ORIGINAL ARTICLE

Adherent *Escherichia coli* in colorectal mucosal biopsies: A histological and ultrastructural evaluation

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ABSTRACT

Background: Colorectal mucosal biopsies occasionally demonstrate the presence of bacteria adherent to the epithelium. This study evaluated the histological and ultrastructural correlates of such bacterial adherence. **Materials and Methods:** Rectal mucosal biopsies from eight patients in whom histopathological examination of biopsies had earlier demonstrated adherent bacteria were examined by electron microscopy and by bacterial culture. Colorectal biopsies of 69 patients with adherent bacteria detected histologically were retrospectively evaluated for histological changes at sites proximal and distant to adherent bacteria. **Results:** *Escherichia coli* of different serogroups were isolated from 7 of 8 rectal biopsies demonstrating bacterial adherence. All isolates showed diffuse or focal adherence to HEp-2 cell monolayers. Ultrastructural changes noted included microvillus damage, pedestal formation, actin web condensation, and protrusions of the apical cytoplasm of epithelial cells into the lumen towards the bacteria. Histological changes noted at light microscopy included reduction in epithelial cell height, focal epithelial cell degeneration, cryptitis and neutrophil infiltration at sites of bacterial adherence whereas these were usually absent at sites distant to adherent bacteria. Bacterial adherence was noted more often in biopsies from Crohn's disease patients than in patients without this diagnosis ($P < 0.001$). **Conclusion:** Adherent *Escherichia coli* in colorectal biopsies were associated with focal epithelial damage and showed an association with Crohn's disease.

KEY WORDS: Colon, Crohns disease, enteroadherent *Escherichia coli*, mucosal biopsy, rectum

INTRODUCTION

Adhesion of bacteria to mucosal surfaces is an important mechanism in the pathogenesis of infections in the gastrointestinal tract. Gram negative bacteria adhering to the colonic epithelium are occasionally noted during routine examination of colorectal mucosal biopsies. However, the literature does not attach any special significance to their presence in mucosal biopsies. Certain pathogenic strains of *Escherichia coli* have the ability to adhere to enterocyte and colonocyte apical membranes and produce characteristic morphologic alterations.[1] Enteroadherent *E. coli* (EAEC) have been implicated in the causation of persistent diarrhoea in children and adults.[2,3] Adherent *E. coli* have also been implicated in the pathogenesis of ulcerative colitis and Crohn's disease.[4,5] The present study identified adherent *Escherichia coli* by culture of rectal mucosal biopsies in patients with a histological finding of bacterial adherence to the surface epithelium. A retrospective analysis of histological and ultrastructural changes in biopsies showing bacterial adherence is also reported.

MATERIALS AND METHODS

Eight patients whose rectal mucosal biopsies showed histological evidence of bacterial adhesion underwent repeat biopsy of the rectal mucosa within a week of the first biopsy. The biopsies were preceded by oral colonic lavage involving the intake of four litres of polyethylene glycol electrolyte solution (PEGLEC, Tablets India, Chennai) along with two tablets of Bisacodyl the previous night. Colonoscopic examinations were not performed unless the feces was clear and watery without flecks of fecal matter. Biopsies were taken using sterilized endoscopic biopsy forceps and two bits were fixed for electron microscopy in buffered glutaraldehyde and processed in Araldite. One-micron sections were stained with toluidine blue to select areas suitable for electron microscopic examination, and ultrathin sections stained with aqueous uranyl and lead salts were examined in a Philips EM 201 electron microscope. Two biopsy bits for these patients were also taken for bacterial culture. These biopsy bits were washed vigorously with sterile saline, ground and plated onto MacConkey agar and blood agar plates and incubated overnight at 37°C. Appropriate biochemical reactions were used for bacterial identification. At least 5 colonies of non-mucoid lactose fermenters on MacConkey agar plates were

serogrouped to identify enteropathogenic serotypes of *Escherichia coli* by slide agglutination using Biotec polygrouping antisera and Wellcome individual EPEC antisera. All *Escherichia coli* that could not be grouped thus were serogrouped at the Central Public Health Laboratory, Colindale, London, U.K. All *Escherichia coli* isolated were tested for adherence using HEp-2 cell monolayers.[6]

