

ORIGINAL ARTICLE

Histomorphology of aberrant crypt foci in colorectal carcinoma

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Abstract

Colorectal carcinogenesis is a complex multistep process that includes changes in histomorphological appearance of the colonic mucosa and changes at molecular level. Aberrant crypt foci (ACF) was first described by Bird in 1987 on examination of methylene-blue-stained colonic mucosa of azoxymethane-treated mice under light microscopy. Since then ACF was considered as the earliest preneoplastic change that can be seen in the colonic mucosa. The aim of this study was to look at the histomorphology and distribution of ACF in colorectal carcinoma. 50 formalin-fixed archival colectomy specimens for colorectal carcinoma were examined under light microscopy after staining with 0.2% methylene blue. ACF was identified by larger and darker crypts with thickened epithelium, and often elevated from adjacent normal mucosa. ACF was found in 41 of 50 colectomy specimens examined. There were 328 ACF consisting of 36 (11.0%) ACF without hyperplasia or dysplasia, 263 (80.2%) ACF with hyperplasia and 29 (8.8%) ACF with dysplasia. Of these 29 ACF with dysplasia, 25 showed low grade dysplasia and four high grade dysplasia. The density of ACF was higher in the left colon, those older than 65 years of age and among males but these findings were statistically not significant. The crypt multiplicity of hyperplastic ACF (30.149, SD 28.395) was larger than dysplastic ACF (20.613, SD 40.128). The spectrum of histological changes observed probably represent the evolution of ACF in colorectal carcinogenesis.

Keywords: Aberrant crypt foci, colorectal carcinoma, dysplasia, hyperplasia

INTRODUCTION

Colorectal carcinogenesis is a complex multistep process that includes changes in both histomorphological appearances of the colonic mucosa and at molecular level. It is believed that there are two pathogenetically distinct pathways for the development of colon cancer.^{1,2} The first pathway is characterised by chromosomal instability resulting in stepwise accumulation of mutations in a series of oncogenes and tumour suppressor genes. The molecular evolution of colon cancer in this pathway can be seen through a series of morphologically identifiable stages: initial localised colonic epithelial hyperplasia, followed by formation of adenomas that progressively enlarge and ultimately develop into invasive cancers i.e. the adenoma-carcinoma sequence. The second pathway is characterised by genetic lesions in DNA mismatch repair genes (microsatellite instability).

Aberrant crypt foci (ACF) was first described by Bird in 1987 on examination of methylene-blue-stained whole-mount preparations of colonic mucosa of azoxymethane-treated mice under light microscopy.³ ACF was characterised by larger and darker crypts, a thickened layer of epithelium which was slightly elevated, having slit-like lumina with an increased pericryptal space from the surrounding normal crypts.⁴ ACF was considered a precursor lesion of carcinoma, reinforced by the presence of dysplasia,^{5 - 9} monoclonality¹⁰ and gene mutations (k-ras, APC),^{11, 12 - 14} This study was to identify this earliest change (ACF), and to see its histological pattern and distribution in colorectal carcinoma specimens.

MATERIALS AND METHODS

Fifty (50) archival formalin-fixed colonic resections of colorectal carcinoma diagnosed

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between January 2003 and December 2004 were retrieved from the Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia. These were specimens that had been kept after histopathology examination and reporting of the carcinomas were completed. Only specimens with intact and adequate normal mucosa were included in the study. Any faecal content present was washed off. Normal appearing mucosa located 3 cm away from the tumour area was dissected from the colonic wall. Excess underlying connective tissue was trimmed off to improve its flattening and for better evaluation under light microscopy. The mucosa was then placed between 2-pieces of cardboard, reimmersed in 70% ethyl alcohol for 24 hours to further improve flattening.¹⁵ The surface area sampled was recorded as width x length (cm²). After refixation with 70% ethyl alcohol, the mucosa was washed in tapwater and then stained with 20-40 mL of 0.2% methylene blue for 3-5 mins. Subsequently the excess stain was washed off with tapwater for five minutes. For observation by light microscopy, the mucosa was cut into small pieces and placed under two glass slides (5.0 x 8.0 cm) and examined under 4 x objectives with mucosal surface up. The methylene blue highlighted the ACF. These ACF and its mucosa border were mapped out on the glass slide before microdissection was done. This map assisted in tracing back the ACF if the mucosa curled back during the procedure. The number of ACF and the number of crypts per ACF was recorded. ACF was microdissected with a rim of normal surrounding mucosa. The samples were then processed for histopathology by the usual manner. The processed ACF was embedded in paraffin wax, perpendicular to the surface and cut vertically at 4 µm in order to visualize the entire length of the crypts and stained with haematoxylin and eosin (H&E).

