

Topic- Study of role of special stains in determination of etiologies of acute appendicitis - A cross sectional study

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Abstract

Background: Acute appendicitis is a very common acute surgical condition of abdomen. If left untreated the condition could be life threatening because of perforation causing peritonitis and shock. When a surgeon comes across an inflamed and red appendix during surgery the diagnosis is quite obvious. However, such typical gross findings may not be present always. Life time risk of appendicitis is about 7% and it is most commonly seen in adolescents and young adults. In spite of advances and new technologies and

imaging techniques, there is a dilemma in the diagnosis of acute appendicitis, and most often the diagnosis is based on the mixture of history, observations, clinical acumen and surgical sense. Whereas prompt diagnosis and surgery may result into negative appendicectomies. Hence histopathological examination of appendicectomy specimens remain gold standard for diagnosis of appendicitis. Not only diagnosis of inflammation but also a clue to the etiology and specific diagnosis can be obtained with help of histopathological examination and special staining.

Aims and Objectives: To assess the various etiologies causing obstruction and inflammation in appendicectomy specimens using special stains

Material and Methods: After receiving approval of Institutional ethics committee the study was conducted in the histopathology section of our department using the specimens of appendicectomy sent for histopathological evaluations during the mentioned study period. Total 150 specimens were evaluated and the slides were stained with routine stains like H and E and special stains like PAS and Alcianblue. Histo-morphological features as seen in the slides stained with the all the 3 stains mentioned above were studied and following observations were made

Observations and results: We studied 150 appendicectomy specimens and the different diagnosis established after thorough examination of H and E, Alcian blue and PAS stained sections were Acute suppurative appendicitis-80 cases (25 of them showed fecolith) (53%), Acute gangrenous appendicitis-25 cases (16%), Acute on Chronic appendicitis-15 cases (10%), Acute appendicitis with lymphoid hyperplasia-25 cases (16%), Acute appendicitis with Mucormycosis-1 case (0.6%), Acute appendicitis with candidiasis-1 case (0.6%), Mucocoele-1 case (0.6%), Hypoplastic appendix-1 case (0.6%), Tumor metastasis-1 case (0.6%)

Conclusions: Acute appendicitis could be due to variety of causes. Histopathological evaluation remains gold standard for accurate diagnosis and though not every time but in many cases special stains help to pinpoint the etiology thereby helping in better management of the patient.

Keywords: Appendicitis, Appendicectomy, Histopathology, Special stains.

Introduction

Acute appendicitis is a very common acute surgical condition of abdomen. (1,2) Most commonly it presents as the pain in abdomen which can be traced to right iliac fossa, accompanied with fever and vomiting. If left untreated the condition could be life threatening because of perforation causing peritonitis and shock. (3) When a surgeon comes across an inflamed and red appendix during surgery the diagnosis is quite obvious. However, such typical gross findings may not be present always.

Life time risk of appendicitis is about 7% and it is most commonly seen in adolescents and young adults (4) Incidence of acute appendicitis is increasing in developing countries probably due to adoption of western diet (5).

Acute appendicitis seems to be the outcome of obstruction of appendicular lumen (6). most commonly due to faecoliths (7) as well as because of other causes like, worms, lymphoid hyperplasia, tumors to name a few (2) In spite of advances and new technologies and imaging techniques, there is dilemma in the diagnosis of acute appendicitis, and most often the diagnosis is based on the mixture of history, observations, clinical acumen and surgical sense (8,9). Whereas prompt diagnosis and surgery may result into negative appendicectomies (3).

Hence histopathological examination of appendicectomy specimens remain gold standard for diagnosis of appendicitis. Not only diagnosis of inflammation but also a clue to the etiology and specific diagnosis can be obtained with help of histopathological examination and special staining. At times unusual findings like incidental tumors may be found too during histopathological examination and hence it becomes important to study every single specimen of resected appendix (8).

Aims and objectives

To assess the various etiologies causing obstruction and inflammation in appendicectomy specimens using special stains.

Materials and methods

Study Design: Cross sectional study.