The retrospective component of the study was the study of 69 colorectal biopsies that had been reported to show histological evidence of bacterial adhesion over a three and a half year period. All the biopsies had been processed routinely and stained with haematoxylin and eosin, and examined by one of three histopathologists who only evaluated gastrointestinal mucosal biopsies. Routinely these three specialist histopathologists reported upon the presence of bacteria adherent to the surface epithelial cells, and all 69 biopsies reported to have adherent bacteria were chosen for the study. Biopsies from these 69 patients were re-evaluated for histological changes related to sites of bacterial adherence. We specifically looked for the following features - epithelial cell degeneration, neutrophil infiltration of surface and crypt epithelium, mucus depletion, regenerative activity, lamina propria cellularity and neutrophil margination in the microvasculature. All changes were noted in relation to areas with adherent bacteria, and were noted also in the same biopsy at sites distant to bacterial adhesion, and in biopsies from the same individual where no bacteria were identified. Epithelial cell height was measured for an average of 10 cells per high power field using a calibrated eye piece graticule. Lamina propria cellularity and neutrophil infiltration were subjectively graded on a scale of 0 to 2. The final clinical diagnosis was recorded from review of the hospital records. The studies were approved by the Research and Ethics Committee of the Christian Medical College, Vellore and informed consent was obtained from the patients.

RESULTS

In all 8 patients (6 with a diagnosis of irritable bowel syndrome and 2 with a diagnosis of Crohn's disease) in whom biopsies were obtained for examination, adherent bacteria were reconfirmed in the second biopsy by light microscopy. Culture of these biopsies grew *Escherichia coli* in 7 of the 8 biopsies. All the isolates showed adherence to HEp-2 cell monolayers. They were all of different serotypes. Adherence pattern of the different serotypes was as follows: Diffuse adherence (O10), localized adherence (O2:K60, O158, O160) or stacked brick pattern of aggregative adherence (O19ab, O86, O200).

Examination of ultrathin sections by light microscopy showed bacteria covering the epithelial surface either diffusely [Figure 1] or focally [Figure 2]. On electron microscopy, degenerative changes were noted in epithelial cells [Figure 3]. Bacteria were noted close to the microvilli [Figure 4] sometimes showing pedestal formation resembling the attaching-effacing lesions of enteropathogenic *Escherichia coli* infection [Figure 5]. Cells at sites of adherence showed excess production of microvillus glycocalyces [Figure 6] reaching towards the bacteria. Microvilli appeared short, irregularly grouped and deformed, or were lost with protrusion of the apical cytoplasm [Figure 6]. Actin

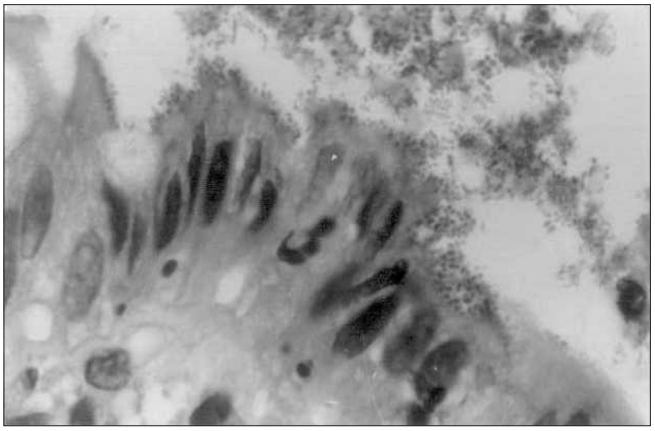

Figure 1: Colonic mucosa with microcolonies of adherent bacteria diffusely covering the surface epithelium, which shows degenerative changes. (EM, × 1218)

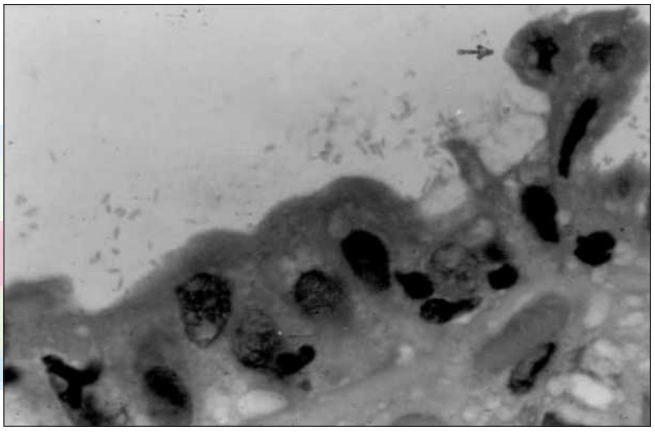

Figure 2: Colonic surface epithelium showing bacteria close to the microvillus border. Note the degenerative change and tufting (arrow) of the cells (EM, × 1137)

