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TRANSITIONAL CHANGES IN THE MUCOSA ADJACENT TO THE COLORECTAL CARCINOMA –A SEM STUDY

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Colorectal cancer is the fourth most common cancer worldwide, with about 677,000 deaths each year. The term transitional mucosa™ was coined by Filipe in 1969 to describe alterations in the morphology and mucin histochemistry of the large intestinal mucosa immediately adjacent to colorectal adenocarcinomas. The TM is considered as an important prognostic marker for patients with large bowel cancer following radical resection because patients with retained TM have a poorer prognosis. Three samples from the proximal margin of a resected colorectal surgical specimen were taken to study the extent of the transitional changes in them. They were selected approximately 2cms, 5cms and 10cms away from the tumor site. The tissues were then processed and viewed under Scanning Electron microscope (SEM) to gauge the extent of the transitional changes. These changes were compared with the normal colon samples taken from normal patients. Transitional changes were found both near as well as farther away from the tumor and were characterized.

Keywords: colorectal cancer, transitional mucosa, scanning electron microscope

INTRODUCTION

Colorectal cancer being the fourth most common cancer worldwide is responsible for about 677,000 deaths each year [1]. It is ranked the fourth most common cancer in men and third most common cancer in women throughout the world [2]. In Peninsular Malaysia, colorectal cancer is the second most common cancer after breast in both sexes. Furthermore, it is ranked the first among the males and the second among the females [3]. The Transitional Mucosa ™ adjacent to the colorectal cancer was first described by Filipe in 1969, as the mucosa surrounding the colorectal adeno carcinoma which showed altered mucus secretions [4]. An association was recognized between mucin histochemical changes at the margins of resection and a poorer clinical outcome of patients with colorectal cancer after surgery. Hence TM at the resected margins is of prognostic value [5]. The most apparent histochemical change in TM is the predominance of sialomucins as opposed to the sulphomucins found in normal human colorectal mucosa. With the high iron diamine-alcian blue (HID-AB stain), the normal colorectal mucosa stained brown and black owing to the predominance of sulphomucins, whereas the sialomucins in TM appeared as a blue staining reaction. The TM is predominantly confined to a zone within 2 cm of a carcinoma [6]. Greaves et al, using HID-AB stain found that the zone of TM extended to an average of 3.4 cm from the tumor site. They ascertained that the minimal and maximal changes were found to be in between 0 cm and 19.5 cm respectively from the tumor site [7]. Both the proximal and distal margins of the carcinoma exhibit the transitional changes in the mucosal layer [5]. Strikingly, the mucosa in the proximal margin of the tumor is liable to show more severe and extensive transitional changes than the distal margin [4]. As a pathology investigating means, the SEM provides the high magnification and great depth of focus to study growth pattern abnormalities on the colonic surface at the level of the individual cell [8]. Zhao Yu Zhen et al concluded that SEM may be a valuable tool in the diagnosis of colorectal carcinoma by taking into account the surface mucosal changes [9].

MATERIALS AND METHODS

Patients who met the inclusion criteria were explained to verbally and in written form on the objectives, procedures and risk of the study, before being asked for consent.

Normal colonoscopy samples

The normal colon sample was collected from patients undergoing normal colonoscopic examination in Dept of Surgery, Tengku Ampuan Afzan Hospital Kuantan. Subjects with no colon cancer of family history of colon cancer were selected for the study. The samples were taken using standard colonoscopy spikes (Brand; Radial Jaw 3). The samples were washed with a jet of saline as soon as they were brought out from the patient's body and

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gently scraped with a #2 nylon painter's brush to remove the mucus layer which may obscure the surface morphology. Then, the samples were fixed in the Mc Dowell fixative in labeled bijoux bottles. The bottles were then chilled in the ice box.