Place of study: After obtaining approval of the institutional ethical committee, this study was conducted in histopathology department of a tertiary care center.

Study duration: Nov. 2020-Nov 2022

Sample size: All the specimens of appendix which fit the inclusion criteria, received during the mentioned study period

Data Collection: All the specimens of appendix received for histopathological evaluation during the mentioned study period.

Inclusion criteria

1) The specimens of resected appendix sent for examination during Nov. 2020-Nov 2022

Exclusion criteria

1) Interval appendicectomy specimens.

Total 150 specimens were evaluated.

Methodology

Study utilized blocks and slides prepared from appendicectomy specimens sent to our department. From each block 3 slides were made and stained with Hand E, PAS and Alcian blue respectively and examined under light microscope thoroughly. Histo – morphological features as seen in the slides stained with the all the 3 stains mentioned above were studied. Results were expressed using tables and charts.

Observations and results

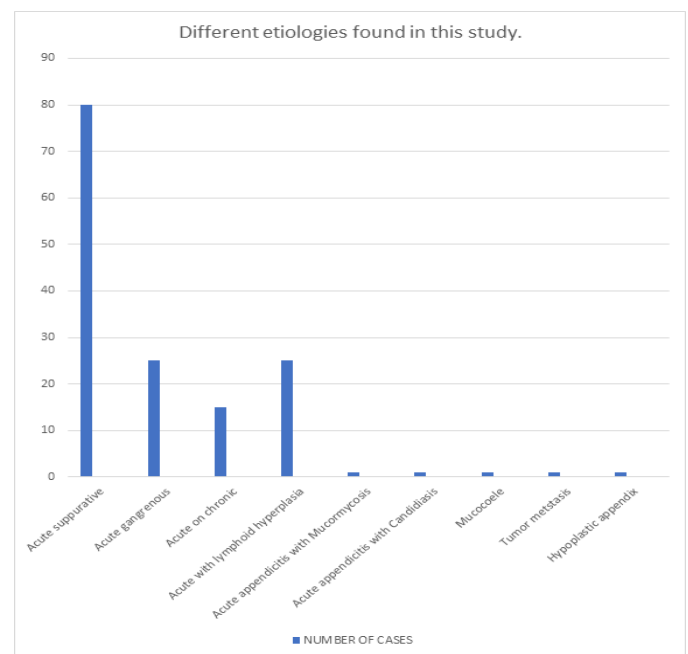
We studied 150 appendicectomy specimens and the different diagnosis established after thorough

examination of H and E, Alcian blue and PAS-stained sections were as follows.

- Acute suppurative appendicitis-80 cases (25 of them showed fecolith) (53%)
- Acute gangrenous appendicitis-25 cases (16%)
- Acute on Chronic appendicitis-15 cases (10%)
- Acute appendicitis with lymphoid hyperplasia-25 cases (16%)
- Acute appendicitis with Mucormycosis-1 case (0.6%)
- Acute appendicitis with candidiasis-1 case (0.6%)
- Mucocoele-1 case (0.6%)
- Hypoplastic appendix-1 case (0.6%)
- Tumor metastasis-1 case (0.6%)

Following chart represents the variety of etiologies found in this study.

Chart1:



Following are some of the images of significant findings as seen on routine as well as special stains.

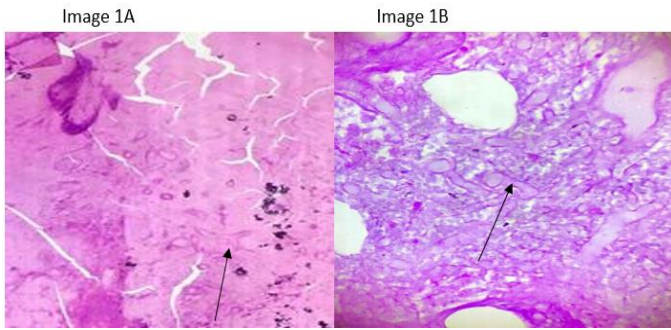


Image 1A: Showing Hand E-stained section from appendix showing fungal hyphae in the lumen

Image 1B: showing PAS-stained section from the same case confirms presence of fungal hyphae suggestive of mucormycosis.

