Research Article

Comparison of Mucin Patterns in Colonic Pathologies by Histochemistry

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Abstract

Introduction: High Iron Diamine Alcian blue (HID-AB) is a well-established technique for demonstrating colonic acid mucins namely sulphomucins (brown- black) and sialomucins (blue). In certain colonic pathologies expression of sulphomucin, the predominant mucin in normal colonic epithelium is altered.

Objective: To compare the changes in the pattern of mucin staining in, adenocarcinoma (AC), normal colonic epithelium, solitary rectal ulcer (SRU), transitional zone mucosa (TZ) (i.e.: normal non dysplastic mucosa adjacent to adenocarcinoma) and in ulcerative colitis (UC).

Method: AC-21, normal colonic epithelium-30, SRU-23, TZ-20, UC-29 biopsies were stained with the HID-AB stain. Two investigators assessed the percentages of mucin staining by counting the number of cells staining for sulphomucin and sialomucin in an area of colonic epithelium measuring 1mm in length. The differences in these 5 categories were assessed for statistical significance using the one-way ANOVA test and a Post Hoc Comparison of the mean values was carried out to establish which groups were different.

Results: ACs showed no sulphomucin or sialomucin staining while normal colonic epithelium showed nearly 100% sulphomucin staining.

The percentage mean value for sulphomucin staining was AC-12.47%, normal colonic epithelium - 96.7%, SRU-35.7%, TZ-48.98%, and UC –75.43%. Hence the differences between these categories reached a level of statistical significance except between TZ and SRU.

Conclusion: The patterns of mucin staining observed with HID/AB staining included relatively similar changes in mucin staining in the TZ and SRU, the loss acid mucin staining in AC and the relatively mild degree of sulphomucin loss in UC. Though there are significant differences in mucin staining between some of these colonic pathologies practical problems were encountered in using HID/AB as a diagnostic tool.

Introduction

Intestinal mucins are carbohydrate rich glycoproteins with a unique molecular structure and chemical properties [1]. The carbohydrate component is arranged in oligosaccharide chains of varying length and degree of branching. The gastrointestinal mucins in general are divided into neutral and acidic mucins. The intestinal mucins of both large and small intestine are acidic with gastric mucins being largely neutral [2]. Further, the colonic mucins are known to be predominantly sulphated mucins while the small intestinal mucins are predominantly sialic in nature [3].

The mucosal surface throughout the gastrointestinal tract needs to resist the aggressive external elements present in food and encountered during its normal function.

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Thus, the gastrointestinal mucins play a role in protection as well lubrication of the gastrointestinal tract. This defensive system is based on the fundamental characteristics shared with barriers found at other mucosal surfaces. The barrier enabling exchange between the epithelial cells of the gut lumen for the purposes of nutrition and protection, is made up of a layer of secreted mucus and a cell-surface membrane glycocalyx. The mucus defensive barrier forms the first line of defense to the external environment and contains both innate and adaptive immune elements [1].

It is therefore not unusual for the composition of mucins to change in various gastrointestinal disease processes either as a primary event or secondary to the disease itself. Mucins appear to be altered in adaptive processes such as metaplastic conditions including Barrett oesophagus, intestinal metaplasia in the stomach as well as disease processes including ulcerative colitis, colonic polyps and colonic and gastric neoplasia [4].

While further studies into the pathogenesis and reasons for the alteration of mucin is required, the current study was carried out to specifically identify whether these alterations could be used for diagnostic purposes. This was deemed important as there are many situations in which diagnostic pit falls are encountered such as in the differential diagnosis of SRU from AC and ulcerative proctitis in biopsy specimens. Since HID/AB stains are relatively cheap they may be considered for routine use in the diagnosis difficult cases.

Method
Wax blocks of thirty cases from each category diagnosed as normal colonic epithelium, AC, SRU and UC were retrieved from the departmental archives. 20 sections from TZ adjacent to malignancies obtained from colectomy specimens were also included. The slides were reviewed, and the original diagnosis was reconfirmed by two co-investigators.

