Original articles

Histological study of macroscopically normal mucosa adjacent to tumour in colorectal resection specimens

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Abstract

Objective: To study the histological features of macroscopically normal mucosa in colorectal carcinoma specimens and attempt to correlate the severity of these changes and the distance of the mucosa from the tumour.

Method: Ninety three resection margins from 52 specimens were categorized according to the distance from the tumour, 0-1cm, 1.1-5cm and more than 5cm. The histological parameters assessed included, lymphoid aggregates, acute inflammation, chronic inflammation, crypt distortion and villous change. These were graded as absent, mild, moderate and severe. The presence of intraepithelial lymphocytes, cystically dilated ducts and epithelial erosions were recorded as absent or present. Results were analyzed using Pearson Chi- square test and Fischer-Exact statistics.

Results: The histological features found included, lymphoid aggregates in 40% acute inflammation in 15% chronic inflammation in 16% crypt distortion 17%, cystically dilated glands 21%, epithelial erosion 13% and villous change 32%. The presence of cystically dilated glands showed a significant correlation with the distance from the tumour.

Conclusion: Varied histopathological features were observed in the mucosa adjacent to the tumour, awareness of which can prevent misinterpretation and over diagnosis of these features.

Key words - Colorectal carcinoma, macroscopically normal mucosa, histological features.

Introduction

The surgical margins are sampled irrespective of their distance from the tumour from all specimens resected for colorectal carcinoma. Depending on the extent of the colonic resection the mucosa may be adjacent or far from the carcinoma. Several studies have shown that there are characteristic changes in the proximal mucosa

adjacent to the carcinoma (transitional zone) compared to the mucosa distal to the carcinoma. These include both morphological and histochemical changes in the mucosa (1,2). In addition several authors have conducted mucin histochemical studies and noted that a transition from sulfomucin to sialomucin occurs in the mucosa adjacent to the carcinoma and that these

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changes are related to neoplastic or preneoplastic changes (1). However others have suggested that these morphological and histochemical changes are secondary changes rather than preneoplastic (2). It is important for the Pathologists to be aware of these histological changes as some may mimic specific disease conditions such as inflammatory bowel disease leading to misinterpretation and management problems.

Objective

To study the histological features of macroscopically normal mucosa in colorectal carcinoma specimens and attempt to correlate the severity of these changes and the distance of the mucosa from the tumour.

Materials and methods

Ninety three resection margins (both proximal and distal), of 52 resected specimens from patients with colorectal carcinoma, who had no history of inflammatory bowel disease, no familial adenomatous polyposis coli and had not undergone neo-adjuvant therapy prior to surgery were included in the study. The specimens which included the ileum and the anus as resection margins were excluded. The distance of the resection margin from the tumour was obtained from the macroscopic description of the specimen in requisition forms. The transitional zone mucosa was defined as the mucosa within one low power field of the tumour. The resection margins were categorized in to 3 groups according to the distance from the tumour to the assessed end resection margin as follows, Group 1 - 0 - 1cm, Group 2 - 1.1 – 5cm, Group 3 -

more than 5.1cm. Haematoxylin and eosin stained sections of the resection margins were analysed for features including lymphoid aggregates, inflammation in the lamina propria (acute and chronic), intraepithelial lymphocytes, crypt distortion, dysplasia, epithelial erosions, villiform change and cystic dilatation of the crypts. Each of these feature was assessed for both proximal and distal resection margin, as follows,

(a) Lymphoid aggregates - significant when there were > 1 lymphoid aggregate/10mm muscularis mucosa.

(b) Intraepithelial lymphocytes - Present when it was >25 intraepithelial lymphocytes/100 enterocytes.

(c) Crypt distortion - semiquantatively graded as mild, moderate and severe. Mild when occasional crypt distortion was present, severe when all the crypts in the section was distorted (branching, shortening and fusion) and moderate when it was in between the above two grades.

