HISTOLOGY OF ABERRANT CRYPT FOCI IN COLORECTAL CARCINOMA

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FACULTY OF MEDICINE
UNIVERSITI KEBANGSAAN MALAYSIA
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HISTOLOGY OF ABERRANT CRYPT FOCI IN COLORECTAL CARCINOMA

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DISCLAIMER

I hereby declare that the work in this dissertation is my own except for quotations and summaries which have been duly acknowledged.

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DR. DAYANGKU NORLIDA AWANG OJEP

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ABSTRACT

**Background:** Colorectal carcinogenesis is a complex multistep process that includes histomorphological changes of the colonic mucosa and changes at the molecular level. The step that currently receives attention is the early focal abnormality seen in grossly normal mucosa, the aberrant crypt foci (ACF). ACF is first described by RP. Bird in 1987 on examination of the methylene-blue stained whole-mount colonic mucosa of azoxymethane-treated mice under light microscope. Currently, ACF is considered as precursor lesion even though they are heterogeneous in microscopic appearance. Majority of ACF are hyperplastic and a small percentage showing dysplasia. Data shows that ACF is mainly seen on the left colon than the right colon. Thus, the aim of this study is to look at the histomorphology and the distribution of ACF in colorectal carcinoma.

**Methods:** This is an observational study of 50 formalin-fixed colectomy specimens for colorectal carcinoma in Hospital Universiti Kebangsaan Malaysia. After refixation with 70% ethyl alcohol the mucosal samples are stained with 0.2% Methylene–Blue and then examined under 4x with standard microscope. Under light microscopy, ACF is characterized by larger and darker crypts with thick epithelium, slit like lumen that is often elevated with increase pericryptal space with adjacent normal mucosa. The ACF is microdissected and embedded in paraffin wax, perpendicular to the surface and stain with Hematoxylin and Eosin. In this study, ACF is classified into three groups namely ‘normal mucosa’, ‘hyperplasia’ and ‘dysplasia’. Within the dysplastic group, ACF is further divided into low grade, high grade, low and high grade and lastly mixed type (hyperplasia and dysplasia).
**Results:** 50 colectomy specimens for colorectal carcinoma was studied with a total surface area of 3225.2 cm$^2$ of normal appearing mucosa sampled. We found ACF in 41 out of 50 (82%) colectomy specimens. There was no ACF identified in 9 (18%) colectomy specimens. From the 41 cases, we identified 328 ACF. 36 (11.0%) ACF showed normal mucosa, 263 (80.2%) were hyperplastic and 29 (8.8%) were dysplastic. ACF with dysplasia were further divided; with 17 (5.2%) ACF showing low grade dysplasia, 1 (0.3%) with high grade dysplasia, 3 (0.9%) showed both areas of low and high grade dysplasia and 8 (2.4%) exhibit mixed type. Density of ACF was higher on the left colon (0.153 ± 0.153) than the right colon (0.050 ± 0.081) but this was not statistically significant. The crypt multiplicity of dysplastic ACF (60.63 ± 48.52) was larger than hyperplastic ACF (43.07 ± 24.27). The occurrence of ACF in colorectal carcinoma was significantly higher compared from normal control with p-value < 0.05.

**Conclusion:** Majority of ACF was hyperplastic (80.2%) and a small percentage was dysplastic (8.8%). ACF histology was heterogeneous and represents a spectrum of evolution; from those showing normal mucosa (11%) to hyperplasia (80.2%), mixed type (2.4%) and to ACF showing low grade dysplasia (5.2%), low and high grade dysplasia (0.9%) and lastly ACF with high grade dysplasia (0.3%) only. We found that dysplastic ACF was larger than hyperplastic ACF that suggested a greater malignant potential of the dysplastic ACF. The density of ACF was higher on the left colon, but this was not statistically significant. And the occurrence of ACF in colorectal carcinoma was significantly higher from the normal control. Therefore, this study further supported the role of ACF as a precursor in colorectal carcinoma.
ABSTRAK


Metodologi: Ini adalah kajian permerhatian ke atas 50 spesimen kolektomi yang telah disimpan dalam formalin daripada Hospital Universiti Kebangsaan Malaysia. Setelah ditetapkan semula dalam 70% etil-alkohol, sampel mukosa kemudian diwarnakan dengan 0.2% Metilena Biru dan diperiksa di bawah objektif 4x dengan mikroskop standard. Di bawah mikroskop, FKA mempunyai kripta yang lebih besar dan gelap serta epitelium yang tebal, lumen yang sempit dan memanjang dan ia biasanya kelihatan timbul dari mukosa normal sekelilingnya serta mempunyai kawasan perikripta yang lebar. FKA kemudiannya dipotong dan diletakkan dalam lilin paraffin pada posisi tegak keatas permukaan dan kemudian diwarnakan dengan Eosin dan Hematoksilin. Dalam kajian ini, FKA diklasifikasikan kepada tiga kumpulan iaitu 'mukosa normal', 'hiperplasia' dan
‘displasia’. Di dalam kumpulan displasia, FKA dibahagai pula kepada gred rendah, gred tinggi, gred rendah dan tinggi dan akhir sekali jenis gabungan (hiperplasia dan displasia).

