Restorative Proctocolectomy with Ileo-anal Reservoir, a Histopathological, Histochemical, and Electron Microscopic Study

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Abstract

This study was conducted to investigate the nature of colonic metaplasia in ileo-anal pouches and incidence / frequency of pouchitis in the same. Biopsy specimens from 8 patients with functioning ileal pouches were studied using routine histology, mucin histochemistry and electron microscopy, over a 2 - year period. All 8 patients had villous abnormalities in the form of blunting of villi and sub total or partial villous atrophy. 6 patients had an increase in the goblet cell population and Paneth cell hyperplasia. These changes were supported by electron microscopic findings of a decrease in number and flattening of ileal type microvilli and their transformed morphologic resemblance to colonic type microvilli. All the ileal pouches also had acquired colorectal type sulphomucin, when sections stained with Alcian-blue and High Iron Diamine - Alcian blue, were studied. However, no case of pouchitis as defined in literature, was found in this study.

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Key Words : Colonic phenotypic change; Ileal pouch-anal anastomosis; Pouchitis

Introduction

Restorative proctocolectomy with ileal pouch - anal anastomosis is now an established alternative to proctocolectomy with an end ileostomy in the surgical management of Ulcerative Colitis and Familial Adenomatous Polyposis. It has also been used in other diseases limited to the colon such as Hirschsprung's disease and idiopathic constipation [1]. The operation consisting of total abdominal colectomy, distal rectal mucosectomy and endorectal ileo-anal anastomosis removes the mucosa of the entire colon and rectum, preserves both the transanal route of defaecation and anorectal continence and eliminates the need for a permanent spout ileostomy.

However, two long term complications have been reported in various series; namely pouchitis and colonic metaplasia (colonic phenotypic change or colonisation). In most large series, the incidence of pouchitis is between 10-20% but seems to occur only in patients with ulcerative colitis [2,3]. The cause is unknown. Possible mechanisms suggested are faecal stasis and reduced volatile short chain fatty acid concentrations in pouch stool, both of which may allow the overgrowth of toxin producing bacteria [4].

There is evidence that pouch mucosa undergoes colonic metaplasia/colonic phenotypic change. Varying degree of chronic inflammation and architectural abnormality such as villous flattening or partial villous atrophy are found in approximately 90% of reservoirs.

When villous atrophy is severe, there is crypt hyperplasia, though no intraepithelial lymphocytic infiltrate that characterizes celiac disease. These adaptive changes are almost universal [5]. Hence, the appearance of ileal pouch mucosa changes from a villous architecture of small bowel to a glandular morphology typical of the colon. Concurrently, it has also been found that in about 50% of the patients, ileal pouch epithelial mucin changes from small bowel type sialomucin to colorectal type sulphomucin. Both histological and histochemical changes occur irrespective of the pre-resection diagnosis (Ulcerative Colitis, Familial Adenomatous Polyposis) [5]. The aim of this study was to determine the occurrence and extent of colonic metaplasia, not only by histological and histochemical methods but also by electron microscopy in mucosal biopsy specimens of ileal pouch-anal anastomosis (IPAA) from 8 patients who had undergone restorative proctocolectomy.

Material and Methods

The study was approved by medical ethics committee and informed consent was obtained from all patients. Biopsy specimens obtained from pelvic ileal pouches of 8 patients were studied. None of the patients had pouchitis at the time of biopsy, which was defined as the occurrence of bloody diarrhoea, with pouch inflammation seen on endoscopy and the absence of specific pathogenic organisms in the pouch stool. Normal (control) ileal mucosa was obtained from two patients either at colonoscopy or from surgically resected specimen (unrelated disease; at least 5 cm away from apparent lesion). Normal colonic biopsy specimens were taken from

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two patients. All the tissues were fixed in buffered formalin, processed routinely and then embedded in paraffin wax. 04 micron thick sections of formalin fixed paraffin embedded tissue were stained with :