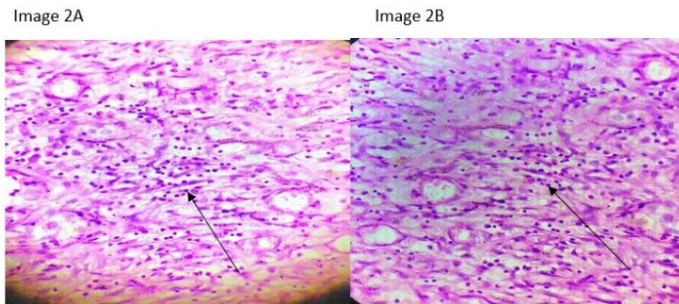


Image 2: A shows H and E-stained section which shows presence of yeast cells (Arrow) in appendicular tissue

Image 2B: shows PAS-stained section from same case which confirms presence of yeast cells (Arrow) suggestive of Candida species.

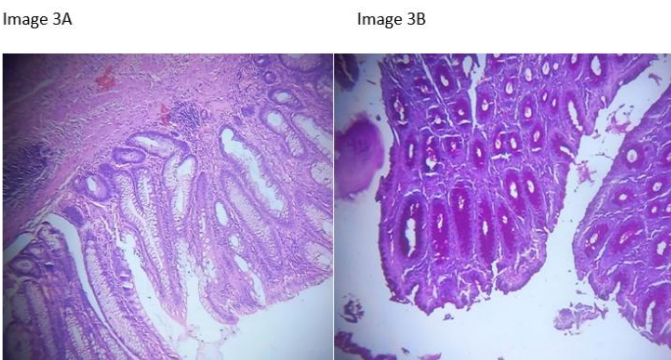


Image 3A: showing a Hand E-stained section from case of mucocoele of appendix which shows hyperplastic mucosa lined by goblet cells.

Image 3B: PAS-stained section from the same case highlighting PAS positive mucin globules of goblet cells

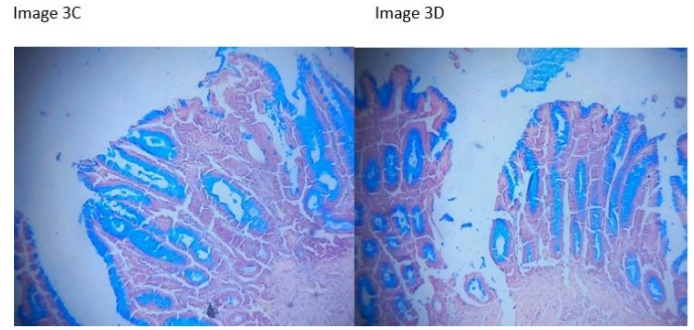


Image 3C: and 3D show Alcian blue stained sections of same case which confirm Alcian blue positive hyperplastic mucosa lined by mucin secreting goblet cells.

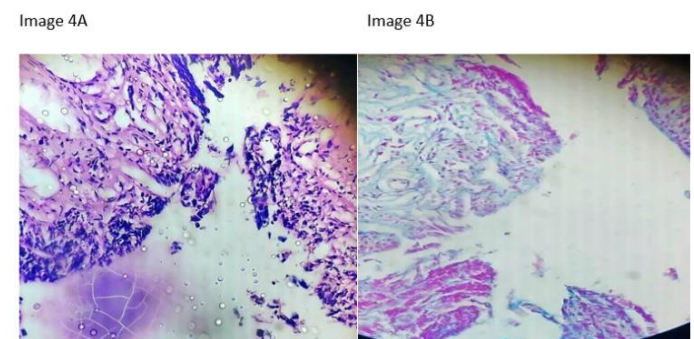


Image 4A: showing a H and E-stained section of appendix in which there is loss of normal mucosa and replacement by metastatic deposition of malignant tumor cells

Image 4B: showing Alcian blue stained section from the same case showing malignant cells negative for Alcian blue.