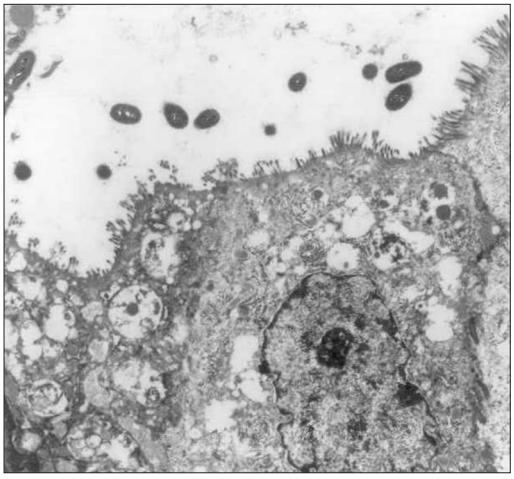

Figure 3: Apical region of surface colonocytes with bacteria adjacent to microvillus border. Microvilli are short, irregular and grouped. Cytoplasm shows vacuolization and damaged mitochondria (EM, × 4063)

polymerization near the terminal web region and swelling of mitochondria and dilatation of rough endoplasmic reticulum were also noted.

Biopsies from the cecum, ascending colon, transverse colon, descending colon, sigmoid colon or rectum did not show significant differences in prevalence of adherent bacteria [Table 1]. Epithelial cell height was significantly reduced at sites of bacterial attachment (9.74 ± 3.15 microns, mean ± SD), compared to sites remote from bacterial attachment (11.87 ± 5.28) or to control biopsies without adherent bacteria (15 ± 3.2) ($P < 0.001$) [Figure 7]. Areas with bacterial attachment showed focal epithelial cell degeneration and neutrophil infiltration of the epithelium [Table 2, Figure 8]. Cryptitis and neutrophilic infiltration of the lamina propria were also more frequent in biopsies demonstrating bacterial adherence compared to biopsies without bacterial adherence [Table 2, Figure 8].

The final clinical diagnoses in these 69 patients included irritable bowel syndrome (22), Crohn's disease (14), ulcerative colitis (4), tuberculosis of the small bowel (2), infective colitis (7), and miscellaneous conditions (20). None of them was HIV seropositive. During this same time period, colorectal biopsies were obtained from a total of 2223 patients. These included biopsies from 40 patients who were diagnosed with Crohn's disease. The distribution of biopsies with bacterial adherence in relation to the patient's diagnosis is shown in Table 3. The proportion of Crohn's disease patients (14/40, 35%) who had biopsies demonstrating bacterial adherence was significantly higher than the proportion of patients with other diagnoses in whom biopsies showed bacterial adherence (Chi squared test, $P < 0.001$).

Table 1: Percentage of biopsies showing bacterial adhesion to the epithelium, in relation to the segment biopsied

Segment	No. of biopsies examined	No. (%) showing bacterial adhesion
Cecum	30	22 (70.0)
Ascending colon	17	12 (70.5)
Transverse colon	27	20 (74.1)
Descending colon	22	14 (63.6)
Sigmoid colon	8	4 (50.0)
Rectum	58	43 (74.1)

DISCUSSION

In this study adherent *Escherichia coli* (EAEC) in colorectal mucosal biopsies were associated with histological evidence of mucosal inflammation and ultrastructural evidence of epithelial cell damage. The ultrastructural changes resemble those reported in *E. coli* (RDEC) infection in rabbits.[7] Pedestal formation, reported in EPEC infection,[1] was noted in 2 of 8 biopsies. EAEC were first implicated in the causation of persistent diarrhoea in children,[1,2] and later in chronic enteropathy in patients with HIV/AIDS.[8,9] Recent studies have implicated adherent-invasive *E. coli* in the causation of ileal Crohn's disease.[10,11] These bacteria exhibited phenomena of both adherence and invasiveness and were largely found in ileal biopsies although similar bacteria could occasionally be found in the colon. Our studies did not demonstrate evidence of invasiveness of the bacteria.

