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A Comparative Study of Mucin Expression in Colorectal Adenocarcinoma and Normal Colonic Mucosa

[1]Dr. Jiya Jaleel, [1]Dr. Sompal Singh, [1]Dr. Namrata Sarin, [1]Dr. Garima Singh, [1]Dr. Vijaya Pandey, [2]Dr. Arsha Narayanan, [2]Dr. Aswathy Gopalakrishnan

[1]Department of Pathology, NDMC Medical College and Hindu Rao Hospital, Delhi

[2]Department of Pathology, KMC, Kannur

Corresponding Author: Dr. Jiya Jaleel**,** Department of Pathology, NDMC Medical College and Hindu Rao Hospital, Delhi.

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Abstract

Background: Colorectal cancer poses a significant global health concern**.** The majority of colorectal carcinomas are adenocarcinomas. Deregulation of mucin expression has been reported in various gastrointestinal lesions progressing to cancer. Mucin expression by the tumour cells influences the prognosis and therapeutic management.

Aim: To determine the type of mucin in normal mucosa and adenocarcinoma of the colorectal region.

Material and Methods: A total of 15 consecutive cases of colorectal carcinoma and 15 controls of normal colonic mucosa were included in each group. The samples were received in 10% neutral buffered formalin, processed, embedded in paraffin and serial sections of 4-5 microns were prepared. The tissue was stained by H & E and special stains such as PAS, Alcian blue (pH -2.5), and combined AB – PAS. The percentage of mucin-positive cells was noted.

Results: In our study, there was a predominance of acidic mucins with traces of neutral mucins in cases (group 1) and a predominance of acidic mucins along with some amount of neutral mucins in controls (group 2). Compared to cases (group 1) the amount of neutral mucins was more in controls (group 2).

Conclusion: The percentage of cells showing acidic mucins on AB-PAS stain was significantly higher in colorectal adenocarcinoma cases as compared to normal colonic mucosa. The results of the present study including mucin content and type may be helpful in understanding the pathogenesis of colorectal carcinoma

Keywords: Colorectal adenocarcinoma, Mucins

Introduction

Colorectal cancer is an intimidating health issue worldwide. It is the third most common cancer in men (1,065,960 cases, 10.6 % of all cancer cases) and the second most common in women (8,65,630 cases, 9.4% of all cancer cases).[1] In India colon and rectal cancer account for 1.8 % and 2.6 % respectively of all cancer cases.[2] The majority of colorectal carcinomas are adenocarcinomas. The grading is done as well, moderately and poorly differentiated depending on the glandular architecture regularity as per WHO classification. Mucin production varies from minimal to abundant (that is 50% or more of the tumour area having the mucin content). Cases with abundant mucin are categorized as mucinous adenocarcinoma.[3,4]

Mucins are histochemically classified into neutral and acidic mucins. Acidic mucins are further classified into sulfated and sialomucins.[5] Sulfated muco substances are found in the deeper colonic tissue and a decline in its production predisposes to malignancy.[6] The predominant mucin in normal colonic mucosa are acidic with scanty neutral mucin.[7] Periodic - acid - Schiff (PAS) reaction and Alcian blue staining are the two basic histochemical techniques used for analysing the changes in the tissue mucin carbohydrate content. PAS technique stains both neutral and acidic (simple non sulfated and complex sulfated mucins). Alcian blue can stain only acidic mucin. Deregulation of mucin expression has been reported in various gastrointestinal lesions progressing to cancer.[8] Mucin expression by the tumour cells influences the prognosis and therapeutic management.[3.4] Hence the aim of our study is to determine the type of mucin in normal mucosa and adenocarcinoma of the colorectal region.

Material and Methods

The present study is a cross - sectional analytical study conducted over a period of one year in the Department of Pathology at NDMC Medical College and Hindu Rao Hospital, Delhi. A total of 15 consecutive cases of colorectal carcinoma and 15 controls of normal colonic mucosa were included in each group. Normal mucosa from colectomy specimens removed for reasons other than the neoplastic lesions was taken as controls. Diagnosed cases of malignant epithelial colorectal neoplasm was taken as cases. Gross detailed examination of the surgically resected specimens and colonoscopic biopsy was done. The samples received in our department was collected in 10% neutral buffered formalin, processed, and embedded in paraffin. Serial sections of 4-5 microns were prepared. The tissue was stained by H & E and special stains such as PAS, Alcian blue (pH -2.5), and combined AB – PAS. The percentage of mucin-positive cells was noted.