(d) Villiform change - graded as mild when minimal irregularity and undulation of the surface epithelium was present and severe when it was adenomatoid in type. Changes in between these two grades was considered as moderate.

(e) Inflammation - semiquantitative assessment for acute and chronic inflammation was carried out.

(f) Acute inflammation was documented as mild when scattered polymorphs were present in the lamina propria, moder-

ate when polymorphs were present in the crypt/surface epithelium and severe when crypt abscess formation was noted.

(g) Chronic inflammation was graded as mild when there was a loss of the normal inflammatory cell gradient between the upper third and lower two thirds of the lamina propria with plasma cells below the crypt epithelium in >2 foci, severe when a diffuse lymphoplasmacytic cell infiltration was noted extending below almost all crypts and moderate when the lymphoplasmacytic cell infiltrate was in between the mild and severe grades.

(h) Other features evaluated as present or absent were, hyperplastic polyps, adenomatous polyps and dysplasia.

The correlation between the presence and severity of each histological parameter and the distance from the tumour were analyzed by the Pearsons Chi- square method using the SPSS statistical software package (version 10). In the case of histological parameters which had >20% of cells with a lower expected value, only 2 groups were utilized for analysis by combining groups 2 & 3 to form group 2 (>1.1cm). Thus the statistical difference between the transitional zone (Group 1) and mucosa more than 1.1cm away from the tumour (Group 2) were compared using Fischer exact statistics.

Results

The histological parameters assessed and their frequency of occurrence in each of the resection margins grouped according to the distance from the tumour along with the chi

square value (X^2) and the p value are given in table 1.

In cases where Chi square value could not be calculated due to low expected values, the resection margins have been grouped in to two groups by combining groups 2&3 as group 2 (ie; Transitional zone or less than 1cm as group 1 and margins more than 1cm away from the tumour as group 2) and the Fischer exact statistics displayed in table 2. Of the features evaluated, the presence of lymphoid aggregates was the most common observed feature in 40% of the margins examined. However variation between the groups (with distance from the tumour) did not reach any statistically significant level. The other two histological features observed commonly were villous change (32%) and cystic dilatation of glands (21%). Only cystic dilatation of the glands showed a statistically significant variation with the distance from the tumour. Cystic dilatation was present in Group 1 - 12, Group 2 - 4 and Group 3- 4. Other histological features noted were acute inflammation 14/93(15%), chronic inflammation 15/93 (16%), crypt distortion 16/93 (17%) and epithelial erosions 13/93 (13%). Acute inflammation, chronic inflammation and crypt distortion were graded as mild in most margins. However in 4 margins acute inflammation was moderate (Group 1-2, Group 2-1, Group 3- 1) and 1 margin showed moderate chronic inflammation in Group 3. None of these histological features showed a significant variation between the two groups ie; the transitional zone and margins distal to 1cm from the tumour. Two other features observed were the microscopic presence of tumour at the resection margin and fibrosis of the lamina propria.