Evaluation of ACF histology

Using 4x, 10x and 40x objectives, the histological sections of ACF were screened. ACF was grouped into the following groups based on histological criteria described from previous reports.^{6,7,9, 16 - 19}

ACF without dysplasia or hyperplasia: Lacking significant modification in the epithelium, enlarged crypts, no crowding or stratification of nuclei, no mucin depletion and no dysplasia.

ACF with hyperplasia: Larger or longer than normal crypts, slightly elevated from normal

mucosa, serrated lumen, some mucin depletion and without dysplasia. Nuclei are enlarged or sometimes crowded without stratification.

ACF with dysplasia: Enlarged and elongated crypts with nuclear stratification. There is depletion of goblet cells and mucin. *Low grade dysplasia:* Nuclei are elongated, cigar shaped, oval with hyperchromasia and fairly basally located. *High grade dysplasia:* Nuclei are large, round with vesicularity and contain prominent nucleoli.

In this study, the colonic site was grouped into 'right colon' which was defined as caecum and the colon from caecum up to the splenic flexure and the 'left colon' defined as colon starting from the descending colon up to the rectosigmoid junction and included rectum. Density was defined as number of ACF per cm². Multiplicity was defined as number of crypts per ACF.

Statistical analysis

The results were analysed statistically by using independent *t*-test and chi-square test with the level of significance at 95% (p-value < 0.05) using SPSS (Statistical Package for Society Study) program version 17.

RESULTS

Fifty colorectal specimens resected for colorectal carcinoma were included in the study. 25 patients were male and 25 female. The patients' ages ranged from 29 to 83 years with a mean of 61 years. The total surface area of normal-looking mucosa examined was 3225.2 cm² with the mean area of 64.5 (SD 75.0) cm². 328 ACF were identified in 41/50 (82%) of the colon specimens (Figure 1). Crypt multiplicity of ACF ranged from 5 to 250 crypts per foci.

The density and the multiplicity of ACF with patients' demographic data are summarised in Table 1. The density of ACF was higher in males (0.160, SD 0.189) than females (0.113, SD 0.092). The older age group above 65 years (0.159, SD 0.175) gave higher density than those of 65 years and younger (0.122, SD 0.131). There was slightly more ACF among Chinese (0.166, SD 0.160) than Malays (0.160, SD 0.155). The left colon (0.153, SD 0.153) had higher density of ACF than the right colon (0.050, SD 0.081). ACF crypt multiplicity followed almost similar pattern with larger crypts observed among males (43.6, SD 34.7), those above 65 years old (44.8, SD 29.0), among Chinese (0.166, SD 0.152)

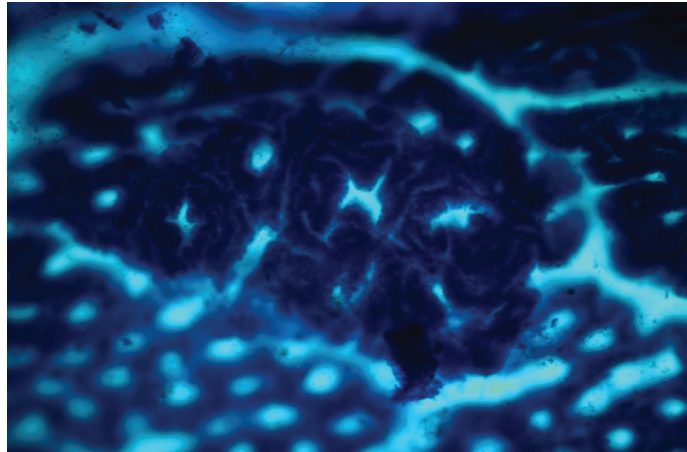


FIG. 1: An ACF after mucosal staining with methylene blue and observed under light microscopy at 40x magnification. The lesion was slightly elevated with darkly stained and larger crypts from the surrounding normal mucosa.

and on the left colon (39.7, SD 29.1). But, statistically these findings showed no significant difference.

