neutrophil count in the normal appendix and early appendicitis: Diagnostic index of real acute inflammation. Ann Diagn Pathol 2002;6:352-6.
11. Pieper R, Kager L, Nδsman P. Clinical significance of mucosal inflammation of the vermiform appendix. Ann Surg 1983;197:368-74.
12. Humes DJ, Simpson J. Acute appendicitis. BMJ. 2006 Sep 9;333(7567):530- 4.
13. Stephenson J, Snoddy WT. Appendiceal lesions. Observation in 4,000 appendectomies. Arch Surg 1961;83:661-6.
14. Aravindan KP. Eosinophils in acute appendicitis. Possible significance. Indian J Pathol Microbiol 1997;40:491-8.
15. Jona JZ, Belin RP, Burke JA. Eosinophilic infiltration of the gastrointestinal tract in children. Am J Dis Child 1976;130:1136-9.
16. Ciani S, Chuaqui B. Histological features of resolving acute, non-complicated phlegmonous appendicitis. Pathol Res Pract 2000;196:89-93.
17. Vijaya V Mysorekar, S Chanda, Chitralekha P Daneeka. Mast cells in surgically resected appendices. Indian Journal of Pathology and Microbiology 2006;49(2):229-33.