Appendicular Histology and Its Clinical Significance in Patients with Ulcerative Colitis: A Prospective Study from North India

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ABSTRACT
Background and aims: The appendix may be involved in ulcerative colitis (UC) to variable extent. Clinical significance of appendicular involvement is not clear and it has long been considered an innocent bystander. This study prospectively investigated appendicular histology and its clinical significance in patients with UC.

Materials and methods: Colonoscopy was performed in 60 patients and biopsies were taken from appendiceal orifice, cecum, and colon. Appendiceal histology was classified as active if there was presence of neutrophils with crypt distortion. Inactive inflammation was defined as positive for crypt distortion and negative for neutrophils. Otherwise the histology was reported as normal or nonspecific changes. The patients were followed for a minimum period of 6 months.

Results: In patients evaluated endoscopically, 63% patients had histological involvement of the appendiceal orifice. Pancolitis was seen more commonly in patients with appendiceal orifice inflammation than in patients with normal appendiceal histology (44.7 vs 9%, p=0.001). Most patients with appendiceal orifice inflammation had involvement of the cecum. Skip lesion of the appendix without cecum involvement was seen in 37% of the patients. The patients with appendiceal orifice inflammation had a significantly higher grade of endoscopic colitis compared to patients with normal histology (p=0.006). Relapse rate was higher in patients with appendiceal orifice inflammation than in patients with normal appendiceal histology (18.4 vs 9%), although the difference was insignificant (p=0.329).

Conclusion: Appendicular involvement in ulcerative colitis is not uncommon. Its involvement may predict the course, extent, and severity of colitis.

Keywords: Appendix, Colonoscopy, Inflammation, Ulcerative colitis.


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INTRODUCTION
Inflammation in ulcerative colitis (UC) is believed to originate in the rectum and spreads in continuity to the proximal portions of the colon. However, several histopathological studies have confirmed that the proximal colon, especially at the base of appendiceal orifice, is frequently involved by inflammatory infiltrates in patients with UC.1-4 Studies on colectomy specimens show that appendiceal involvement is present in 15 to 86% of patients in the form of skip lesions.5,7 and other reports suggest that appendiceal involvement in UC always occurs in continuity with adjacent-involved cecum and the rest of the colon.8,9 The clinical significance of appendiceal involvement in UC has been elucidated in some studies and it was shown that patients with a periappendiceal inflammation had a more aggressive course with more frequent relapses.10

Studies have suggested an inverse relationship between appendectomy and the subsequent development of UC.11-15 The mechanisms for this protective effect of appendectomy on UC are unknown. It is speculated that removal of appendiceal-associated lymphoid tissues causes certain alterations in mucosal immune responses that prevent the onset of UC.

To evaluate the clinical and prognostic significance of appendiceal histology in patients with idiopathic ulcerative colitis (IUC), this study was conducted at a tertiary care hospital in North India.

MATERIALS AND METHODS
Study Design
This prospective study was conducted between July 2012 and December 2013 at a large tertiary care hospital in North India. All patients of IUC seen at our unit...
during the study period were prospectively assessed for inclusion in the study. We included patients of >12 years of age diagnosed as UC based on a typical history of diarrhea and/or blood/pus in stools, endoscopic appearance, and histological features compatible with UC.\textsuperscript{16} We excluded patients with previous appendectomy or bowel resection, pregnancy, known malignancy, or Crohn’s disease.

All patients underwent a detailed history and physical examination and the findings were recorded on a predesigned proforma, with a special emphasis on details of duration of disease, extent of disease, and treatment history. Detailed hematological and biochemical investigations were done. Clinical severity was determined by Truelove and Witts Classification of the Severity of Ulcerative Colitis.\textsuperscript{17} After obtaining informed consent colonoscopy was performed in all patients at the time of entry into the study. Colonoscopy was performed with flexible colonoscopy after administering intravenous midazolam as a premedication. Details of endoscopic findings were recorded. Endoscopic appearance of the appendiceal orifice was also noted (Fig. 1). Endoscopic severity of colitis was determined by using the Mayo score.\textsuperscript{18}

During colonoscopy, apart from biopsies of colon, biopsies were also obtained at the base of appendiceal orifice and cecum and were transported in a 10% formalin vial to the histopathology department for serial sections. Biopsies were examined by a histopathologist under light microscopy after staining with hematoxylin-eosin. The histopathologist was blinded to the clinical details.