Statistical Analysis

Age was classified in appropriate class intervals and was tabulated. Mann-Whitney U test was used to compare the age differences between cases and controls. To assess the significance of difference in gender between cases and controls, Z test for difference in proportion was used. Mann-Whitney U test was used to compare the number of mucin positive cells between cases and controls. P-value <0.05 was taken as statistically significant.

Results

A total of 15 consecutive cases of colorectal carcinoma and 15 controls of normal colonic mucosa were included in each group in the study. The age of the cases (group 1) included in the present study ranged from 15 to 75 years, with a mean of 47 years. In our study maximum

number of cases were in the age group of 46 to 55 years (40%) followed by 36 to 45 years (20%). The age of the controls (group 2) included in the study ranged from 15 to 75 years, with a mean age of 45 years. In our study maximum number of controls were in the age group of 36 to 45 years followed by 46 to 55 years.

Out of 15 cases (group 1), eight (53%) were males and seven (47%) were females. Out of 15 controls (group 2), eight (53%) were males and seven (47%) were females. The difference in gender distribution between cases and controls was statistically insignificant (z test, z = 0, p =1). In the present study out of 15 cases, six (40%) were clinically diagnosed as Carcinoma colon, four (27%) as carcinoma rectum, and five (33%) as rectal growth. Out of 15 cases (group1), seven cases (46.7%) were well-differentiated, four (26.6%) were moderately differentiated and three (20%) were poorly differentiated adenocarcinoma. One case (6.7%) showed features of mucinous adenocarcinoma.

Table 1: Histologic differentiation of cases (group 1)

	Well-differentiated adenocarcinoma	Moderately-differentiated adenocarcinoma	Poorly-differentiated adenocarcinoma
Number of cases	7 (46.7%)	4 (26.6%)	3 (20%)

Out of 15 cases (group 1), the majority (33.3% of the cases) showed mucin positivity in the range of 66 –70% and 76 – 80% of tumour cells each. The mean (±SD) percentage of mucin-positive cells in cases was 76.6% ± (6.7%). Out of 15 controls (group 2), the majority (26.6%) showed 66 – 70% mucin-positive cells. The mean (±SD) percentage of mucin-positive cells in controls was 79.6%± (9.3%). The difference in the percentage of mucin-positive cells in cases and controls was statistically insignificant (p-value – 0.4).

In our study out of 15 cases (group 1), the majority (46.6%) showed AB-positive cells in the range of 66 – 70%. The mean (±SD) percentage of AB positive cells in cases was 70% ± (9.0%). Out of 15 controls (group 2), the majority (33.3%) showed AB-positive cells in the range of 66 – 70%. The mean (±SD) percentage of AB-positive cells in controls was 67% ± (7.9%). The difference in the percentage of AB-positive cells in cases and controls was statistically insignificant (p-value – 0.2).

Out of 15 cases, the majority (26.6% each of the cases) showed PAS-positive cells in the range of 66 – 70% and 71 – 75% each. The mean (±SD) percentage of PAS-positive cells in cases was 73.6% ± (8.9%). In our study out of 15 controls, the majority (20% each of the cases) showed 81 – 85%, 71 – 75%, and 61 – 65% PAS-positive cells each. The mean (±SD) percentage of PAS-positive cells in controls was 75% ± (9.0%). The difference in the percentage of PAS-positive cells in cases and controls was statistically insignificant (p-value – 0.7).

Out of 15 cases, the majority (40%) showed AB-PAS positive cells staining blue in the range of 51 – 60%. The mean (±SD) percentage of AB-PAS positive cells staining blue in cases was 59% ± (8.0%). Out of 15 controls, the majority (66.7%) showed 30 – 40% blue staining cells. The mean (±SD) percentage of AB-PAS positive cells staining blue in controls was 43.6% ± (9.3%). The difference in the percentage of cells staining blue color with AB-PAS in cases and controls was statistically significant (p-value – 0.0002).

Out of 15 cases, the majority (60%) showed AB-PAS positive cells staining magenta in the range of 6 – 10%. The mean (±SD) percentage of AB-PAS positive cells staining magenta in cases was 6.6 % ± (4.4%). Out of 15 controls, the majority (66.6%) showed 6 -10% magenta staining cells followed by three controls (20%) showing

16 - 20% magenta staining cells. The mean (±SD) percentage of AB-PAS positive cells staining magenta in controls was 12.6% ± (5.9%). The difference in the percentage of cells staining magenta color with AB-PAS in cases and controls was statistically significant (p-value – 0.005).