**Keputusan:** 50 spesimen kolektomi untuk karsinoma kolorektal dikaji dengan jumlah keluasan permukaan mukosa normal 3225.2 cm². FKA dijumpai dalam 41 daripada 50 (82%) spesimen kolektomi. Tiada FKA dijumpai pada 9 (18%) spesimen kolektomi. Daripada 41 kes, kami kenalpasti 328 FKA. 36 (11.0%) FKA menunjukkan mukosa normal, 263 (80.2%) menunjukkan hiperplasia dan 29 (8.8%) mempunyai displasia. FKA displasia dibahagikan kepada; displasia gred rendah sebanyak 17 (5.2%), displasia gred tinggi iaitu 1 (0.3%), 3 (0.9%) FKA menunjukkan kedua-dua displasia gred rendah dan tinggi dan 8 (2.4%) FKA menunjukkan jenis gabungan. Ketumpatan FKA adalah lebih tinggi di kolon kiri (0.153 ± 0.153) berbanding kolon kanan (0.050 ± 0.081) tetapi pemerhatian ini adalah tidak signifikan. Dalam kajian ini, kami dapati gandaan kripta pada FKA displasia (60.63 ± 48.52) adalah besar berbanding FKA hiperplasia (43.07 ± 24.27). Kejadian FKA adalah lebih tinggi dan signifikan dalam karsinoma kolorektal berbanding kontrol normal dengan nilai p < 0.05.

**Kesimpulan:** Kebanyakan FKA menunjukkan hiperplasia (80.2%) dan peratusan kecil menunjukkan displasia (8.8%). Histologi FKA adalah heterogenus dan mungkin menggambarkan spektrum evolusi daripada FKA mukosa normal kepada hiperplasia (80.2%), jenis gabungan (2.4%) kepada FKA displasia gred rendah (5.2%), displasia gred rendah dan tinggi (0.9%) seterusnya FKA displasia gred tinggi (0.3%) sahaja. Kami
dapat bahawa FKA displasia adalah lebih besar berbanding FKA hiperplasia yang mencadangkan potensi malignan adalah lebih tinggi di kalangan FKA displasia. Ketumpatan FKA adalah lebih tinggi di kolon kiri tetapi keputusan ini adalah tidak signifikan. Kami juga dapat, kejadian FKA dalam karsinoma kolorektal adalah lebih tinggi dan signifikan berbanding kontrol normal. Dengan itu, kajian ini menyokong fungsi FKA sebagai prekursor dalam karsinoma kolorektal.
ABBREVIATIONS

ACF - Aberrant Crypt Foci
CRC - Colorectal Carcinoma
APC - Adenomatous Polyposis Coli
FAP - Familial Adenomatous Polyposis
PCNA - Proliferating Cell Nuclear Antigen
WHO - World Health Organization
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HISTOLOGY OF ABERRANT CRYPT FOCSI IN COLORECTAL CARCINOMA

INTRODUCTION

Colorectal carcinogenesis is a complex multistep process that includes changes in both histomorphologic appearance of the colonic mucosa and changes at the molecular level. It is now believed that there are two pathogenetically distinct pathways for the development of colon cancer. The first pathway is characterized 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 occurs through a series of morphologically identifiable stages: initially localized colon epithelial hyperplasia, followed by formation of adenomas that progressively enlarge and ultimately develop into invasive cancers. This is referred to as the adenoma-carcinoma sequence. Whereas the second pathway is characterized by genetic lesions in DNA mismatch repair genes (Microsatellite Instability).

The process of carcinogenesis takes time to reach the invasive stage. Therefore, there should be time to take measures on to prevention of cancer onset. Unfortunately, at present time colorectal cancer remains one of the major causes of death in most developed countries. In Malaysia, colorectal carcinoma is the commonest cancer among men and the 3rd most common cancer among women.
In the last few years, human colon carcinogenesis has been extensively investigated in trying to understand the early event in their development. The search for the earliest morphological precursors has led researchers to the findings of early focal abnormality seen in normal mucosa, the aberrant crypt foci (ACF).\textsuperscript{13}