Histology

All 328 ACF were histologically examined. The histological patterns of ACF according to gender, age and colonic site are summarised in Table 2. Of 328 ACF, 36 (11.0%) was without hyperplasia

or dysplasia, 263 (80.2%) were hyperplastic (Figure 2) and 29 (8.8%) were dysplastic (Figure 3). 25 (86.2%) of the dysplastic ACF showed low grade dysplasia and 4 (13.8%) high grade dysplasia (Figure 4). Generally, the density of hyperplastic ACF was highest among the three histological patterns. In terms of demography, both gender and all age groups had more ACF with hyperplasia than the other histology groups.

TABLE 1: Density and crypt multiplicity of ACF according to gender, age, race and colonic site

	<i>n</i>	Density Mean (S.D)	<i>p</i> ^a	Crypt multiplicity Mean (S.D)	<i>p</i> ^a
Sex					
Men	25	0.160 (0.189)	0.269	43.6 (34.74)	0.137
Women	25	0.113 (0.092)		31.6 (19.83)	
Age (yrs)					
≤ 65	31	0.122 (0.131)	0.398	33.1 (27.9)	0.163
> 65	19	0.159 (0.175)		44.8 (29.0)	
Race					
Malay	18	0.160 (0.155)	0.904	39.9 (25.47)	0.122
Chinese	21	0.166 (0.152)		52.3 (23.43)	
Indian	1	*		*	
Others	1	*		*	
Colorectal site					
Right	8	0.050 (0.081)	0.071	26.1 (24.2)	0.094
Left	42	0.153 (0.153)		39.7 (29.1)	

Density was defined as number of ACF per cm²

Multiplicity was defined as number of crypts per ACF.

S.D = Standard Deviation

^aIndependent *t*-test

*Density and multiplicity was not calculated because each has only one ACF.

TABLE 2: Density of ACF histological pattern according to gender, age and colonic site

	Without dysplasia or hyperplasia Mean (S.D)	Hyperplasia Mean (S.D)	Dysplasia Mean (S.D)
Sex			
Men	0.013 (0.020)	0.140 (0.176)	0.007 (0.018)
Women	0.019 (0.029)	0.086 (0.074)	0.015 (0.236)
Age (yrs)			
≤ 65	0.017 (0.021)	0.094 (0.109)	0.010 (0.021)
> 65	0.015 (0.031)	0.144 (0.170)	0.012 (0.022)
Colorectal site			
Right	0.009 (0.012)	0.047 (0.088)	0.018 (0.033)
Left	0.018 (0.027)	0.126 (0.141)	0.009 (0.018)

Density is defined as number of ACF per cm²
S.D = Standard Deviation

ACF with hyperplasia outnumbered the other histological patterns regardless of colonic site.

DISCUSSION

Aberrant crypt foci identification was reproducible with methylene-blue staining on formalin-fixed colonic specimens. It has been reported that ACF can be seen in fresh mucosa, formalin-fixed specimens, and even *in vivo* using magnifying colonoscopy highlighted by methylene-blue.^{15-17,20} Thus surveillance for this precursor lesion in

high-risk groups and healthy individuals before the onset of cancer is feasible.

We confirmed that the majority (41 of 50) of colonic mucosa examined harbour ACF. Nine mucosal samples did not show any ACF. There were no statistical difference among these two groups in terms of age, sex, ethnicity and colonic site (result not shown). Although there was no statistical difference, a higher density of ACF was observed in the older age group, males, Chinese and left colon. This is in agreement with