Ultrastructural examination showed evidence of pedestal formation in areas and evidence of epithelial cell damage including mirovillus damage, mitochondrial damage, actin condensation in the terminal web region, and interestingly showed cytoplasmic processes extending towards the adherent bacteria. Whether these processes are responsible for sampling

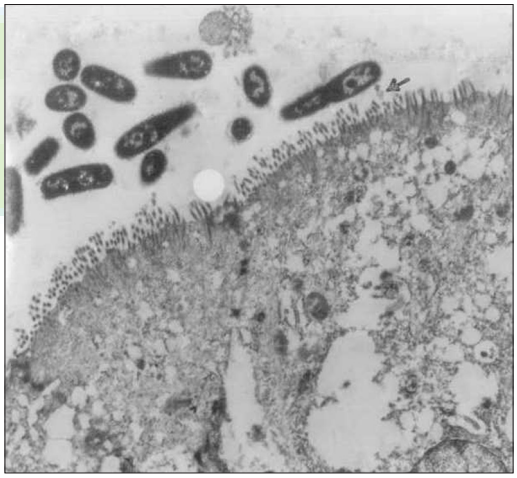

Figure 4: Bacteria close to the microvilli with glycocalyx attached to them (arrow). Vacuolization of apical cytoplasm and mitochondria is seen (EM, × 9856)

Table 2: Prevalence of histological abnormalities associated with bacterial attachment. Comparison of areas with and without bacterial attachment

	Prevalence at		
	Site of bacterial attachment (%)	Areas without bacterial attachment	*P* value
Focal epithelial cell degeneration	111/115 (96)	72/162 (44)	<0.001
Neutrophil infiltration of epithelium	33/115 (28.6)	6/162 (3.7)	<0.001
Epithelial regeneration	39/115 (33.9)	11/47 (23.4)	NS
Mucus depletion	34/115 (29.5)	10/47 (21)	NS
Cryptitis	33/115 (28.6)	6/47 (12.7)	0.0419
Neutrophil margination in adjacent lamina propria vessel	30/115 (26)	7/47 (14.8)	NS
Lamina propria neutrophil infiltration	64/115 (55.7)	17/47 (36.2)	0.0371

Values shown are number of biopsies with the observed feature in the numerator, and total number of biopsies examined for those features (denominator), with the percentage given in parentheses. Focal epithelial cell degeneration and neutrophil infiltration of the epithelium were looked for in all biopsies in areas without bacterial attachment, whereas the remaining histological features were looked for only in biopsies in which no adherent bacteria were noted. Fisher's exact test was done to calculate significance of difference

bacterial molecules such as the pathogen associated molecular patterns is speculative and was not examined. Histological examination showed that there was epithelial damage localized to sites of bacterial adherence, without evidence of damage at sites distant from bacterial adherence or in biopsies without adherent bacteria. This suggests that these bacteria are not merely incidental findings in colonic biopsies but are likely to be associated with pathology in the bowel wall. Adherent bacteria were detected on light microscopy by experienced pathologists who dealt only with gastrointestinal mucosal biopsies. We did not use special stains to detect the bacteria on light microscopy in the first instance. While the detection rate is very high when specialist pathologists examine mucosal biopsies, it is possible that the use of special stains may have increased the yield of adherent bacteria in the study. The preliminary finding of an association with Crohn's disease in this study is very interesting.

Table 3: Frequency of detection of adherent bacteria in relation to the clinical diagnosis

Disease condition	Total number of patients	Number with adherent bacteria	Percentage
Crohn's disease	40	14	35.00
Ulcerative colitis	94	4	4.25
Irritable bowel syndrome	793	22	2.77
Tuberculosis	88	2	2.27
Infective colitis	212	7	3.30
Other diagnoses	996	20	2.00

Other diagnoses included patients investigated for rectal bleeding, colorectal cancer, colorectal polyposis, chronic diarrhoea and iron deficiency anaemia

In summary, adherent bacteria (EAEC) were detected in 3.1% of colorectal mucosal biopsies and were associated with epithelial cell degeneration and shortening, cryptitis and inflammatory infiltration of the lamina propria as well as the epithelium. These bacteria showed a positive association with Crohn's disease. Characterisation of the strains by O antigen serotyping