**Histological Assessment of the Appendicular Orifice**

The inflammation in the histological specimen obtained by endoscopy from appendicular orifice was classified and graded as active or inactive disease or nonspecific/normal histology (Figs 2A and B and 3A and B).\textsuperscript{6,19} Active inflammation was defined as presence of neutrophils with crypt distortion. Inactive inflammation was defined as presence of crypt distortion and negative for neutrophils. When a biopsy specimen was negative for neutrophils and crypt distortion, the grade of inflammation was defined as minimal or normal histology. The severity of active inflammation was determined based on the density of infiltrating inflammatory cells and was classified as either mild, moderate, or severe. The other histological findings of the appendix noted were the presence of cryptitis, crypt abscess, goblet cell depletion, crypt branching/shortening, basal plasmacytosis, eosinophilic infiltration, ulceration, and Paneth cell metaplasia.

*Ulcerative appendicitis:* Ulcerative appendicitis was defined as the presence of a focal area of superficial inflammation containing either cryptitis or crypt abscess, a distorted crypt architecture, goblet cell depletion, and basal plasmacytosis.

*Histology of colon:* Histological disease activity was assessed and graded using Truelove and Richard criteria.\textsuperscript{17,20}

*Clinical remission:* Clinical remission was defined as normal frequency of bowel movements with formed stools, no abdominal pain, and no blood in the stools.
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**Endoscopic remission:** Endoscopic remission was defined as score of 0 on Mayo endoscopic score, i.e., normal mucosal appearance on endoscopy.

**Clinical relapse:** Clinical relapse was defined as worsening of bowel function and reappearance of rectal bleeding.

**Clinical and/or Endoscopic Follow-up**

All the patients included in the study were followed up for at least 6 months. During follow-up the number of relapses was noted. The treatment given to the patients was recorded.

**Data Analysis**

We compared patients with appendiceal orifice inflammation (AOI+) vis-à-vis patients with normal appendiceal orifice histology (AOI−). Differences in clinical features, laboratory parameters, endoscopic features, histopathological features, and relapse rates were analyzed.

**STATISTICAL METHODS**

The quantitative variables were estimated using mean, median, and standard deviation. Qualitative or categorical variables were described as frequencies and proportions. The categorical variables were compared using chi-square or Fisher’s exact test whichever was applicable, whereas means of two groups were compared using Student’s t-test. Correlations were calculated using Spearman’s r-test. All statistical tests were two-sided and were performed at a significance level of \( p < 0.05 \).

**RESULTS**

Sixty patients of IUC were prospectively enrolled in the study. They had a minimum of 6 months follow-up following enrolment in the study. Among the 60 patients with UC in the current study, 38 (63%) patients had inflammatory involvement of the appendiceal orifice inflammation (AOI+), whereas 22 (36.7%) patients had a normal appendiceal orifice histology (AOI−). In 38 patients with AOI+, 20 (33.3%) patients had an active disease whereas 18 (30%) patients had an inactive disease.

**AOI+ vs AOI−**

We compared patients with AOI+ with those having normal appendiceal orifice histology (AOI−).

**Comparison with Baseline Characteristics**

There was no significant difference between the two groups with respect to age, gender, body mass index, duration of disease, hemoglobin levels, liver function tests, extra-intestinal manifestations, current treatment, previous use of steroids, or other immunosuppressants (Table 1). However, mean ESR was significantly higher in patients with AOI+ than in patients with normal appendiceal histology (AOI−).

**Comparison of Endoscopic Extent of Colitis**

Among the 38 patients with AOI, 17 (44.4%) patients had pancolitis and left-sided colitis was seen in 11 (28.9%) patients. Nine (23.6%) patients had proctosigmoiditis, whereas among the 22 patients with normal appendiceal histology, only 2 (9%) patients had pancolitis. Three (13.6%) patients had left-sided colitis, and proctosigmoiditis was seen in 11 (50%) patients. The difference between the two groups with respect to extent of colitis was significant \( (p=0.001) \).