Samples from colorectal cancer resected specimens

Samples were taken from a patient who underwent laparoscopic anterior resection for a rectosigmoid mass. Three sites were selected i.e. 2cms, 5cms and 10cms from the tumor border in the proximal resected margin as shown in Fig. 1. Samples of size 1 cm^2 were removed from the above three sites. The surface mucus was removed and the samples were immediately fixed in the Mc Dowell fixative in labeled bijoux bottles. The bottles were then chilled in the ice box.

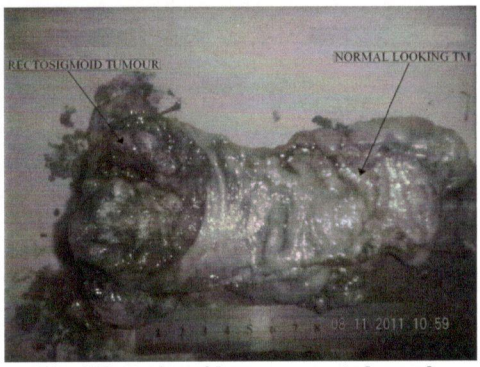

Fig. 1 Rectosigmoid cancer resected sample

Methodology

The processing of the samples was carried out in the Electron Microscopy Unit of International Islamic University campus in Indera Mahkota, Kuantan.

The samples were first washed with 0.1 M Sodium phosphate buffer thrice, followed by post-fixation with Osmium Tetraoxide at 4 °C for 2 hours. Then the samples were washed with 0.1M sodium Phosphate buffer twice for followed by washing with double distilled water thrice. Samples were then dehydrated through graded alcohol series, 50%, 75%, 95% and 100% followed by Acetone. The samples thus dehydrated were transferred into the Critical Point Drying specimen holder submerged in Acetone. After critical point drying, the samples were mounted onto a SEM specimen stub with a double-sided sticky tape. The stub was then put into the Gold Coating Machine and samples were coated with gold. Finally, the samples were viewed under the Scanning Electron Microscope (Zeiss Evo 50).

RESULTS AND DISCUSSION

Normal mucosa

The normal large bowel mucosa was characterized by the presence of long furrows running predominantly transversely across the mucosa. These are the in foldings of the mucosa and known as primary crypts. The SEM image of a normal large bowel mucosa is shown in Fig. 2-3. In between these primary crypts the surface epithelium was studded with openings of the secondary crypts which are the glandular units called crypts of Liberkuhn. Fig. 1 shows that the surface has been divided into territories separated by furrows of variable size.

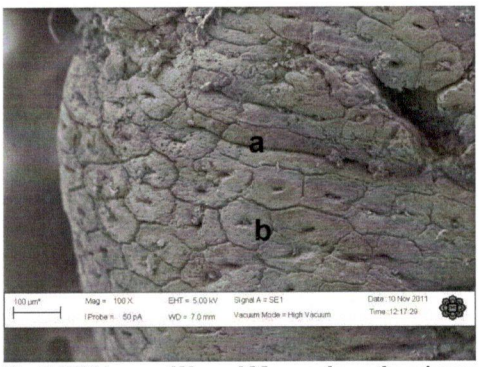

Fig. 2 SEM image of Normal Mucosa shows the primary crypt (a) and Crypt of Liberkuhn (b).

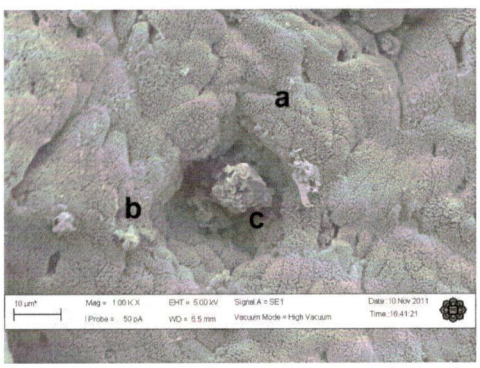

Fig. 3 SEM image of normal mucosa shows the absorptive cell (a), goblet cell (b) and the crypt opening through which mucus is secreted (c).