1. Haematoxylin Eosin (H&E)
 2. Periodic Acid Schiff (PAS)
 3. Alcian Blue at pH 2.5 & 1.0
 4. High Iron Diamine - Alcian Blue (Spicer's method)
1. The H&E sections were studied for evidence of colonic phenotypic change and were graded for the degree of villous atrophy (none, villous abnormality, sub total and total atrophy); the presence of acute and / or chronic inflammation were noted. Epithelial dysplasia was looked for.
 2. PAS +ve substances e.g. mucins, stain magenta and though consistent results are observed with neutral mucin, the results with sialo and sulpho mucins are known to be inconsistent. Test and control studies were of limited utility.
 3. Alcian Blue at pH 2.5 stains sialo and sulphomucin but at pH 1.0 stains sulphomucin specifically. These results were confirmed in the controls.
 4. HID-AB stains small bowel type sialomucin blue, indicating the presence of sialic acid and colorectal type sulphomucin brown indicating the high degree of sulphation. The stained sections were assessed for blue predominance, a mixed pattern or brown predominance. Consistent results were obtained for both tests and controls.

Tissues retrieved from paraffin blocks were deparaffinised and post fixed in 2% osmium tetroxide (OsO_4); specimens were dehydrated and embedded in Epon 812. Silvery white sections of thickness 50-70 nm range were cut perpendicular to the mucosal surface and contrasted with uranyl acetate and lead citrate.

Normal small intestinal mucosa has remarkably uniform microvilli both in size and shape with straight fine filaments running through their length to mingle with the terminal web in the apical cell cytoplasm. In the normal colonic mucosa, microvilli have a very prominent filamentous core which extends into the cell cytoplasm as a "filamentous core rootlet". Some parts also show glycocalyx bodies i.e small spherical bodies enmeshed in the glycocalyx on the surface of the microvilli. With colonic phenotypic change, the microvilli may decrease in number and length with an uneven distribution and prominent filamentous core rootlet.

Results

In the 2 year period from Oct 1999 to Oct 2001 clinical data and endoscopic biopsies of 8 patients with ileal pouch anal anastomosis were collected. Table 1 summarizes the clinical data and endoscopic findings of 8 patients. There was an equal ratio of males to females with a mean age of 50 years and 53 years respectively.

All the patients were asymptomatic at the time of biopsy, but 4 of them showed hyperaemic mucosa on endoscopy. However, no ulcer was visualized in any pouch. 6 cases were studied within one year and 2 in the third year after pouch construction. Fig 1 describes routine microscopic features in the 8 patients.

In the 2 patients who showed total villous atrophy, the duration since the pouch construction was more than 2 years and these patients did not show Paneth cell hyperplasia. In the 2 patients who did not show an increase in the proportion of goblet cells, pouch construction had been performed within the preceding 6 months.

Mucin Histochemistry

All the biopsy specimens were positive for PAS, Alcian blue at pH 2.5 (which stains both sulpho and sialomucin), Alcian blue at pH 1.0 (specific for sulphomucin) and showed a predominant brown black staining on Spicer's High Iron Diamine - Alcian blue which is again specific for sulphomucins. All the stains were setup with the appropriate controls.

Electron Microscopy

When compared with normal ileal and colonic controls, 4 of the test biopsies revealed blunting of the microvilli, a decrease in their number, prominent core rootlets and glycocalyxal bodies, features more suggestive of large intestinal microvillous type of transformation.

Discussion

Several studies have shown that the ileal pouch mucosa undergoes morphological changes and that most probably it reflects an adaptive response to the new luminal environment [4,5,6]. Transformation to a glandular morphology resembling colonic mucosa is common [7]. Histochemical changes in pouch mucin

Table 1
Summary of clinical data and endoscopic findings of 8 patients

Age	Sex	Diagnosis (Reason for surgery)	Duration since surgery	Asymptomatic/ Symptomatic	Endoscopic findings
68	F	Recurrent carcinoma colon	3 years	Asymptomatic	Normal
38	F	Familial adenomatous polyposis	7 months	Asymptomatic	Normal
50	M	Ulcerative colitis	6 months	Asymptomatic	Hyperemia
52	M	Ulcerative colitis	3 months	Asymptomatic	Hyperemia
66	F	Carcinoma rectum and carcinoma colon	3 years	Asymptomatic	Normal
40	F	Familial adenomatous polyposis	8 months	Asymptomatic	Normal
48	M	Ulcerative colitis	6 months	Asymptomatic	Hyperemia
57	M	Ulcerative colitis	4 months	Asymptomatic	Hyperemia

Fig. 1 : Details of the microscopic features observed in the 8 patients

Fig. 2 : Photomicrograph of ileoanal pouch mucosal biopsy showing blunting of villi and increased goblet cells (H&E x 40)

from sialo to sulphomucin are seen in pouches with villous atrophy [5]. These findings have led to the hypothesis that the pouch mucosa undergoes colonic metaplasia as an adaptive change but it may become vulnerable to immune damage or polyp formation, which eventually affects colonic and not ileal mucosa in the susceptible population [5,6].