HID/AB staining was performed on sections from these blocks as follows.
The HID solution was prepared by using N, N-Dimethyl – meta - phenylene diamine-hydrochloride 120mg and N, N-Dimethyl–para-phenylenediamine-dihydrochloride 20mg dissolved in 50ml of distilled water with 50ml of 60% ferric chloride solution added in a Coplain jar. This fresh Diamine solution was then added to tissue sections that were brought to distilled water and left covered for 24 hours. The sections were then washed well in running water and were counterstained with 1% Alcian blue in acetic acid for 5 minutes. The slides were then washed again and counterstained with 0.5% aqueous neutral red. Slides were further washed dehydrated, cleared, and mounted.

The stained slides were then evaluated by the two investigators with no knowledge of the original pathology of these cases. The mucin staining was assessed by counting the number of cells stained for sulphomucin (brown) and sialomucin (blue) and expressed as a percentage in an area of colonic epithelium measuring 1mm in length.

There were 21 ACs, 23 SRU, 30 normal colonic epithelium, 20 TZ mucosa and 29 UC slides that were deemed satisfactory for assessment. There were several cases that had to be excluded from the original number of thirty in some categories due to lack of tissue during further sectioning or inadequate tissue for evaluation of staining.
When there was a lack of concordance of > 10% between the two investigators the scoring was done at a multi-headed microscope and a consensus score was achieved.

ANOVA test and a Post Hoc Comparison of the mean values for each category was carried out to establish significant differences in staining patterns in each group.

Results
The concordance between the two investigators was high and <1% of the cases needed review.

The results for the percentage of sulphomucin and sialomucin staining in each of the biopsies of the five categories investigated are tabulated in Table – 1.

The AC showed little or no mucin staining (Figure 1) with only three cases showing sulphomucin staining. These were cases of AC arising in adenomatous polyps, where the adenomatous areas showed sulphomucin staining whilst the invasive component lacked both sulpho and sialomucin staining.

Table 1: Percentage of sulphomucin and sialomucin staining in different colonic pathologies.

