ORIGINAL ARTICLE

Association of Alterations in Mucin Expression in the Transition Zone with the Histological Grade of Colorectal Carcinoma

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ABSTRACT

Background: Worldwide colorectal cancer accounts for 9.7% of all incident cancers. Despite the increase in incidence in colorectal carcinoma in developed and developing world, mortality in both sexes has slowly declined, due to early diagnosis. Overtime there has been devised a number of clinical and morphological parameters and one of these histological features of colorectal carcinoma with a significant impact on screening, early diagnosis and prognosis is alterations in mucin expression.

Methods: A total of 50 cases of CRC were selected. The present study was done to evaluate the association of alterations of mucin that is from sulphomucin to sialomucin with the histological grade. The specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin. The special stains for the mucins were mucicarmine, PAS Alcian blue, HID.

Results: Out of the 50 cases 34 cases including the mucinous and signet cell carcinomas were evaluated for mucin histochemistry. Out of these 34 cases 26 cases showed an alteration in the mucin expression which was directly proportional to the advancing grade of the tumor showing a significant p – value.

Conclusion: Our study proved the predominance of sialomucin with poor histological grade.

Keywords: Colorectal carcinoma, mucin, PAS alcian blue

INTRODUCTION

Worldwide colorectal carcinoma accounts for 9.7% of all incident cancers. Despite the increase in incidence in colorectal carcinoma in developed and developing world mortality in both sexes has slowly declined due to early diagnosis. Overtime there have been devised a number of clinical and morphological parameters, and one of these histological features with significant impact on screening, early diagnosis and prognosis is alterations in mucin expression. Therefore the transitional mucosa of colon harboring a carcinoma has conclusively demonstrated to be morphologically and histochemically abnormal[1].

The Mucin molecule is highly glycosylated, with long sugar side chains attached to a protein backbone (especially the amino acids serine and threonine) through oxygen bonds. These bonds prevent the breakdown of mucin by proteases, and provide density and viscosity. Mucin can therefore efficiently fulfill its role as the main constituent of the mucus-protecting layer in the gastrointestinal tract. The tandem repeat peptide, rich with serine and threonine and containing the dense sugar side chains, is typical of various Mucin species. All mucins share some general characterisitics, for example, they have repetitive domains of peptides rich in serine, thereonine, and proline in their backbone. Serine and threonine are sites for O-linked and N-linked glycosylation. Presence of the tandem repeat domain which varies in number, length and O-glycosylation is the common structural component of all mucins[2].

The general structure and biochemical composition of mucins provides protection for the cell surface where as specific molecular structures regulate the local microenvironment near the cell surface. In addition, mucins also help in communication the information of the external environment to the epithelial cells via cellular signaling throught membrane-anchored mucins[3].

Studies, by means of high iron diamine-alcian blue (HID-AB) staining, have shown that when normal colonic mucosa is compared with the epithelium adjacent to carcinoma, changes in Mucin composition occur. These changes can be both qualitative and quantitative. The amount and histochemical characterisitics of Mucin secreted by carcinoma of large gut vary from case to case and even within different regions of the same tumor [4].

Mucins are broadly classified into neutral and acidic chemotypes. The proportion between acidic and neutral mucins is constant and appears to be modified in IBD. Such changes are through to proceed the development of Neoplasia. Neutral

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mucins stain magenta with PAS stain in contrast to acid mucins which stain blue with alcian blue [5]. Acid Mucins are subdivided into sialomucins or sulphomucins, based respectively on the presence of terminal sialic acid or sulphate groups on the oligosaccharide chain [6]. It has been observed that sulphomucin, which stains black-brown by HID, is mainly present in normal colonic mucosa, whereas sialomucins, which gets blue in color by AB staining, is mainly seen in transitional mucosa. Data in literature suggests that sulphomucin reactive cells are inversely related to the degree of dysplasia [7]. In normal mucosa sulphomucins are see localized in the cytoplasm, in association with the cell apex, luminal surface or secretory products, whereas in carcinoma, there is loss of such sulphomucins from the cytoplasm[8]. In adenocarcinoma there seems to be reduction in the total mucous output, reduction in sulphation and increase in sialylated mucin whereas, mucinous carcinoma shows hypersecretion of mucin with no significant loss of sulphation but it is accompanied by changes in sialic acid content and oacetylation. Thus, sulphomucin depletion occurs in colorectal adenocarcinoma but not in mucinous carcinoma where Sulhpomucin displays a progressive decrease from early to advanced disease[8, 9].

