

# IS MUCIN CONTENT A PROGNOSTIC INDICATOR IN COLORECTAL CARCINOMA?

NAILA IRUM HADI, KAZI ABDUL SHAKOOR, BUSHRA WASEEM

## ABSTRACT

*Objective* To evaluate the mucin profile of colorectal adenocarcinoma by means of a histochemical study and to correlate mucin content and histological grade vis-à-vis prognosis.

*Study design* Descriptive study

*Place & Duration of study* Jinnah Postgraduate Medical Centre Karachi, from August 2004 to August 2007

*Patients and Methods* Seventy patients who underwent surgical resection of sporadic colorectal adenocarcinoma over a 3-year period were evaluated for histological classification as to mucinous (MUC) or a non-mucinous (nMUC) subtype. They were further divided by histological differentiation into low-grade and high-grade tumors. The two groups were compared in terms of mucin content by histochemical techniques. Relationship between mucin content and histological grade was also analyzed.

*Results* On the basis of mucin content all cases were divided into mucinous (MUC; n = 14; 20%) and non-mucinous (nMUC; n = 56; 80%) adenocarcinomas. The predominant mucin on histochemistry of colorectal adenocarcinoma (nMUC and MUC) was sialomucin. Tumors were further graded histologically into low-grade (85.71%) and high-grade (14.28%). MUC adenocarcinomas showed a higher proportion of high grade tumors.

*Conclusions* Both MUC and nMUC are distinct histological subtypes of colorectal adenocarcinoma. No relationship could be established between pattern of mucin secretion and histological differentiation, however there is a definite relationship between mucin content and histological grade. It could be concluded from this study that mucin content vis-à-vis histologic grade have an influence on prognosis. Further elucidation and follow-up is needed.

*Key words* Colorectal adenocarcinoma, Mucin content, Histological grade.

## INTRODUCTION:

Colorectal carcinoma is a major cause of cancer associated morbidity and mortality in North America,

Correspondence  
Dr. Naila Irum Hadi  
Department of Pathology  
Jinnah Postgraduate Medical Centre  
Karachi.

Europe and other regions with similar life styles and dietary habits.<sup>1</sup> It is considered a disease of western world with the general thinking that population in Pakistan lacks the high risk factors for colorectal carcinoma. However, rising incidence of colorectal carcinoma has been observed in Pakistan and other developing countries, possibly as a result of industrialization and gradual westernization; the more so in younger age group compared to the western figures.<sup>2</sup>

Certain pathologic features have been considered important prognostic determinants in adenocarcinoma of colon and rectum. A distinctive cytologic trait is mucin production, usually extruded into the stroma with the formation of a gelatinous matrix variously described as colloid, mucoid and mucinous carcinoma.<sup>3</sup> Mucin content has a definite relationship to prognosis. This fact was confirmed by Umpleby et al who found that colorectal carcinomas of high mucin content require wide excision, tend to recur locally and carry a poor prognosis.<sup>4</sup>

The amount and histochemical characteristics of mucins secreted by carcinoma of large intestine vary from case to case and even in different regions of the same tumor. In most cases, secretion is scanty or absent. It may be intracellular, in gland-like lumina or in large extracellular pools, usually as a mixture of neutral mucins, sialomucins and sulfomucins. Sialomucins generally predominate in mucoid carcinomas, and differ from those in the normal colonic mucosa in relative proportions of different O-acetylated sialic acids and a greater susceptibility to neuraminidase digestion.<sup>5, 6, 7, 8</sup>

There has been a widespread interest in the area of mucin histochemistry as an aid to diagnosis of diseases of the intestinal tract.<sup>9</sup> Particular attention has been paid to alteration in relative amounts of sulphated and non-sulphated mucins present within epithelial cells.<sup>10</sup> In the present study, an attempt has been made to define the mucin profile of colorectal adenocarcinoma by means of a histochemical study in our patients and to point out a relationship between mucin content and histological grade.

#### **METHODOLOGY:**

Surgical specimens / paraffin blocks of 70 cases of colorectal carcinoma were retrieved from files of Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi. There were 50 male and 20 female patients (mean age of 46 years; age range 20 to 71 years), who had undergone surgical resection like colectomy, hemicolectomy, subtotal colectomy, segmental resection and abdomino-perineal resection. Gross features of the specimens received during the period of study were recorded. Representative sections taken and fixed in 10% formalin, processed and paraffin blocks prepared. 2-3 micron sections were cut for routine H & E (Hematoxylin and Eosin) staining. PAS (Periodic Acid Schiff) was done for staining mucin, PAS / AB (Periodic Acid Schiff/ Alcian Blue) for nature of mucin i.e. neutral and acidic. HID (High Iron Diamine) staining was done to find out sulfomucins and HID / AB (High Iron Diamine/ Alcian Blue) staining was done to differentiate between sulfomucin and sialomucin.<sup>11, 12</sup>

Colorectal carcinomas were graded into high-grade and

low-grade according to the recommendations of College of American Pathologists (CAP).<sup>13</sup> Only non-mucinous adenocarcinomas (nMUC) and mucinous adenocarcinomas (MUC) were included in this study. They were divided into following groups on the basis of mucin content, which were marked from '+' to '++++' as follows: mild (<20%) mucin = +, moderate (20-40%) = ++, marked (40-60%) = +++, extensive (> 60%) = +++. Tumors with mucin content < 60% were labeled as non-mucinous adenocarcinoma (nMUC) while tumors with mucin content > 60% were included in mucinous adenocarcinoma (MUC). Sections from normal colon and rectum were taken as controls.