| Normal (n=31)		Adenocarcinoma (n=21)		Ulcerative colitis (n=29)		SRUS (n=23)		Transitional Zone (n=20)	
Sulpho%	Sialo%	Sulpho%	Sialo %	Sulpho%	Sialo%	Sulpho%	Sialo%	Sulpho%	Sialo%
98	2	0	0	93	7	5	95	50	50
100	0	0	0	75	25	0	0	35	65
100	0	0	0	55	45	0	0	60	40
100	0	0	0	10	90	30	70	100	0
100	0	0	0	65	35	3	93	10	90
100	0	0	0	90	10	0	100	100	0
100	0	70	30	60	40	20	80	50	50
100	0	0	0	100	0	10	90	55	45
100	0	0	0	5	95	15	85	50	50
100	0	0	0	95	5	60	40	25	75
70	30	0	0	45	55	7	93	55	45
100	0	0	0	95	5	30	70	20	80
90	10	100	0	45	55	35	65	40	60
100	0	80	20	60	40	25	75	5	95
70	30	0	0	100	0	0	0	5	95
85	15	0	0	100	0	30	70	90	10
100	0	0	0	45	55	45	65	3	97
100	0	0	0	55	45	45	55	40	60
100	0	0	0	95	5	95	5	50	50
100	0	0	0	100	0	45	55	90	10
100	0	0	0	100	0	90	10		
100	0			100	0	97	3		
100	0			100	0	100	0		
100	0			100	0				
100	0			100	0				
100	0			93	7				
100	0			95	5				
100	0			97	3				
90	10			95	5				
100	0			95	5				
93	7								
MEAN VALUE 96.65	3.37	12.47	2.49	75.43	17.93	35.70	68.64	48.98	56.01
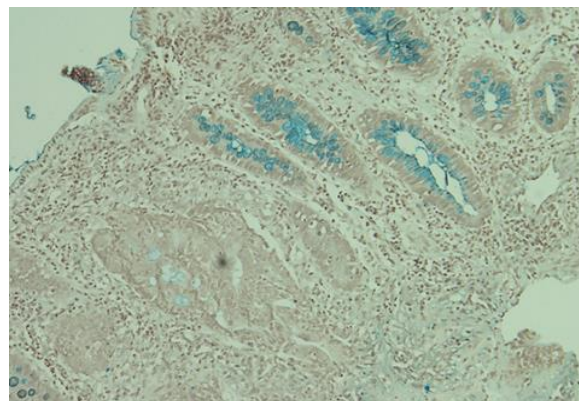
Figure 1: Adenocarcinoma focus showing negative mucin staining (right lower) and sialomucin positivity (left upper) in the TZ (HID-AB stain x200)
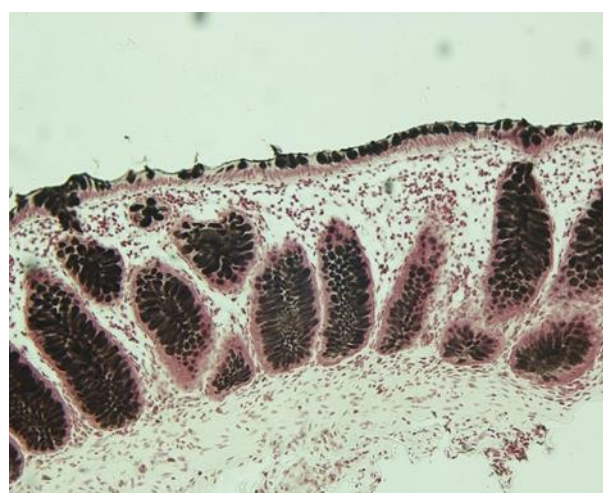

Figure 2: 100% sulphomucin positivity in normal colonic mucosa (HID-AB stain x200)

The normal colonic mucosa showed an overwhelmingly predominant sulphomucin staining with a percentage mean value of staining reaching 96.7%. (Figure 2)