The term trasitional mucosa was first used by filipe in 1969 to describe changes in morphology and mucin biochemistry of colonic mucosa, immediately adjacent to colorectal carcinoma. Trasitional mucosa has two chracterisitic features, elongation and branching of mucosal crypts with dilation of goblet cells and, alteration in the ratio of sulphomucins to sialomucins as observed with HID-AB stain. Brown-black sulphomucin, the predominant secretion of normal mucosa is decreased in transitional mucosa which exhibits an increase in non-sulphated blue sialomucin[10]. This predominance of sialomucins (and reduction of sulfated ones) resembles the pattern of mucous secretion seen in the human fetal gut. Such changes could thus be evidence of reversion to an embryonic state characterizing the early stages of colorectal carcinogenesis, increasing the chances of detection of early stages of colon cancer[11].

It has also been observed that the preponderance of sialomucins in the mucosa of the left colon is more extensive and intense as compared to that seen in tumors of the right colon. This can partially be explained by a prolonged exposure and an increased concentration gradient of carcinogens in the left colon and rectum, versus the right colon[12].

Mucin histochemical features of the transitional zone seem to play a role in predicting metastasis and prognosis of CRC. Transitional zone showing sulphomucin appears to have greater lymphoid infiltration and is associated with favourable prognosis. On the contrary, sialomucin type of mucin in transition zone shows low grade lymphoid infiltration and seems to have grave prognosis[12].

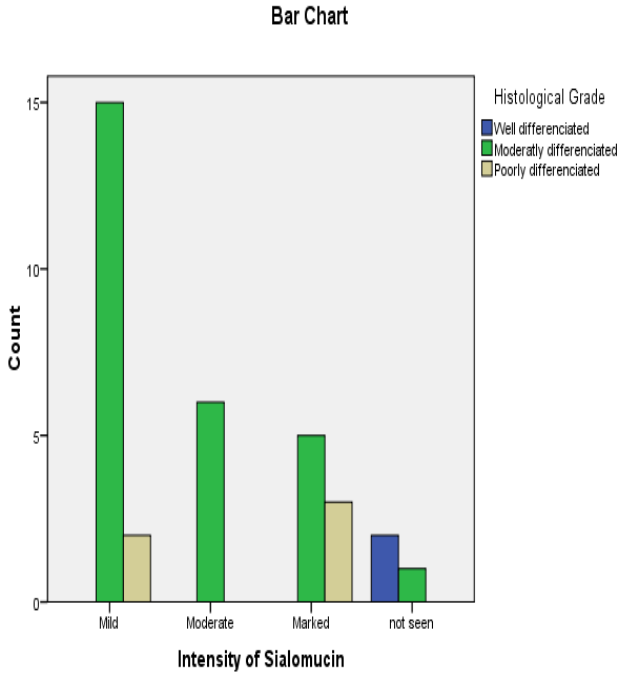
MATERIALS AND METHODS

A descriptive study consisted of samples received between 1st of October 2010 and September 2011 diagnosed in the department of morbid anatomy and histopathology of UHS, Jinnah hospital Lahore, Mayo hospital Lahore and Services hospital Lahore. Total member of cases were 50. A total 10-15 blocks from each specimen were examined Paraffin embedded sections were prepared using automatic tissue processor, followed by preparation of paraffin block using our embedding station. The sections were stained with H&E stain to determine the histological diagnosis for selecting tissues with mucin production. Slides were stained with PAS, Alcian blue, high iron diamine-Alcian blue, Meyer's mucicarmine and Alcian blue-PAS to demonstrate the mucin production. Positive and negative controls were run for quality assurance. Here is a brief account of various mucin stains summarized along with their characteristics. Meyer's mucicarmine is most frequently used histochemical stain to demonstrate acidic mucin[13,14]. These stains are useful in demonstrating both intracellular and extracellular mucins. PAS (peroidic acid-Schiff) stains glycogen as well as mucins, but tissue can be pre-digested with diastase toremove glycogen rendering PAS an important mucin stain[15]. The Alcian Blue stain is primarily used to stain acid mucopolysaccharides. The alcian blue stain at a pH of 2.5 stains for both sulfated (sulphomucins) and carboxylated (sialomucins) mucopolysaccharides which are found in the goblet cells located in the intestine. Alcianblue-PAS staining is used to differentiate between acid and neutral mucin15. High iron diamine-Alcian blue detects sulphomucins (brown) and sialomucins(blue) in the tissue[16,17].