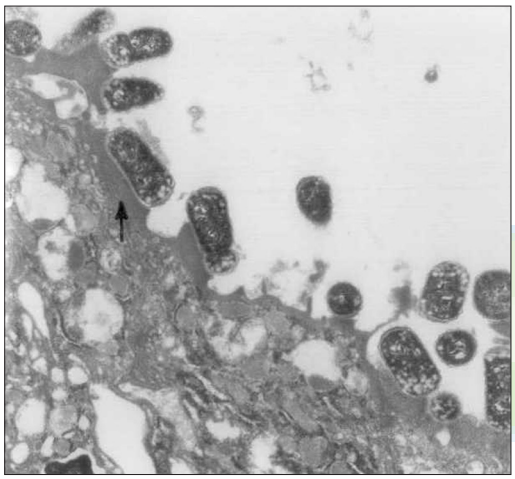

Figure 5: Apical border of colonocyte with bacteria adherent to cell border showing characteristic pedestal formation (arrow). Dilated endoplasmic reticulum and altered mitochondria are seen (EM, × 19710)

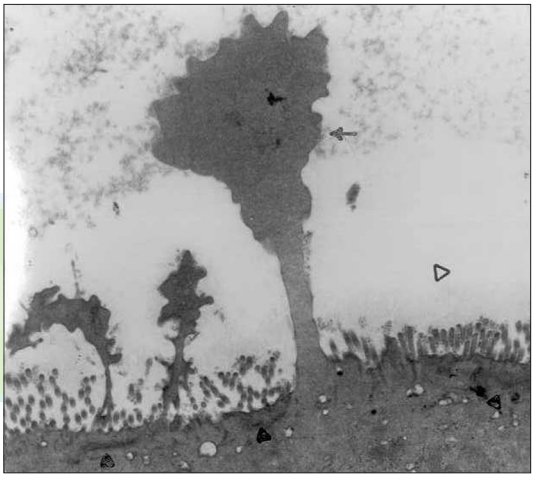

Figure 6: Apical border of colonocyte with protrusion of cell borders (arrow). There is actin polymerization at the rootlets of microvilli (arrow head). Prominent glycocalyx can be noticed (open arrow) (EM, × 11519)

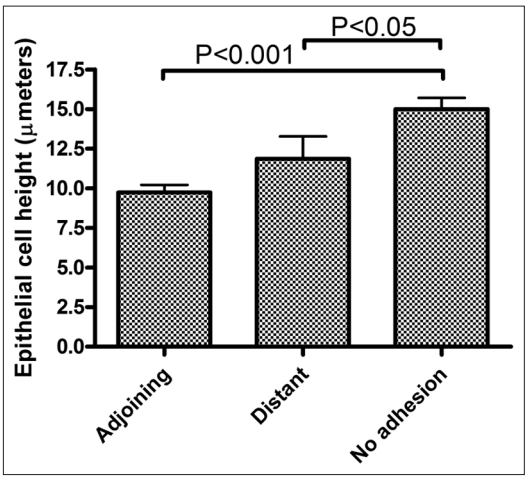

Figure 7: Epithelial cell height in microns (mean and SD are shown) of cells adjacent to adherent bacteria, cells in areas distant to adherent bacteria in the same biopsy, and cells in biopsies from the same patient without adherent bacteria. Significant *P* values are shown (ANOVA with post-hoc Tukey's test)

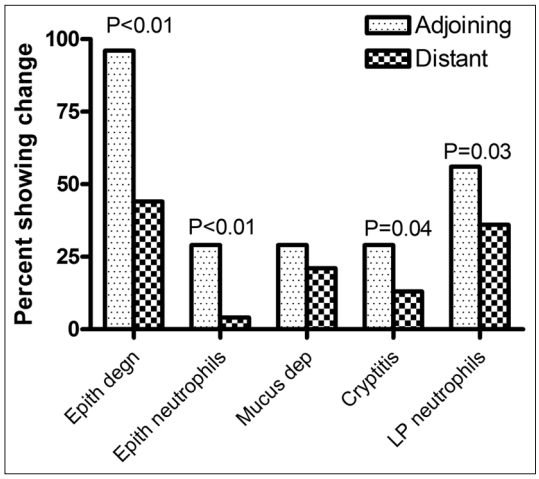

Figure 8: Mucosal changes in the biopsies in sites adjoining adherent bacteria and at sites distant from adherent bacteria. Changes are shown as percentages of sites examined. Significant *P* values (Fisher's exact test) are shown

showed that they belonged to different strains, but they all exhibited the characteristic adherence patterns of enteroadherent *Escherichia coli* in cell line adherence assays. Further, studies are necessary to confirm these associations and to elucidate their role in disease pathogenesis.

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