UC biopsies showed a mild degree of depletion of sulphomucin staining with a mean percentage value of 75.43%. (Figure 3) However, complete or marked degree of mucin depletion was not evident except in two cases. Interestingly the TZ mucosa and the SRU biopsies showed moderate degrees of sulphomucin staining which reached mean values of 48.98% and 35.70% respectively. (Figure 4) Hence there was a significant difference in the percentage mean values AC, normal colonic mucosa and UC groups. However, the difference between SRU and TZ mucosa was not significant. (Figure 5)
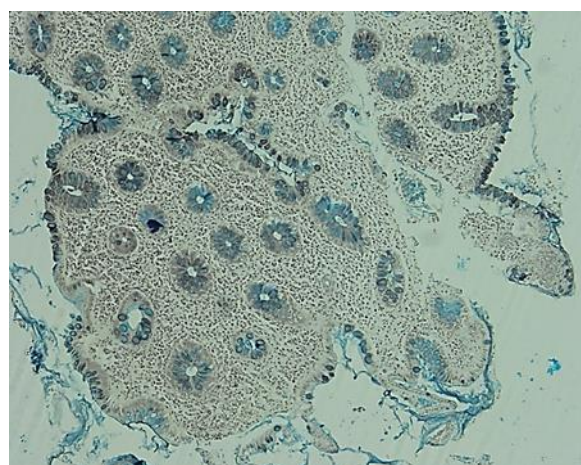
Figure 3: Mixed sialomucin and sulphomucin staining in UC (HID-AB stain x200)
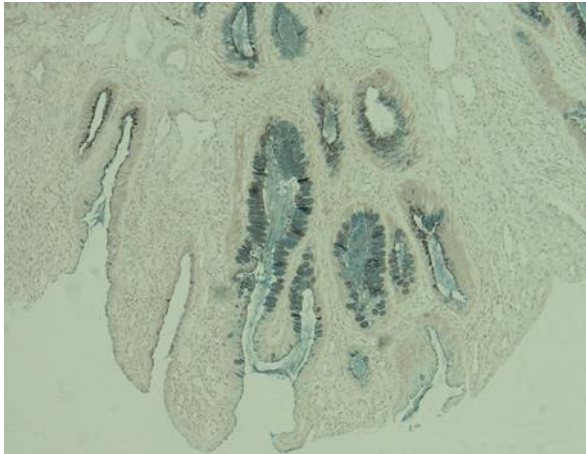
Figure 4: Mixed sialomucin and sulphomucin staining in SRU (HID-AB stain x200)
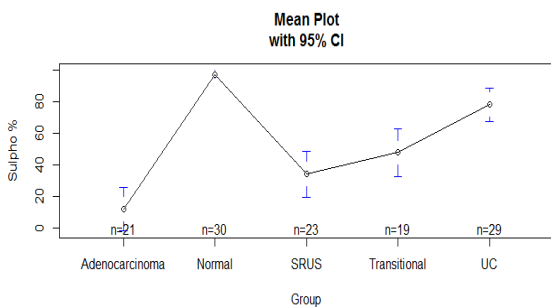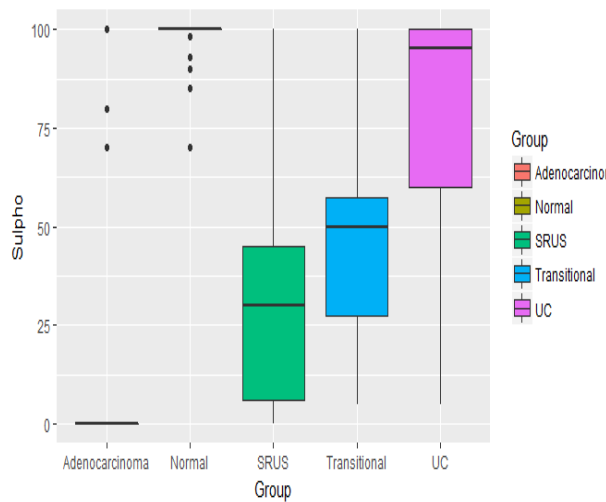
Figure 5: The difference in the percentage mean values among the five groups.

Discussion

Mucins are well documented in playing essential physiological roles, including protecting epithelial surfaces against damages [5], suppressing inflammatory activity by preventing direct exposure of commensal bacteria to the epithelium (6) and transmitting information from the external environment to the epithelium referred to as cell signaling [7].

The biopsies of AC used in this study showed little or no staining for either sialomucin or sulphomucin. In the three cases where staining was demonstrated it was seen mainly in the adenomatous component of the tumour rather than the invasive component.

This draws an interesting parallel with a study conducted on the expression of mucin in different histological grades of colonic carcinoma in a Ghanian population that showed a decrease in the acid mucin pattern with a concomitant increase in the neutral mucins when progressing from low grade well differentiated carcinoma to high grade poorly differentiated carcinoma [8]. In this study unlike ours the mucin patterns studied included acid and neutral mucins using a Alcian blue / PAS staining technique. It has been therefore postulated that acid mucins may inhibit tumour growth as well as be involved in cell division and that down regulation of acid mucin expression may drive or predispose the colonic tissue to malignancy.

Previous studies have also described the findings of increased mucin heterogeneity in both intra- and intertumoral regions of gastric cancers. Well-differentiated cancers typically contain more sulphomucin, whereas sialomucins predominate in moderately and poorly differentiated cancers [9].

This data indicates that further research regarding mucin expression especially mucin genes and biological behavior of gastrointestinal carcinomas are warranted. Hence, the possibility that mucins play a much greater role in the pathogenesis of malignancy should be further investigated.