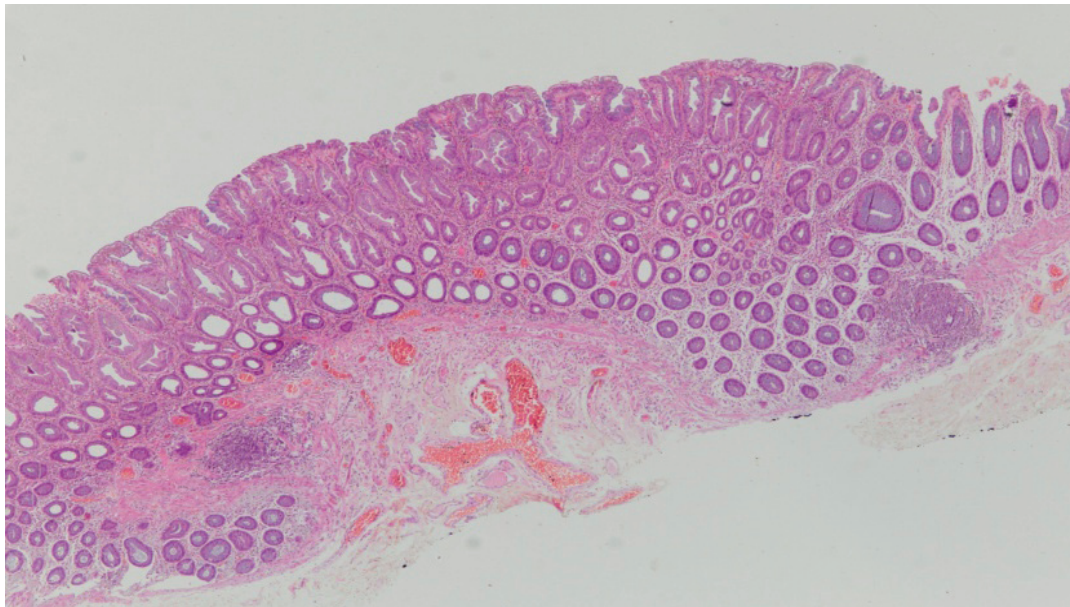


FIG. 2: ACF with hyperplasia and adjacent normal mucosa. The ACF displayed enlarged and elongated crypts. The crowded epithelial lining gave rise to the serrated or “saw-tooth” appearance. The epithelium showed a mixture of absorptive cells as well as goblet cells with some degree of mucin depletion. (H&E X4)

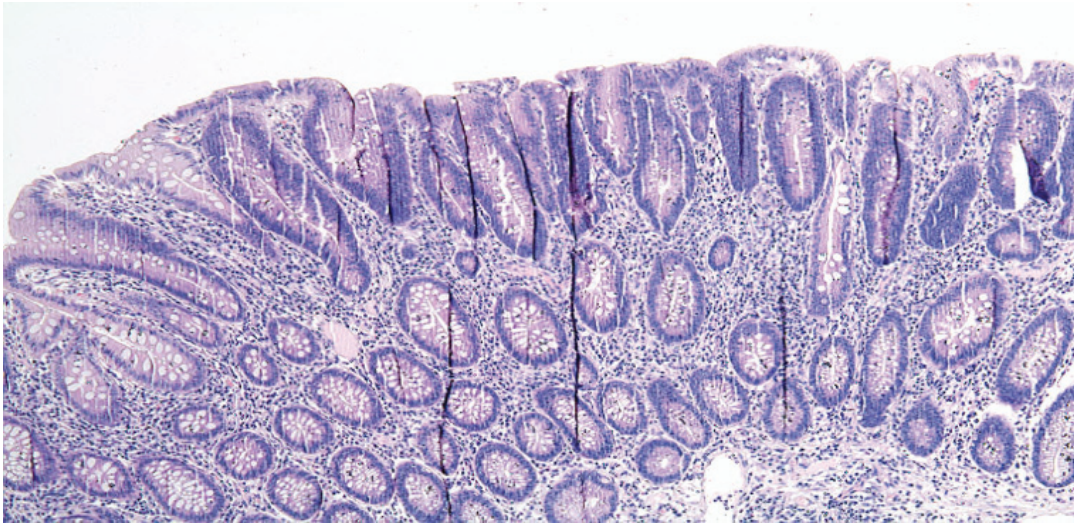


FIG. 3: ACF with dysplasia in enlarged and elongated crypts. There is marked depletion of goblet cells in the dysplastic crypts. The epithelium displays elongated to oval hyperchromatic nuclei with loss of nuclear polarity.

previous reports and reports of National Cancer Registry of Malaysia (2003) where colorectal carcinoma was found more frequently in these groups.^{9, 17, 21}

ACF crypt multiplicity ranged from 5 to 250 which is within the range of previous reports.¹⁷ The pattern of crypt multiplicity was similar with ACF density, where larger crypts were seen among males, age older than 65 years and from the left colon. This was also observed in a previous study, except for colonic site

where it was reported that ACF multiplicity was lower in the left colon and the rectum than the right colon.¹⁶ These findings were probably related to different pathogenetic mechanisms at different colonic locations²² and the geographical differences of the studied groups.