Furthermore, the correlation between the AOI and the extent of colitis was found to be positive and significant \( (r=0.519, p=0.000) \) (Graph 1).
Comparison of Cecal Involvement

Among the 38 patients with AOI, 24 (63%) patients had cecal involvement on histology. Fourteen (37%) patients had no cecal involvement, considered as skip inflammation of the appendix. Whereas, among the 22 patients with normal appendiceal histology, only 3 (14%) patients had cecal involvement and 19 (86%) patients had no cecal involvement. The cecal involvement was significantly higher in the patients with AOI than in the patients with normal appendiceal histology (p = 0.000). On further evaluation in patients with AOI, there were no significant differences observed between patients with skip lesions vs nonskip inflammation of the appendix.

Comparison of Endoscopic Severity of Colitis

Among the 38 patients with AOI, 1 (2.6%) patient was found to be in endoscopic remission, while 21 (55.2%) patients had a mild colitis. Moderate disease was seen in nine (23.6%) patients and seven (18.4%) patients had endoscopically severe disease. Whereas, among the 22 patients with normal appendiceal histology, 6 (27.2%) patients had endoscopic remission and 14 (63.6%) patients had a mild disease. Moderate disease was seen in one (4.5%) patient. Endoscopically severe colitis was seen in one (4.5%) patient. The patients with appendiceal orifice inflammation had a significantly higher grade of endoscopic colitis compared to patients with normal histology (p = 0.006) (Graph 2).

Comparison of Relapse Rates

The patients were followed up for a minimum period of 6 months. Relapse rate was higher in patients with AOI as compared to patients with normal appendiceal orifice histology (18.4 vs 9%) but it was statistically not significant (p = 0.329). The majority of the patients in both the groups maintained remission (81 vs 91%) (Graph 3).

DISCUSSION

Several studies have confirmed that the base of appendiceal orifice is frequently involved by inflammatory
patients.9-11 Whereas, some studies using endoscopic patients show that appendix is involved in 15 to 86% of aggressive course and more frequent relapses.

There is a wide variability in the reported rates of caecal involvement in patients of UC, especially who have appendiceal involvement. In our study, 63% of the patients had a cecal involvement on histology and 37% of the patients had histologically normal cecum, i.e., appendiceal involvement as “SKIP” lesion was seen in 37% of the patients with AOI. However, in patients with AOI, there were no significant differences observed between patients with SKIP lesions vs nonskip inflammation of the appendix. There appears no biological explanation as to why appendiceal inflammation may occur more frequently in patients with distal colitis and as skip lesions as shown in various studies.9-11

Embryologically, the appendix arises from the cecum. Therefore, it would be anticipated that a disease state that causes cecal inflammation would also involve the appendix and vice versa may hold true. The variability in the results may be due to different operational definition of AOI and the type of patients included in the study. There is no worldwide consensus on definition of AOI in UC. Matsumoto et al15 have defined appendiceal inflammation based on endoscopic appearance while Ladefoged et al21 have defined AOI based on endoscopic appearance and histological evidence of inflammation. Moreover, AOI may be nonspecific and can occur in otherwise normal colon, patients with irritable bowel

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We also classified the appendiceal inflammation as active and inactive. It was seen that in the 38 patients with AOI, 53% of the patients had an active disease whereas 47% patients had an inactive disease. The inflammation was mild in majority (75%) of patients. These results are consistent with the results shown by Kroft et al9 where they have seen that 20 of 39 appendices (51%) showed active disease, 4 (10%) had quiescent disease, and 9 (23%) had either normal or nonspecifically inflamed appendix.

With respect to the extent of colitis, the patients with appendicular orifice inflammation were noted to have more frequency of pancolitis. A recent prospective endoscopic study by Ladefoged et al21 has shown different results. In their study of 83 patients with UC, among the 48 patients with microscopic inflammation of the appendiceal orifice, 12 (25%) patients had pancolitis whereas 23 (47.9%) patients had left-sided colitis. Proctitis was seen in 15 (31.2%) patients with AOI. These differences may be due to the finding of a higher frequency of pancolitis in our overall study patients. Among the 60 patients in our study, 19 (32%) had pancolitis as compared to Ladefoged et al21 study where pancolitis was seen only in 12 (16%) of the overall 75 patients. In our study, when statistically analyzed, the correlation between the AOI and the extent of colitis was found to be positive and significant (r = 0.519, p = 0.000). This signifies, i.e., the extent of disease increased, the chance of finding AOI also increased.