Though the epithelium of the crypt seemed to merge with its neighbor, the glandular units were separated from their fellow counterparts by narrow clefts. The crypts were composed of the cells which have migrated from the crypt opening on to the

surface. The secondary crypt openings were predominantly round and symmetrical in shape through which mucus was discharged as strands.

At high magnification, the cells on the crypt surface could be identified. The absorptive cells were predominant and were polygonal in shape, mostly pentagonal, with dense, surface microvilli as shown in Fig. 4. The microvilli often adhered to each other by a thin coating of mucus on their glycocalyces. Microvilli were closely packed, short and somewhat club like with rounded tips. The absorptive cells on the surface were similar, being regular but the goblet cells were protruding from the surface. The goblet cells can be differentiated with the sparse but longer microvilli than that of the absorptive cells and mucus extruding from their surface. Other goblet cells which have presumably discharged their contents appeared either as rounded holes or demonstrated a depressed irregular surface. These goblet cells were randomly distributed at an approximate ratio of 1 to every 5-10 absorptive cells.

TM 2 cm from the rectosigmoid tumor

The most striking feature of the transitional mucosa was the thickened mucosal surface compared to the normal mucosa. The thickened mucosa is due to the dilatation, elongation and branching of crypts. The surface also showed changes in the contour of the crypt openings which ranged from mild distortion to markedly distorted primary crypts. Sometimes these primary cryptal arrangements were lost and replaced by irregular nodules demarcated by residual primary crypts. The destruction of the crypt architecture thus, produced a villous appearance of the surface of the mucosa as shown in Fig. 4. At high power the secondary cryptal structure was also largely lost although the crypt orifices could be discerned with scrutiny. The crypt orifices also showed marked irregularities wherein they were incomplete and surrounded by an incomplete whorl of cells as shown in Fig. 4. The crypts also exhibited a total disorganized pattern of growth, with columns of cells proliferating in an apparently random manner to give rise to hyperplastic crypts as shown in Fig. 5. There was a disproportionate increase in the number of goblet cells when compared to the absorptive columnar cells. In some areas, some absorptive cells showed features of microvillar denudation as shown in Fig. 6.

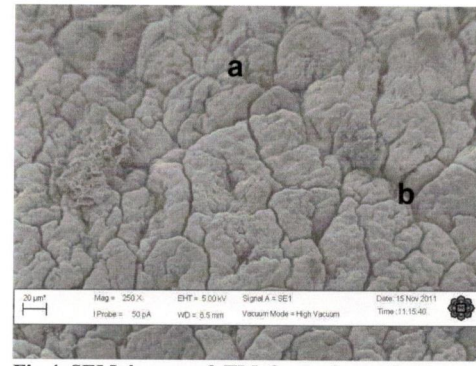

Fig.4 SEM image of TM 2 cm from the tumor shows the villi like mucosa (a) Features of an incomplete crypt is shown in (b)

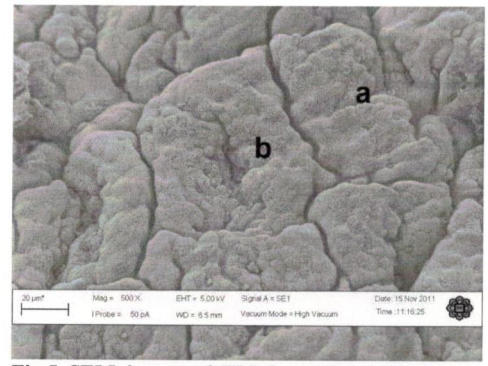

Fig.5 SEM image of TM 2 cm from the tumor shows features of distorsion of crypts (a) and hyperplasia of crypts (b).

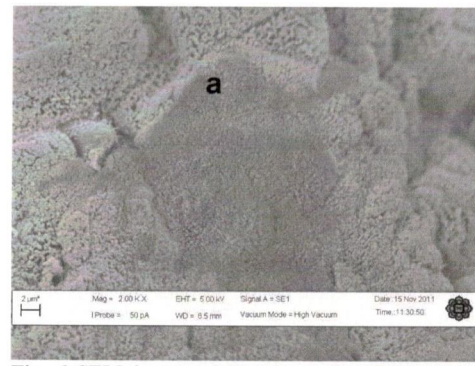

Fig. 6 SEM image of TM 2 cm from the tumor shows features of microvillar denudation (a).