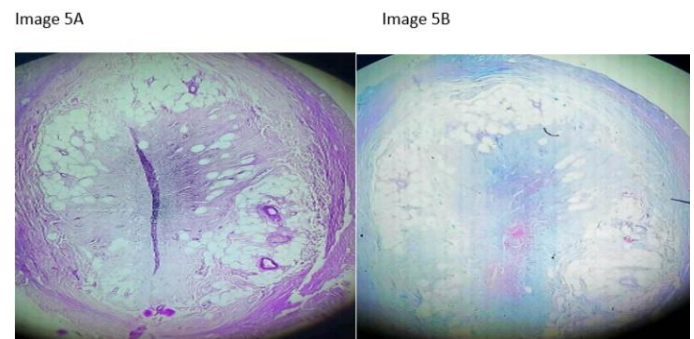


Image 5A: shows a H and E-stained section of a hypoplastic appendix from a case of malrotation of gut.

Image 5B: is an Alcian blue stained section of the same case showing total absence of mucosa.

Discussion

Comparison with various studies

- In the recent study by Aneel myageri et al (10) titled Clinicopathologic Study of Appendix Specimens- A Two Year Retrospective Study at a Tertiary Care Center, they studied histopathological features and utility of USG in case of acute appendicitis.

In their study they studied 472 appendectomy cases out of which 283 (59.95%) were males and 189 (40.04%) were female patients. They found that most of the patients presented in the 3rd decade of life. e. Mean age was 29.25± 15.09 years (median age-26)

- In our study we found that out of the 150 cases 80 were male (53.6%) and 70 were female (46.4%) and maximum cases were from the age group of 21-30 years (that is the 3rd decade) followed by 11-20 years and 31-40 years respectively. So we can see that these findings are similar to the findings of the study by Aneel myageri.

- Mohammadayub Jat et al (11)-Histopathological records of 480 resected appendices submitted to histopathology department were studied to determine acute appendicitis, complication rate, histopathological diagnosis and unusual finding on histology. Out of 480 specimens of appendix ,466 (97.0%) accounted for Appendicitis, with peak occurrence in the age group of 11-50 years in male and 11-40 years in female.

- The study supports routine histological examination of all the appendectomy specimens to avoid missing of any clinically important and treatable condition.

Following table represents their findings.

Histopathological findings	Number of cases with percentage
Acute appendicitis	250 (52%)
Suppurative appendicitis	135 (28%)

Gangrenous appendicitis	60 (12.5%)
Perforated appendicitis	19 (2%)
Chronic appendicitis	9 (2.5)
Normal appendix	14 (3%)

Table 3: Representing total number of cases classified as per the etiology in the study by Mohammadayub et al (11)

In comparison with this following are the etiologies found in our study which included unusual findings such as fungal infection, tumor metastasis etc. as well

Diagnosis	Number of cases	Percentage
Acute suppurative appendicitis	80	53%
Acute gangrenous appendicitis	25	16%
Acute on chronic appendicitis	15	10%
Acute appendicitis with lymphoid hyperplasia	25	16%
Acute appendicitis with Mucormycosis	01	0.6%
Acute appendicitis with Candidiasis	01	0.6%
Mucocoele	01	0.6%
Tumor metastasis	01	0.6%
Hypoplastic appendix	01	0.6%

Table 4: Number of cases showing different etiologies along with percentage.

- Appendix is a site for different neoplastic lesions.
- Medha Kulkarni et al (12) did a two years study to determine the incidence of various non-neoplastic and neoplastic lesions of the appendix. clinical findings were correlated with histopathological features, in each case.

Out of 436 appendectomy specimens, non-neoplastic lesions involved 99.34% cases and 0.66 % by neoplastic lesions.

- Acute appendicitis with/without perforation, chronic appendicitis, worm infestation and fibrous obliteration were included in Nonneoplastic lesions whereas 1 case each of neuroendocrine neoplasm (NET), adenoma & Non Hodgkin lymphoma were included in Neoplastic lesions (12).
- However, we did not come across any primary malignancy of appendix in our study. Only one case showed metastatic deposit from unknown primary as an incidental finding.