When considering the pathogenesis of inflammatory bowel disease and UC in particular, a complex interplay of factors is implicated. Chief amongst them are host immune factors due to the underlying genes

and environmental factors such as diet, nutrients and the gut microbiome [10]. Further, there is evidence that the epithelial mucosal barrier that normally helps to isolate the lamina propria from luminal bacteria, is reduced in patients with Inflammatory Bowel Disease (IBD) [11].

Changes in the composition of colonic mucin in IBD may also provide some information on the underlying mechanism of disease and in assessing the cancer risk in colitis. As described, the mucus in the normal colon contains a high proportion of sulphomucins. In UC increased production of sialomucins, decreased sulphomucins and reduction or loss of O-acetylation are observed. These changes appear to be related to the degree of inflammation and are particularly marked in dysplastic epithelium in the absence of inflammation, most frequently in colitis patients at risk of cancer [12,13].

Epidemiological studies in Leicestershire have shown that South Asians in Britain, that is, those families who have migrated to Britain from India, Pakistan and Sri Lanka, particularly Hindus, have a greater incidence of UC but a lower incidence of colorectal carcinoma [14, 15, 16]. The same is true of South Asians living in their country of origin as shown by studies carried out in the Indian subcontinent [17].

It was also interesting to note that the TZ mucosa and SRU biopsies showed some degree of similarity in the sulphomucin and sialomucin content as demonstrated in this study.

The TZ mucosa is defined as colonic mucosa displaying morphological variations such as increased crypt length, gland branching and tortuosity and an increase in the size and number of goblet cells seen on light microscopy. The electron microscopic features include immaturity and heterogeneity of mucin droplets whilst histochemistry demonstrates the predominant staining of sialomucins. Such mucosal alterations are seen in association with a wide variety of conditions including mucosa adjacent to colorectal carcinoma, adenomatous polyps and in familial polyposis coli. This, along with the absence of

sulphomucin in the mucosa beside non adenocarcinomatous primary neoplasms tend to suggest the possibility that this is a premalignant event [18]. However, it is also believed to be a secondary phenomenon related to regeneration, ischaemia or mucosal prolapse being demonstrated in conditions such as solitary rectal ulcer, ischaemic colitis, juvenile, inflammatory and hyperplastic polyps. The current study therefore contributes to the latter view as the histochemical changes seen in transitional zone mucosa and solitary rectal ulcer have been shown to be relatively similar.

Though the current study demonstrates a varying pattern of staining between the different colonic pathologies studied, especially the near complete loss of mucin staining in invasive adenocarcinomas, the use of this technique as a differential diagnostic tool is possibly limited for various reasons. During the study we encountered several technical difficulties in its use such as importance of suitable storage of diamine salts, the need for fresh preparation of solutions, ideally by a single person familiar with the technique performing it and the need for immediate assessment of staining. A fume cupboard was also necessary when preparing the solutions as the diamine salts are deemed toxic.

Further it is best that the normal left and right colon variation in sulphomucin and sialomucin content is established in each laboratory or region which was not carried out in our setting. The spatial distribution of sulphomucins and sialomucins along the crypt length was also not addressed in this study. Scoring of each mucin type along the surface epithelium reveals a greater number of sialomucin-containing goblet cells in the upper crypts of the rectum. The increase in sulphomucin abundance measured in the rectum compared to right colon was due mainly to increased sulphomucin in the lower crypts rather than in the upper crypts or the surface epithelium. This fine-scale mapping is noteworthy as mucins in surface and upper crypt goblet cells may be more important in mediating host microbe interactions, while disease related

changes in mucin types may initially be observed in the lower crypt with less differentiated goblet cells [2]. The variation of mucin chemo types within the crypts and between the different sites of the colon was not addressed in our study.

However, this study did help in describing the variation in the patterns of mucin staining with HID/AB in different colorectal pathologies. These included [1] the parallel changes in mucin staining patterns observed in the TZ mucosa and SRU, [4] the loss of acid mucin staining in AC and [2] the relatively mild degree of sulphomucin loss in UC.

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