Many studies until now state that the great majority of functioning ileal reservoirs develop chronic inflammation and villous abnormality regardless of the initial indication for pouch surgery [1,5] as also found in our study (Fig 1 refers). It is important that these common-place pathological changes in the pouch are not equated with a diagnosis of pouchitis, which should

Fig. 3 : Photomicrograph of ileoanal pouch mucosal biopsy showing colonic phenotypic change and increased inflammatory cells (H&E x 40)

Fig. 4 : Electron micrograph of a long time interval ileal pouch mucosal specimen showing prominent filamentous core rootlets, a feature suggestive of large intestinal microvilli (uranyl acetate, lead citrate x 27,000)

be restricted to a clinical syndrome with specific clinical, endoscopic and histopathological features (Table 2), occurring in 6-20% of pouch patients and representing a relapsing chronic inflammatory bowel disease of the pouch [8].

Table 2
Criteria for diagnosis of pouchitis

Clinical	- Pain, increased stool frequency, fever, urgency, discharge and other systemic effects
Endoscopic	- Increased vascularity, contact bleeding and ulceration
Histological	- Acute inflammation, superficial erosions and ulcerations

In this study, no case of pouchitis was found. A study by HJ de Sliva et al [1] reported 8 cases of pouchitis in a sample size of 25 (32%). The adaptive changes of the pouch have been termed colonic phenotypic change / colonisation on account of morphological and histochemical resemblance to colonic mucosa [8]. It is likely that these changes are the result of environmental influences on the ileal mucosa and a neo faecal flora. This adaptation occurs in the form of villous atrophy-as seen in all 8 of our cases and an increase in the goblet cells as seen in 6 of our cases. Some studies have related the degree of villous atrophy to pouchitis [1] but we find no such co-relation and none of our cases had pouchitis according to the laid down criteria.

Similar to other studies by Nasmyth et al [4] and de Silva et al [1] our observation was that there is no correlation between the degree of villous atrophy and the intensity of both acute and chronic inflammation.

Another interesting feature noted in 6 out of 8 cases was that of Paneth cell hyperplasia in the base of the crypts. The reason suggested is a mucosal reaction to injury by an altered milieu [9]. Mucin histochemistry showing a change in the epithelial mucin to colorectal type of sulphomucin has been reported by various workers [1,10,11] and was a consistent finding in our study.

None of these cases showed dysplasia, adenomas or carcinomas the incidence of which is very low in literature. Lofberg et al [12] reported a single case of mild epithelial dysplasia. Nugent et al have reported mildly dysplastic tubular adenomas in 7 of 38 patients (18.4%) of Familial Adenomatous Polyposis, in the ileal reservoir at a medial interval of 2 years from restorative proctocolectomy [13]. Vuilleumier et al reported a case of invasive columnar cuff carcinoma in a patient of Familial Adenomatous Polyposis 7 years after a pouch surgery [14]. However, the present study period was of two years only and further follow-up period would be required to identify the occurrence of dysplasia, adenoma or carcinoma in the IPAA patients. The other reported complications of ileal pouch - anal anastomosis for chronic ulcerative colitis, namely small bowel obstruction, pouch failure and sexual dysfunction [15], were not observed in our study.

Eight cases of IPAA, from one to three years post surgery, were analyzed for available clinical data, endoscopy and mucosal histomorphology, which included stains for epithelial mucin types and electron microscopic studies.

Changes of "colonisation" of the ileal pouch mucosa were observed in all the cases and confirmed by altered

mucin histochemistry in all cases, as well as electron microscopic evidence of microvillous structural alterations in 4 cases. No cases of "pouchitis" as defined in literature, or occurrence of adenomas or of dysplastic epithelial alterations, were noted.

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