We noticed that role of PAS in diagnosing fungal elements and that of Alcian blue in diagnosing mucinous conditions is well known, however in cases of appendicitis, high level of suspicion (especially in cases with uncontrolled Diabetes mellitus or immunocompromisation) and through histopathological evaluation using these stains helps in arriving at most accurate diagnosis there by helping the clinician to plan the care in a better manner.

As far as other etiologies/diagnosis that we mentioned, special stains do not appear to play any specific role, but further research can be done with respect to histochemical reactivity in acute appendicitis using these findings.

Comparison of various case reports.

Mucocoele

- Zazademetrashavilli et al (13) reported a case of mucinous cystadenoma which showed mucin in the lumen and mucosa lined by uniform layer of goblet cells.
- We found similar features in our study where we reported two cases of mucocoele (Mucinous cystadenoma). Diagnosing these becomes very essential

because if left untreated pseudomyxoma peritonei can develop.

Mucormycosis

- Appendiceal mucormycosis is a rare infection seen in immunocompromised patients which can be fatal. It is usually seen in chemotherapy induced neutropenia in patients with leukemias. Clinically, the symptoms and signs may be suppressed due to ongoing corticosteroids. The condition may appear as bacterial appendicitis or typhlitis. The disease requires surgical debulking and aggressive antifungal treatment.
- A case was reported by Sali P. et al (14) where routine histopathology showed gangrenous perforated appendicitis with Angio invasive mucormycosis. 105
- Heavy mucosal ulceration and necrosis were seen in wall of appendix with scattered small and large fungal colonies in submucosa, infiltrating the serosa and peri appendicular tissue.
- Broad aseptate hyphae with right angle branching was seen with PAS staining.
- In our study we reported one case of appendiceal mucormycosis where both of the specimen showed broad aseptate hyphae branching at right angles in the submucosa and around vessels along with all the features mentioned in the case report quoted above.
- This was well visualised by PAS stain and the diagnosis was confirmed.

Candidiasis

- Noppadol Larbcharoensub(15) reported a case series of fungal appendicitis where they mention that although it is uncommon, the disease can occur among immunosuppressed patients who have developed classical signs and symptoms of appendicitis. Early diagnosis and prompt surgery with medical treatment are associated with a better survival.

- They reported two cases of candida associated appendicitis where the histopathology of the appendix showed fungal organisms with suppurative inflammation and secondary peritonitis.
- In our study we came across one case of candidiasis of appendix and budding yeast cells were identified and confirmed by PAS staining. Surrounding area showed suppuration, inflammatory exudate and other features of acute appendicitis. There are many other case reports by Eleni Efraim Dou et al(16) , Sharat Chandra et al(17), Ken Liu(18) et al who have reported many parasitic conditions like *Enterobius vermicularis* infection, amoebiasis etc. However we did not come across any such pathologies in our study even though they are known causes of infection related appendicitis.

Limitations of this study

- Many special stains like GMS, Van Gieson, and others have been used by different researchers which help to cover a wide spectrum of etiologies. However, in our study we used only Alcian blue and PAS due to technical constraints.
- Many ancillary studies like Immunohistochemistry, Molecular pathology and cytogenetics when combined with routine histopathological examination will increase the scope and outcome of the study.

Overcoming these discrepancies would surely improve outcome of such research.

Conclusion

We conclude that

- Acute appendicitis could be due to variety of causes.
- Histopathological evaluation remains gold standard for accurate diagnosis and though not every time but in many cases special stains help to pinpoint the etiology thereby helping in better management of the patient.

- With regards to etiologies for confirmatory diagnosis of fungal appendicitis PAS staining is essential and should be done routinely in the case scenarios where patient is immunocompromised, has uncontrolled diabetes mellitus etc.
- For mucocoele and other mucin associated etiologies like mucinous cystadenoma, myxoglobulosis etc. Alcian blue stain is essential.
- In case of metastatic deposits in appendix these stains may help in identifying the type of malignancy and thereby helping in locating the primary focus.

We hope that our findings help pathologists to understand structural pathology in appendicitis better and thereby help in coming to most accurate diagnosis thus helping the clinicians in planning a better post-operative care.

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