Histological parameter	Group1 (1cm) (n=28)	Group2 (>1-5cm) (n=23)	Group3 (>5cm) (n=42)	Total (n=93)	X²	p value
1)Lymphoglandular complexes						
Absent	20 (21%)	10 (11%)	26 (28%)	56 (60%)	4.209	0.122
Present	8 (9%)	13 (14%)	16 (17%)	37 (40%)		
2)Acute inflammation						
Absent	22 (24%)	19 (20%)	38 (41%)	79 (85%)	2.132	0.711
Mild	4 (4%)	3 (3.5%)	3 (3.5%)	10 (11%)		
Moderate	2 (2%)	1 (1%)	1 (1%)	4 (4%)		
Severe	0	0	0	0		
3)Chronic inflammation						
Absent	24 (26%)	20 (22%)	34 (36%)	78 (84%)	1.440	0.837
Mild	4 (4%)	3 (3.5%)	7 (7.5%)	14 (15%)		
Moderate	0	0	1 (1%)	1 (1%)		
Severe	0	0	0	0		
4)Crypt distortion						
Absent	22 (24%)	19 (20%)	36 (39%)	77 (83%)	0.602	0.740
Mild	6 (6.5%)	4 (4%)	6 (6.5%)	16 (17%)		
Moderate	0	0	0	0		
Severe	0	0	0	0		
5)Cystically dilated glands						
Absent	16 (17%)	19 (20%)	38 (42%)	73 (79%)	11.364	*0.003
Present	12 (13%)	4 (4%)	4 (4%)	20 (21%)		
6)Epithelial erosion						
Absent	22 (24%)	20 (22%)	38 (41%)	80 (87%)	2.002	0.367
Present	6 (6%)	3 (3%)	4 (4%)	13 (13%)		
7)Villous hyperplasia						
Absent	18 (19%)	15 (16%)	30 (33%)	63 (68%)	0.481	0.786
Mild	10 (11%)	8 (9%)	12 (12%)	30 (32%)		
Moderate	0	0	0	0		
Severe	0	0	0	0		
8)Intraepithelial lymphocytes						
Absent	27 (29%)	23 (25%)	39 (42%)	89 (96%)	1.894	0.388
Present	1 (1%)	0	3 (3%)	4 (4%)		

Table 1. Histological features observed in end resection margins grouped according to the distance from the tumour. * p value <0.05.

Histological Parameter	Group1 (<1cm) (n=28)	Group2 (>1.1cm) (n=65)	Total (n=93)	P value
1)Acute inflammation				
Absent	22	57	79	0.4869
Mild	4	6	10	
Moderate	2	2	4	
Severe	0	0	0	
2)Chronic inflammation				
Absent	24	54	78	1.000
Mild	4	10	14	
Moderate	0	1	1	
Severe	0	0	0	
3)Crypt distortion				
Absent	22	55	77	0.5524
Mild	6	10	16	
Moderate	0	0	0	
Severe	0	0	0	
4)Epithelial erosion				
Absent	22	58	80	0.2004
Present	6	7	13	
5)Intraepithelial lymphocytes				
Absent	27	62	89	1.000
Present	1	3	4	

Table 2. Histological features observed in the transitional mucosa and mucosa distal to tumour

Of the features evaluated, the presence of lymphoid aggregates (Figure 1) was the most common observed feature in 40% of the margins examined. However variation between the groups (with distance from the tumour) did not reach any statistically significant level. The other two histological features observed commonly were villous change (32%) (Figure 2) and cystic dilatation of glands (21%) (Figure 3). Only cystic dilatation of the glands showed a statistically significant variation with the distance from the tumour. Cystic dilatation was present in Group 1 - 12, Group 2 - 4 and Group 3- 4. Other histological features noted were acute inflammation 14/93(15%), chronic inflammation 15/93 (16%), crypt distortion 16/93 (17%) and epithelial erosions 13/93 (13%). Acute inflammation, chronic inflammation and crypt distortion were graded as mild in most margins. However in 4 margins acute inflammation was moderate (Group 1-2, Group 2-1, Group 3- 1) and 1 margin showed moderate chronic inflammation in Group 3. None of these histological features showed a significant variation between the two groups ie the transitional zone and margins distal to 1cm from the tumour. Two other features observed were the microscopic presence of tumour at the resection margin and fibrosis of the lamina propria.

Discussion

A spectrum of histological features were observed at the resection margins. The three most commonly observed histological features were the presence of lymphoid-aggregates, cystically dilated glands and villous change and of all the features assessed, a statistically significant variation