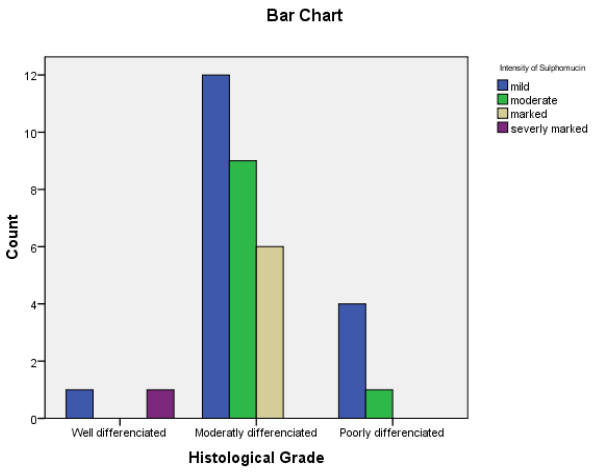
RESULTS

The present study was done to evaluate the alteration of mucin that is from sulphomucin to sialomucin with the histological grade. A total of 50 cases were evaluated for histological classification as mucinous and nonmucinous subtypes on the basis of mucin content. They were divided as follows mild (<20%)=+moderate (20%-40%)=++ marked (40-60%=+++, and severely marked=+++. Sections from normal colon were taken as controls. First H&E staining was done and out of 50 cases, 32 cases were mucinous. After H+E, PAS staining was done in

which we located the mucinous. In Alcian blue staining we differentiated the acidic mucins from other type of mucins. In high iron diamine Alcian blue staining we further differentiated between sulphomucins (brown) and sialomucin (blue). On the basis of mucin content in high iron diamine alcian blue staining 50% cases showed mild sulphomucin, 29.4%cases showed moderate sulphomucin, in 17.6% cases they showed marked sulphomucin, cases showed mild sialomucin, 17.6% cases showed moderate sialomucin and 33.3% cases showed marked sialomucins. Histological grading was also done and compared with the intensity of sulphomucins and sialomucins. 5.8% cases were well differentiated with 50% showing mild and 50% severely marked intensity of sulphomucins.79.4% cases were moderately differentiated with 44.4% showing mild 33.3% moderate and 22.2% severe intensity of sulphomucins, 14.7% cases were poorly differentiated with 80% showing mild 20% severe intensity of sulphomucins,5.8%cases were well differentiated with none showing mild moderate or severe intensity.79.4% cases were moderately differentiated with 55.5% showing mild 22.2% moderate and 18.5% severe intensity of sialomucins.



14.7% were poorly differentiated with 40% showing mild and 60% severe intensity of sialomucins.



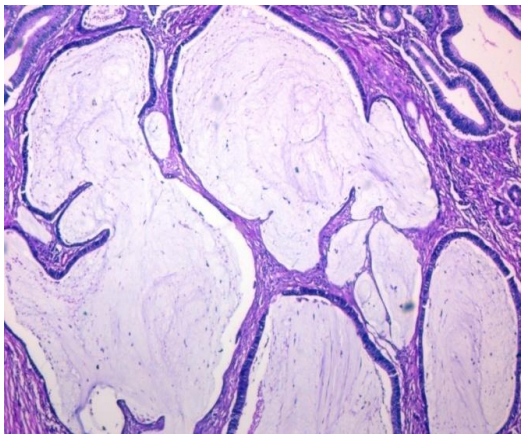


Fig 1. Photomicrograph shows mucin (pink) in mucinous adenocarcinoma of colon.

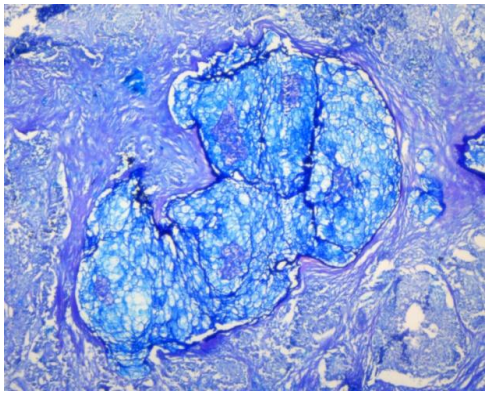


Fig – 2. Photomicrograph shows acidic mucin (blue) and arrow shows neutral mucin (megenta) after Alcian blue-PAS stain in mucinous adenocarcinoma of colon.

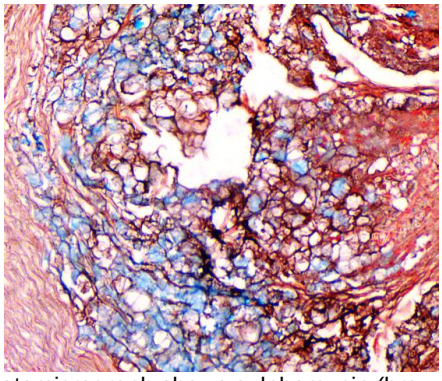


Fig.3. Photomicrograph shows sulphomucin (brown) and arrow shows sialomucin (blue) after HID – Alcian blue stain in mucinous adenocarcinoma of colon