TM 5 cm from the rectosigmoid tumor

From Fig. 7-8, the SEM image showed similar changes as above when compared to the normal mucosa. There was a predominant villous pattern of the mucosa as shown in Fig. 7.There were no areas of microvillar denudation identified though.

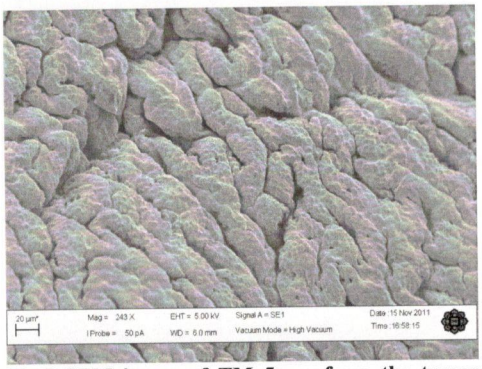

Fig.7 SEM image of TM 5 cm from the tumor shows extensive villi like changes.

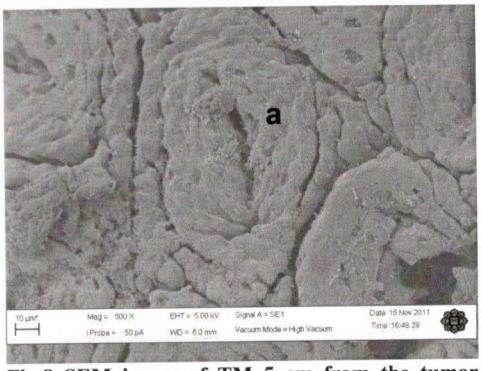

Fig.8 SEM image of TM 5 cm from the tumor shows a compressed crypt orifice (a).

TM 10 cm from the rectosigmoid tumor

Fig. 9 shows a low power view of the transitional mucosa which is nearly similar to the normal mucosa. But in high power as shown in Fig. 10, there is a loss of secondary crypt architecture as well as a disproportionate increase in the number of goblet cells. There were features of microvillar denudation identified as in Fig. 11. The crypts were cracked and fissured with the loss of the secondary crypt architecture.

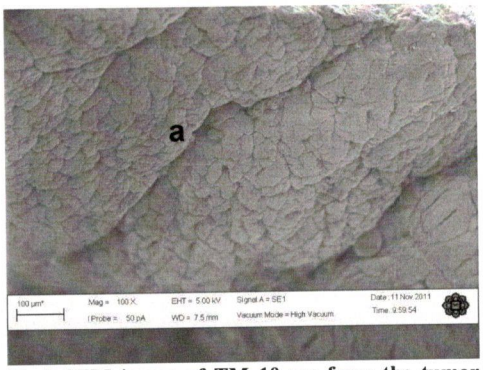

Fig.9 SEM image of TM 10 cm from the tumor shows the thickened mucosa, the 'step sign' (a).

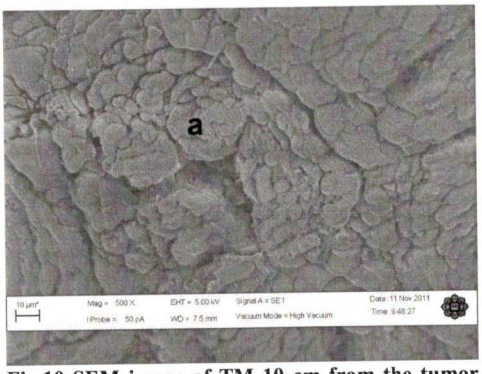

Fig.10 SEM image of TM 10 cm from the tumor shows a fissured crypt (a)