ACF is first described by Ranjana P. Bird in 1987\textsuperscript{1} on examination of the methylene-blue-stained whole-mount preparations of colonic mucosa of azoxymethane-treated mice under light microscope. The assumption of ACF as precursor lesion in murine colon is put forward one year later, coming up with the methodological approach to detect ACF.\textsuperscript{33} Microscopically, the crypts in ACF are two to three times larger than normal crypts, have a thickened layer of epithelium that stains darker than normal crypts, they are slightly elevated, usually have slit-like lumina and have an increase pericryptal space between them and the surrounding normal crypts.\textsuperscript{7,8} As the time progress ACF contains more than one crypt\textsuperscript{27} and one focus can consist from one to hundreds of aberrant crypts.\textsuperscript{3,24}

ACF are characteristically (1) induced by colon specific carcinogen in a dose dependent manner (2) each ACF evolves from one altered crypt (3) exhibit precursor features (dysplasia, abnormal proliferative pattern, K-ras mutation) (4) size and crypt multiplicity increase with time (5) number and growth features predict tumour outcome and quantify risk. (6) Present in individuals with high risk for developing colon cancer.\textsuperscript{8,33}
Later, ACF are identified and quantified in section from the normal appearing human colonic mucosa in colectomy resection for sporadic or familial colorectal carcinoma (CRC) or nonneoplastic colonic disease.\(^2,3,4,5,6\)

ACF can also be easily detected in vivo by magnifying colonoscopy after spraying the colonic mucosa with methylene-blue. Thus, the observation of ACF is considered feasible, not only in patients with colorectal carcinoma but also in healthy individuals.\(^26\)

ACF is a precursor lesion with direct evidence of the existence of an ACF-adenoma-adenocarcinoma sequence in a rodent model in vivo\(^9\). The association between aberrant crypt foci with colonic neoplasia is reinforced by the occurrence of dysplasia in substantial proportion of human ACF, varying from 5%-60%\(^2,3,10,11,12,13\).

Rat ACF and colon neoplasm share similar genetic alterations such as mutations in K-ras\(^15\), changes in enzymatic activity\(^7,16\) and increase in proliferation\(^17\). Similarly ACF identified in human colorectal carcinoma exhibit mutations in k-ras, APC\(^15,18,19,20\), enzymatic changes\(^2\) and increases in proliferative activity\(^21,28\) as to those observed previously in human colon tumours. Microsatellite instability is also shown in a low fraction of human ACF\(^22,23\).

From the earlier observations, ACF are heterogeneous in terms of histology and genetic characteristic in both human and experimental studies\(^2,12,15,16,18,20,24\).
Histologically, ACF can be normal or show various alterations, from hyperplasia to severe dysplasia. Most ACF are hyperplastic and not associated with dysplasia and reported in 19 to 90% of cases. Only a minor fraction of the ACF examined are defined as dysplastic, with a wide range of reported figures (5-60%). In ACF, dysplasia may be focal and even in a single aberrant crypt, suggesting that a transition from hyperplasia to dysplasia in ACF is possible, maybe through cell proliferative alterations. It is noteworthy that carcinoma in-situ in ACF was documented from patient with sporadic colorectal carcinoma. Dysplasia seem to be more frequent in larger ACF, although others found the opposite. Dysplastic ACF is seen commonly in FAP (Familial Adenomatous Polyposis) patients compared from sporadic patients.

**Histopathological characteristics of ACF**

The final histological criteria for ACF are generally accepted as 'nondysplasia', 'dysplasia' and 'mixed type'.

**ACF without dysplasia**

ACF with normal mucosa: lacks significant modification of the epithelium lining the crypts, enlarged crypts (at least 1.5 times larger than normal) with only slightly enlarged and elongated nuclei, but no crowding or stratification, and no mucin depletion. Crypt cells with positive staining of PCNA (Proliferating Cell Nuclear Antigen) and Ki-67 remain at the lower part of the crypts.
ACF with hyperplasia: analogous to the manifestation of hyperplastic polyps of colon. The crypts are larger or longer than normal crypts sometimes showing apical branching. The luminal opening is serrated and slightly elevated from the surrounding normal mucosa, but without dysplasia. Goblet cells are mixed with absorbing cells, with partial mucin depletion. Nuclei are enlarged or sometimes crowded without stratification. Cells with positive staining of PCNA and Ki-67 remain at the lower and middle parts of the crypts.

*ACF with dysplasia (microadenoma)*

Both crypts and cells have different degree abnormalities, with enlarged, elongated and sometimes stratified and depolarized nuclei. The number of goblet cells is decreased obviously and mucin is depleted. The major site of positive staining cells of PCNA and Ki-67 is extended to the upper part of the crypts. ‘Serrated adenomatous ACF’ could also be found to have similar histopathological manifestations of serrated adenomas.