#### **RESULTS:**

On the basis of mucin content, 70 cases of colorectal adenocarcinoma were divided into non-mucinous adenocarcinoma (nMUC; n = 56; 80%) and mucinous adenocarcinoma (MUC; n = 14; 20%). The tumor tissue in all cases of non-mucinous adenocarcinoma showed considerably less mucin than normal tissues. On histochemistry, no mucin was detected in 22 (31.43%) cases. Of the 48 (68.57%) cases showing varying degrees of mucin secretion, 3 (6.25%) cases showed only sulphomucin and 5 (10.42%) cases only sialomucin secretion. There was a mixture of sulphomucin and sialomucin in 40 (83.33%) of these cases with most of them showing a predominantly sialomucin secretion (30 cases; 75%). An equal distribution of sulphomucin and sialomucin was seen in 9 cases (22.5%) and only one of these (2.5%) showed predominantly sulphomucin.

The predominant mucin secreted by tumor cells was sialomucin (35 cases; 72.9%), followed by an equal distribution of sulphomucin and sialomucin (9 cases; 18.75%) and sulphomucin seen only in 4 cases (8.33%). No relationship could be established between the pattern of mucin secretion and the degree of differentiation of tumor.

There were 60 (85.71%) low-grade tumors and 10 (14.48%) high-grade tumors. We found out that mucinous adenocarcinoma (MUC) showed a higher proportion of high-grade tumors i.e. 28.57% as compared to non-mucinous adenocarcinoma (nMUC) which were 10.71%.

#### **DISCUSSION:**

Recently there has been considerable interest in detecting alterations of gastrointestinal mucins in various disease states with a view to using them as criteria for differential diagnosis and prognosis. Most investigators have applied histochemical methods to this problem and in particular the high iron diamine /alcian blue staining technique, which permits the simultaneous recognition of sulfated and non-sulfated sialomucins been widely used.<sup>10</sup> Combination of stains such as

alcian blue / PAS and HID / AB as was done in the present study also enabled us to simultaneously assess the proportions of various mucin types in the epithelium examined.

Mucin changes are a reflection of a more wide spread cellular response to unknown stimuli rather than local secondary effect of tumor growth. Longer exposure to carcinogens and a concentration gradient from caecum to rectum may explain why predominance of sialomucins in the mucosa of the left colon is more extensive and intense. The predominance of sialomucins and reduction of sulfated ones, which have been demonstrated in the goblet cells of mucosa both around carcinomas and in more distant patches, resembles the mucus secretion pattern of human foetal gut. Such changes could therefore be further evidence of the reversion to an embryonic state, which may characterize the early stages of carcinogenesis. A positive result will lead to a more thorough examination of the patient and increases the chance of an early diagnosis of cancer.<sup>14</sup> In the present study also relatively small amount of sulphomucin secretion (4 cases; 8.33%) and a significant increase in sialomucin production were recorded with 72.9% of the colorectal adenocarcinoma producing predominantly sialomucins.<sup>15</sup>

Mucinous adenocarcinoma is a special type of colorectal carcinoma in which large lakes of extracellular mucin are formed, mixed with collections of tumor cells.<sup>16</sup> Different series define mucinous carcinoma as the presence of at least 50% – 60% mucin in the extracellular matrix.<sup>3,4,17,18</sup> In some cases there is an admixture of extracellular and intracellular mucin, the latter resulting in signet ring configuration.<sup>18</sup> Previous studies have shown that mucinous adenocarcinoma comprises 15% of colorectal carcinomas and occurs most commonly in the rectum.<sup>19,20</sup> Recent studies however have reported an increase in the incidence of mucinous adenocarcinoma with figures of 38.5% in one study.<sup>18,21</sup> We grouped 70 cases of colorectal adenocarcinoma into mucinous and non-mucinous adenocarcinomas on the basis of mucin content. In this study incidence of 20% of MUC adenocarcinoma is in agreement with that observed by Suma and Nirmal<sup>17</sup> and Enriquez et al.<sup>22</sup>

The variation between observers in histological grading of colorectal carcinomas found a highly significant level of agreement, however disagreements between observers were in many instances “haphazard”. The wide discrepancies in parameters used for histological diagnosis between some pathologists’ means that studies on treatment and prognosis of colorectal cancer which utilize histological grade for comparison purposes must be viewed with considerable skepticism.<sup>23</sup> However based on the recommendations of College of American Pathologists,<sup>13</sup> we followed the 2-tiered grading system

(high-grade and low-grade) and found it quite useful in reducing inter-observer variation. Although Hilska et al has shown a worse prognosis for tumors with mucin as compared to tumors with no mucin content, the author advocates the use of staging as superior to histological grading as a criteria for prognosis.<sup>24</sup> Some authors consider mucin content to be an independent adverse prognostic factor, but certain other researchers still debate the fact that it cannot be regarded as an independent prognosticator. According to Sanjay et al the current consensus of college of American Pathologists (CAP) and American Joint Committee for Cancer (AJCC) is that mucinous differentiation is not proven as a statistically significant prognostic factor independent of histologic grade.<sup>25</sup> In studies by Enriquez et al<sup>22</sup> and Mirna et al,<sup>26</sup> MUC adenocarcinoma showed a higher tumour grade than non-MUC adenocarcinoma as we found in the present study.

The prognostic value of tumor grade in colorectal adenocarcinoma has not been adequately investigated in matched studies between MUC and non-MUC types. Although a lot of work has been done on mucin content in other countries, to our knowledge this was the first study carried out on this subject in Pakistan. However, larger studies in patients with colorectal adenocarcinoma (MUC and non-MUC) with matching for both grade and stage have to be undertaken to determine the impact of mucin content on the patient’s survival.

#### CONCLUSIONS:

Mucin histochemistry can effectively determine the mucin profile of colorectal adenocarcinoma. Mucin content when considered together with histological grade can be regarded as important prognostic indicators.

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