Recently, results of a multicenter case-controlled study22 in Japan, highlighting that appendectomy protects against the development of UC, have shown that the frequency of pancolitis was 51% in the nonappendectomized as compared to 38.1% in the appendectomized patients. The incidence of proctitis was higher in the appendectomy group than in the group that did not undergo appendectomies (38.1 vs 18.1%). Their results signify that merely the presence of appendix in an individual relates to more extensive colitis.

There is a wide variability in the reported rates of appendiceal inflammation (AOI+) and normal appendiceal orifice histology (AOI−) in patients with UC.9-11 Some studies have shown that appendix is involved as a skip lesion while other investigators have suggested that appendiceal involvement in UC always occurs in continuity with adjacent involvement of the caecum and the rest of the colon.11-13 Most of the studies have been conducted in surgical colectomy specimens while few studies have evaluated the appendix endoscopically. Moreover, the clinical significance of appendiceal involvement is not clear. Some studies have found no difference with disease activity or clinical course while other studies have shown that patients with a periappendiceal patch had a more aggressive course and more frequent relapses.

Various studies using the colectomy specimens of UC patients show that appendix is involved in 15 to 86% of patients.9-11 Whereas, some studies using endoscopic biopsy specimens, instead of surgical specimens, also documented AOI in 26 to 58% of the patients with UC.4,15 In the present study, appendicular inflammation was seen in 63% patients that were evaluated endoscopically.

Graph 3: Relapse rates in patients with (AOI+) and normal appendiceal orifice histology (AOI−)
syndrome (IBS), colonic malignancies, and microscopic colitis. So to show that AOI is associated with UC, it is necessary to show that the histological inflammation in the area is similar to histology of UC.

With regard to this, a prospective endoscopic study was conducted to evaluate the incidence of inflammation of the appendiceal orifice in patients undergoing colonoscopy for diagnosis or surveillance of colonic disease (CD). The histological inflammation of appendiceal orifice was graded as 0: No inflammation, 1: Infiltration of inflammatory cells, 2: Cryptitis, and 3: Ulcerations. Histological inflammation of the appendiceal orifice was demonstrated in 62% of the patients with UC, 64% with CD, 80% with indeterminate colitis, 100% with microscopic colitis, 29% with IBS, and 43% of patients with other diseases. But histological grades 2 and 3 inflammations of the appendiceal orifice were only seen in patients with inflammatory bowel disease. Our study has used the stringent histological definition of AOI using cryoprobe changes and inflammatory infiltrates. It suggests that appendiceal inflammation is actually a part of the colitis process rather than nonspecific inflammation, which may be seen in various other conditions.

The present study has further shown that the patients with appendiceal orifice involvement had a higher grade of endoscopic severity of colitis than patients without appendiceal orifice inflammation. With regard to severity of colitis, various published literatures have shown results similar to ours. Ladefoged et al have also shown that the frequency of AOI was higher in active colitis than in patients with inactive colitis. Another study by Matsumoto et al has shown that there was a trend toward higher values of disease activity in the colon in patients with AOI. Thus it appears from our study and various recent studies that appendiceal involvement in UC indicates a more extensive and active disease in the colon.

To define the prognostic significance of AOI, we followed our patients for a minimum period of 6 months and compared the relapse rates between patients with AOI and normal appendiceal histology. The relapse rate was higher in patients with AOI as compared to patients with normal appendiceal orifice histology (18.4 vs 9%) but it was not statistically significant as majority of the patients in both groups maintained remission (81 vs 91%). Very few studies have prospectively looked into the prognostic significance of appendiceal inflammation. One such prospective endoscopic study by D’Haens et al has shown that patients with periappendiceal inflammation had a more aggressive course and a more frequent relapses.

The small size of the study population thus underlining the power of the study and heterogeneous study population with majority of the patients being on some form of treatment which might have altered the clinical and histological parameters are important limitations of the present study. Moreover, short follow-up period is inadequate to determine the natural history of the disease in patients with disease like UC.

In conclusion, appendicular involvement in UC is not uncommon. Its involvement may predict the course, extent, and severity of colitis. Thus, appendix may be a potential therapeutic target in patients with UC.

REFERENCES