*ACF with mixed type of hyperplasia and dysplasia*

ACF with mixed hyperplastic and adenomatous components histologically showed the combination of various proportions of pure adenomatous pattern with dysplasia and pure hyperplastic pattern without dysplasia.⁶

The WHO (World Health Organization) classifications are simplified as hyperplastic and dysplastic ACF.
The density of ACF in human colon (i.e. the number of ACF per square cm of mucosal surface) is higher in patients with familial adenomatous polyposis and with colorectal carcinoma. And lower in patients with diverticulosis or other benign diseases of the large bowel. ACF density is also significantly and progressively higher from proximal colon toward distal colon, being the highest in sigmoid and rectum, which was in accordance with the location of colorectal carcinoma. The density of dysplastic foci was higher in the colon, especially the right colon, whereas hyperplastic foci were more frequent in the rectum.

According to experimental models, ACF grow after induction. The mechanism by which they increase in size seems to be a process of crypt fission beginning at the base of the crypt and then proceeding upwards until two new crypts are generated. Thus, the number of crypt per ACF also termed "crypt multiplicity" in experimental studies, would be an important parameter in order to evaluate ACF progression. It has been demonstrated that crypt multiplicity was significantly lower from proximal toward distal colon, which was opposite to that of ACF density, and was significantly larger when it was associated with carcinoma or adenoma than with nonneoplastic diseases.

In this study, our aim is to describe the histomorphology and the distribution of aberrant crypt foci from the colonic resection among patient with colorectal carcinoma from Hospital Universiti Kebangsaan Malaysia. With this study we hope to create a data set on the features of ACF in a better understanding of colorectal carcinogenesis.
OBJECTIVES

The general objective of the study was:

1. To study the histomorphology of ACF in colorectal carcinoma in HUKM

The specific objectives of the study were:

1. To identify and quantify ACF in colorectal carcinoma.
2. To study the histomorphologic types of ACF.
3. To analyze the distribution of ACF.
RESEARCH HYPOTHESIS

There is a correlation between ACF histomorphological findings with colorectal carcinoma.
MATERIALS AND METHODS

STUDY DESIGN

This is an observational study of aberrant crypt foci histology sampled from normal appearing mucosa in 50 colectomy specimens for colorectal carcinoma. The specimen is obtained from the archival specimen of the Department of Pathology, Hospital Universiti Kebangsaan Malaysia (HUKM), Kuala Lumpur.

STUDY POPULATION

The samples consist of 50 colonic resections diagnosed for colorectal carcinoma in Hospital Universiti Kebangsaan Malaysia (HUKM), Kuala Lumpur from the year 2003 to 2004.

SELECTION ELIGIBILITY

Inclusion criteria

We studied 50 archival colectomy specimens for colorectal carcinoma done in the year 2003 to 2004 in HUKM. All 50 colectomy specimens must contain areas of normal appearing mucosa for ACF examination.

Exclusion criteria

Those archival colectomy specimens for colorectal carcinoma with inadequate or too distorted remaining normal mucosa are excluded.
MUCOSAL SAMPLING

We studied 50 formalin-fixed colectomy specimens for colorectal carcinoma obtained from the archive of the Department of Pathology, Hospital Universiti Kebangsaan Malaysia (HUKM). The specimen was cut open longitudinally and washed off from fecal content.

As formalin-fixed specimen was not fixed flat, the mucosa was first dissected from the colonic wall. The dissected mucosa was taken as those normal appearing mucosa that was located 3 cm away from the tumour area. The dissected mucosa had to be thin and trimmed from the underlying excess connective tissue in order to improve flattening of mucosa as well as for better evaluation under light microscopic examination. The dissected mucosa was then placed in between 2-pieces of cardboard, reimmersed in 70% ethyl alcohol for 24 hours to improve flattening of the mucosal surface.34

The area of the normal appearing mucosa sampled was recorded as width x length (cm²).

After refixation with 70% ethyl alcohol, the mucosal samples were then washed in tapwater and then stained with Methylene –Blue (20-40 mL of 0.2% methylene-blue for 3-5 mins.) with mild shaking and then the mucosa was washed with water for 5 mins.

To further improve flattening of the mucosa and better observation by light microscopy, the mucosa was cut into small pieces and placed under 2 glass slides (5.0 x 8.0 cm).
To obtain ACF:

The mucosa was then examined under 4x with standard microscope with mucosal surface up. Under light microscopy, the methylene blue was seen concentrated in the nuclei of the ACF enabling their identification. These ACF was then marked on the glass slide and the mucosal border was mapped out. This was done so since during microdissection of the formalin-fixed colon mucosa, they have the tendency to curl back. With the help of the mark made on the glass slides ACF was able to be traced back. There can be more than one ACF in one glass slide preparation. The number of ACF was recorded and the number of crypts per ACF recorded.

ACF sampling:

ACF were identified as:
- darkly stained than the surrounding mucosa
- thickened layers of epithelium
- enlarged crypts
- irregular or slit-shaped lumina
- slightly elevated above the surrounding mucosa

With the help of microscope this ACF was microdissected from the colonic mucosa with a rim of normal surrounding mucosa.

The selected ACF were then processed with the usual manner.