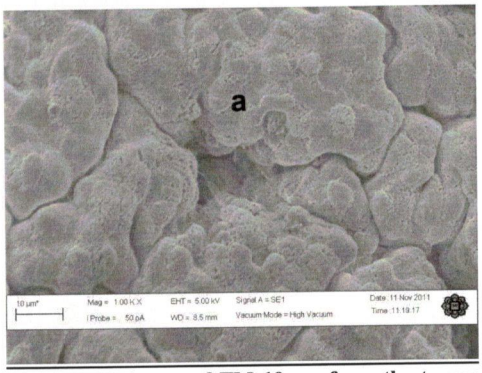

Fig.11 SEM image of TM 10 cm from the tumor shows a distorted crypt with features of microvillar denudation (a).

The most important morphologic prognostic parameters are the tumor extent, lymph node status, tumor grade and the assessment of venous and lymphatic invasion (TNM). Adding to this, researchers suggest that tumor budding, and tumor border configurations are important, additional histological parameters to be regarded in the prognosis [10]. Surgical resection is the preferred choice of treatment for colorectal cancer, and the pathologic assessment of the resection specimen provides data that is essential for postoperative outcome and the rationale for adjuvant therapy [11]. The debate whether the TM adjoining the colorectal carcinoma being a precancerous change or not is still not given a verdict.

This is because patients with retained TM at the margins of resection have a poorer clinical outcome after radical resection in spite of sections of intestinal margins when stained with HE were free of malignant cells. Local recurrence, considered as a catastrophe, has been attributed to the presence of occult metastases, inadequate resection, or implantation at the margins of resection tumor cells exfoliated during surgery. Hence, the evaluation of resection margins for biological changes of colorectal cancer specimens may be of high clinical value [12]. *Habib et.al(1985)* believed that a possible continuing process of carcinogenesis might occur in the remaining colorectum, particularly if there are widespread transitional changes at or near the anastamotic area. This can be due to higher levels of sialic acid in the retained TM, thus depressing tumor immunogenicity, and making it more sensitive to carcinogenic stimuli, and further predisposing it to a malignant transformation [13]. Wang et al confirmed a correlation between the presence of sialomucins at the resection margins and subsequent development of tumor recurrence in those patients [14]. Kim et al in their multivariate analysis concluded that patients with a positive distal margin, on postoperative pathology, should be considered as high risk for anastamotic recurrence [15].

TM, in this study was characterized by abnormalities in the crypt architecture and surface morphology and the changes were found in all three sections, i.e. 2cms, 5cms and 10cms from the tumor in the proximal tumor margin. These changes confirm the fact that the cell proliferation kinetics is abnormal in the TM. Previous studies have ascertained that the transitional mucosa was found anywhere between 3.4cm and 19.5cms and this was mainly proved by histochemical studies and not by morphological studies [7]. Moreover, previous studies using SEM to study the transitional mucosa only found the transitional changes 2cms away from the tumor margin whereas 10cms away from the tumor margin was considered as the normal mucosal counterpart [6]. The findings from this study suggest that the transitional changes were found up to 10cms away from the proximal tumor margin. This may predict that it might be a locally invasive tumor with an aggressive phenotype and poor prognosis. Moreover, this finding also emphasizes on the periodic surveillance of the patient to predict anastamotic recurrences in the future.

CONCLUSIONS

The present study is an attempt to characterize the SEM findings of the TM adjoining the colorectal carcinoma. The extent of TM and its presence at the margins of resection appear to be related to the prognosis of the patients with colorectal carcinoma after curative resection. A study including more number of patients may give us enough statistical data to ascertain a safe and specific cut off score for surgical resection in colorectal cancer patients. Moreover, further prospective studies on a larger scale and on the biological and molecular significance on the transitional changes of the otherwise normal looking mucosa are certainly needed to increase our understanding on TM and its implications on tumor recurrence for a better outcome for patients undergoing surgery for large bowel carcinoma.

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