The identification of dysplastic ACF is crucial to support the theory of ACF as a precursor in colorectal carcinogenesis. There is a wide range of ACF with dysplasia, ranging from 5-60% has been reported.^{5-9, 16-19} In this study

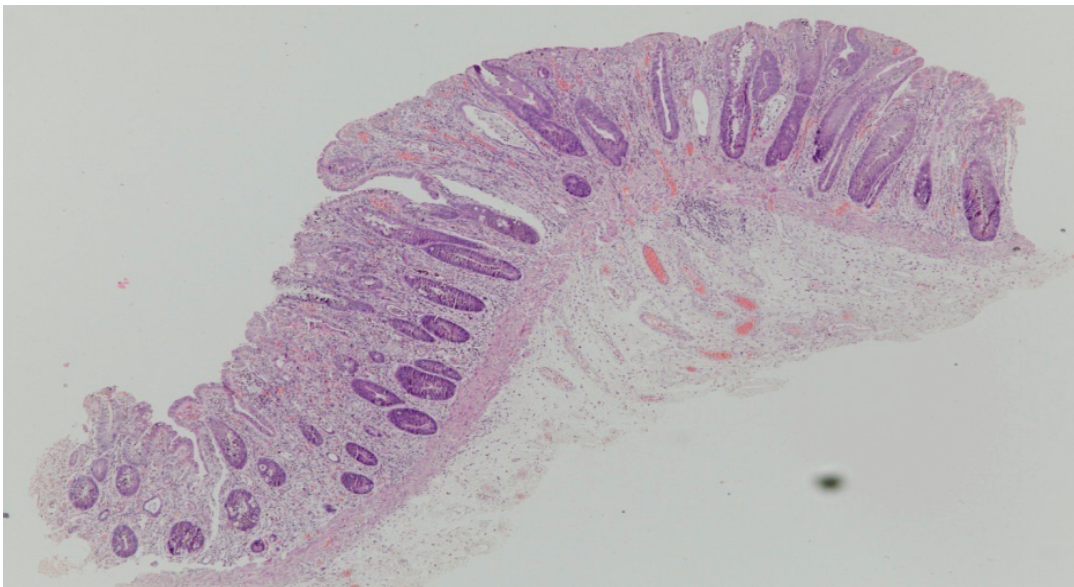


FIG. 4: ACF with high grade dysplasia. The crypts are elongated and large. The epithelium displays nuclear stratification with oval to rounded nuclei and prominent nucleoli. There is marked mucin depletion..

we found majority of dysplastic ACF were low grade dysplasia (25/29) and four showed high grade dysplasia. A few ACF showed areas of both dysplasia and hyperplasia but this data was not included since we took the more advanced histological pattern for tabulation of ACF type. However the presence of such foci suggested that some ACF evolved from hyperplasia into dysplasia and may eventually progress to cancer. Furthermore, ACF with dysplasia showed larger crypt multiplicity (60.63, SD 48.52) than ACF with hyperplasia (43.07, SD 24.27) which was in agreement with previous data.^{7,18} The larger dysplastic ACF reflected their greater proliferation and malignant potential.²³

The density of dysplastic ACF was seen most in the left colon whereas hyperplastic ACF was more dense in the right colon. This could be related with the two pathways in colorectal carcinogenesis. This observation needs to be explored further. A constant finding in previous studies showed ACF with hyperplasia as the majority type.^{7,21} Nonetheless, there were 36 (11.0%) ACF without hyperplasia or dysplasia, which was lower than previous report.¹⁷ However, it was noted that even without significant epithelial change, these ACF expressed monoclonality and thus are likely to be neoplastic.¹⁰

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