of the histological parameter with the distance from the tumour to the resection margin was noted only in the case of cystically dilated glands. Twenty (21%) resection margins revealed cystically dilated glands. All these dilated glands were lined by an attenuated epithelium. Some contained intraluminal neutrophils. None revealed dysplasia of the lining epithelium. Cystically dilated glands in the transitional zone with similar histological features has been observed in other studies (1,3). In these the authors have considered this histological change to be a secondary change due to mucosal injury following obstruction to the outlets of the crypts. The most commonly observed feature was the presence of lymphoid aggregates. However this did not show a statistical significance with regard to the distance from the tumour. The presence of 0.09 lymphoid aggregates within 1mm of muscularis mucosa is considered normal (4). Increase in the lymphoid aggregates in the colon is a well recognized feature observed in colorectal carcinoma. The other disease processes displaying this feature include ulcerative colitis, Crohn's colitis, diversion colitis (5) and gastroenteritis (4). In all these cases not only the number of lymphoid aggregates but also the normal variation in the density is increased, the normal variation being, the density of lymphoid aggregates increase three fold from caecum to the rectum (4). Though this generalized increase in the lymphoid aggregates could be explained for diffuse lesions such as inflammatory bowel disease, the underlying pathogenesis in the case of localized lesions such as colorectal carcinoma is difficult to explain. O'Leary et al (4) have suggested that this is possibly an immunological response to tumour antigens.

The other most frequently observed feature was the presence of mild villous change in 31 cases (32%) in our study.

Fig 1. Closely packed lymphoid aggregates (H&EX20)

Fig 2. Transitional mucosa exhibiting focal villiform arrangement. (H&EX40)

Fig 3. Cystically dilated crypts lined by an attenuated epithelium (H&EX40)

However this feature also did not show a statistically significant correlation with the distance from the tumour. Villous change in the mucosa adjacent to colorectal carcinoma is a feature documented in some studies (2). It has been shown that hyperplastic changes in the adjacent mucosa is induced by colonic carcinoma cells possibly mediated by EGFR and its ligands acting in a paracrine or autocrine manner to increase tumour cell proliferation and production of pro angiogenic molecules, which explains the high vascularity in the mucosa adjacent to colonic carcinoma (6). Acute and chronic inflammation, intraepithelial lymphocytes, crypt distortion and epithelial erosions were the other histological features observed. Some studies have noted both erosions and ulcerations in the mucosa adjacent to and away from colorectal carcinoma (3). All 13 cases in this study featured only erosions and none revealed deep ulcerations. This is also considered a secondary change due to damage caused by obstruction of the carcinoma. The acute and chronic inflammation noted in the resection margins of this study was mainly of a mild degree. A study conducted by Lev et al also revealed that inflammation either acute or chronic is not a prominent feature in the colonic mucosa either adjacent to or distal from the colonic carcinoma (2). Fibrosis in the lamina propria was evident only in one resection margin in group 3. This feature was noted in 3 of the 18 cases studied by Robey - Cafferty et al (7) using trichrome stain. The low number of positive cases in the current study could be due to non detection, as we did not use any special staining techniques. Additional features observed in other studies included overcrowding and stratification of the crypt epithelium (1),

adenomatous polyps (3), metaplastic polyps (3) and aberrant crypt foci (8). These features were not observed in the resection margins we analysed. The presence of histological features other than involvement of the margin by tumour was considered in this study. Most of the studies focus on the transitional zone comparing it to the mucosa more distal from the tumour. By definition transitional zone mucosa is the non polypoidal non neoplastic mucosa that lies within a few centimeters of a colorectal adenocarcinoma (7). Others have classified this as the area within one low power field of an adenocarcinoma (1). Several such studies focusing on the transitional zone have revealed characteristic and significant morphological, histochemical and ultra structural changes. This has raised the question as to whether these are field changes. Some authors have questioned the specificity of these changes to colorectal carcinoma, as such changes are also present in colectomy specimens even in the absence of colorectal carcinoma (9).

In conclusion a varied pattern of histological features have been observed in the resection margins of colorectal carcinoma specimens. However the presence of these changes varied with the distance from the tumour only in the case of cystically dilated glands. Different pathogenetic mechanisms have been suggested for these morphological changes (10, 11). More importantly some of these may resemble specific disease entities such as inflammatory bowel disease or post neo adjuvant therapy. This is important for the histopathologists in daily practice to recognise these morphological features seen at the resection margins and prevent incorrect interpretation.

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