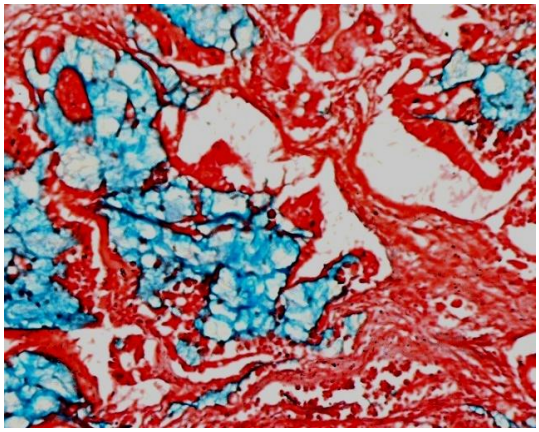


Fig – 4 Photomicrograph showing mixed pattern sulphomcin (brown) and sialomucin (blue) in moderately differentiated mucinous adenocarcinoma of colon

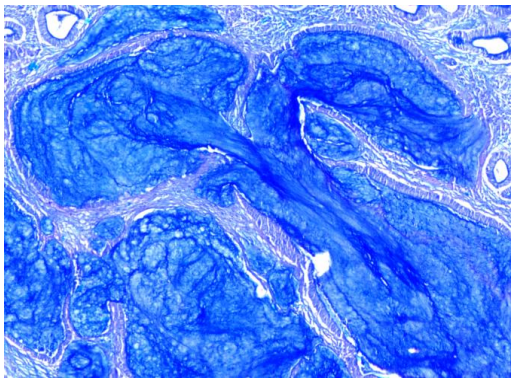


Fig 5: Photomicrograph showing mixed pattern sulphomcin (brown) and sialomucin (blue) in moderately differentiated mucinous adenocarcinoma of colon

DISCUSSION

Recently, there has been lot of interest in the transitional zone of colorectal carcinoma. Many investigators have used different histochemical stains for the detection of sulphated and non sulphated mucins. Compared with nonmucinous carcinoma, mucinous carcinoma has a high incidence for venous, lymphatic invasion and lymph node metastasis. Consequently, prognosis is worse[19]. The transitional zone has characteristic histological picture although it is normal looking mucosa but on staining with specific stains reveals characteristic changes from sulphomucins to sialomucins. In the present study quantitative analysis of sialomucins and sulphomucins in colon biopsies was preformed [20]. The predominance of sialomucins and reduction of sulphated ones which have been demonstrated in the goblet cells of mucosa around carcinoma which pattern is similar to human fetal gut[21]. Transitional mucosa was a term first coined by filipie 1969 to describe the histochemistry of mucosa adjacent to colorectal adenocarcinoma. Alteration in ratio of sulphomucins sialomucins was demonstrated in our study by HID/AB technique[22]. The mucin expression was quantified into different categories of mild, moderate marked and severely marked according to criteria as mentioned in detail in material and methods. Similar to our study the vast majority of histochemical studies carried out for colon showed the presence of acid and neutral mixed sections. The prognostic significance of mucin content in colorectal cancer remains a controversial issue. It is suggested that increased sialomucins reflect a transformation to fetal epithelium since in embroyonic and early fetal life large bowel mucosa mainly secrets sialomucins. In the present study 32(64%) were mucinous. Previous studies have shown that mucinous carcinoma comprises 15% of colorectal carcinoma whereas recent studies have reported an increase in incidence of mucinous adenocarcinoma which is 38.5% in one study. In studies by Enrique et al and Mirna at al, mucinous adenocarcinoma showed a higher tumour grade as we found in our study[23]. Out of the 34 cases 26 cases showed an alteration in the mucin expression which was directly proportional to the advancing grade of the tumors. Some studies have highlighted the predominance of sialomucins contrary to these we found different results that is predominance of both sulphomucins and sialomucins followed by sulphomucins whereas sialomucins was observed only in few cases[24]. In our study on the basis of histological grading majority of cases were moderately differentiated whereas the poorly differentiated had the greatest intensity of sialomucins. According to Sanjay et al the current consensus of college of American pathologist (CAP) and American joint committee for cancer (AJCC) is that mucinous differentiation is not proven as a statistically significant prognostic factor of histological garde[25]. It was seen that mucin content in our study was an independent and important prognostic factor, however researchers still believe the fact it is not

regarded as independent prognostic factor[25, 26]. However, larger studies in patients with colorectal adenocarcinoma (MUC and non-MUC) with for both grade and stage have to be under taken to determine the impact of mucin content as a prognostic marker.

CONCLUSION

The study proved the predominance of sialomucins with poor histological grade .Mucin content when considered with histological grade can be regarded as important prognostic indicator. Staining with HID/Alcian blue is simple, practical tool of low cost and high sensitivity.

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