Out of 15 cases, the majority (60%) showed AB-PAS positive cells staining purple in the range of 36 – 40%. mean (±SD) percentage of AB-PAS positive cells staining purple in cases was 34.3% ± (8.2%). Out of 15 controls, the majority (33.3%) showed 36 – 40% and 46 -50% purple stained cells each. The mean (±SD) percentage of AB-PAS positive cells staining purple was 43.6% ± (8.1%). The difference in the percentage of cells staining purple color with AB - PAS in cases and controls was statistically significant (p-value – 0.005)

Table 2: Comparison of mucin histochemistry in cases and controls

		AB (%)	PAS (%)	AB-PAS: blue (%)	AB-PAS: magenta (%)	AB-PAS: purple (%)
Mean ± SD	Cases	70 ± 9	73.6 ± 8.9	59 ± 8	6.6 ± 4.4	34.3 ± 8.2
	Controls	67 ± 7.9	75 ± 9	43.6 ± 9.3	12.6 ± 5.9	43.6 ± 8.1
P-value		0.2	0.7	0.0002	0.0005	0.0005

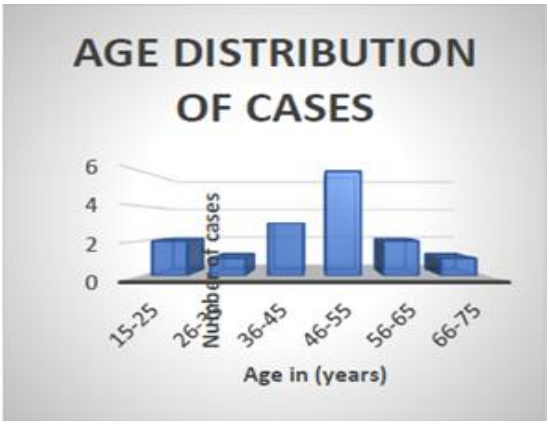

Fig.1: Age distribution of cases (group 1)

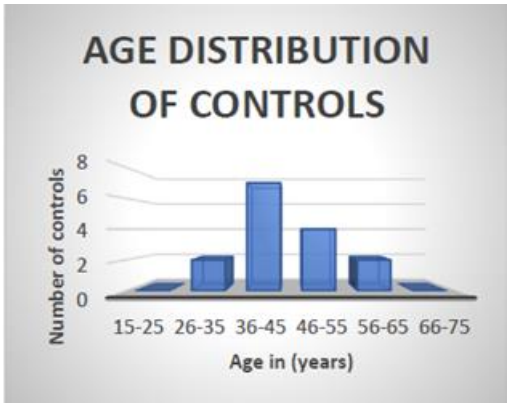

Fig.2: Age distribution of controls (group 2)

Fig.3: Gender distribution of cases

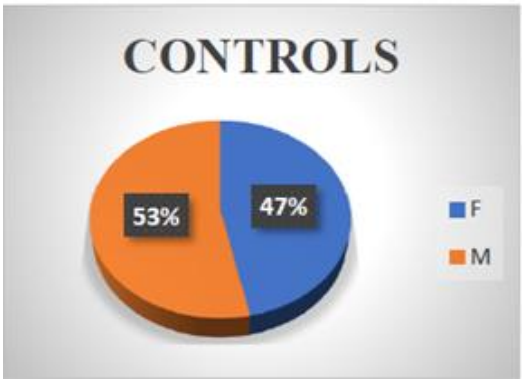

Fig.4: Gender distribution of controls

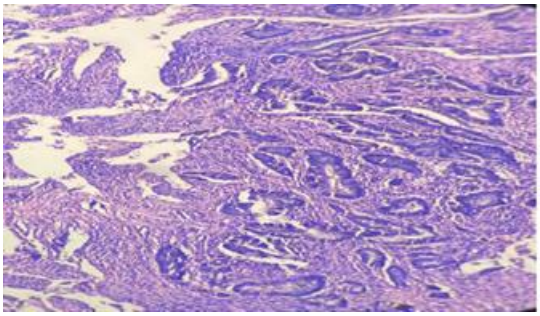

Fig.5: Photomicrograph of Well-differentiated colorectal adenocarcinoma (group 1) [H&E, 100X]

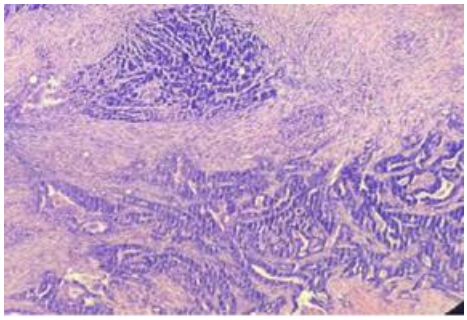

Fig.6: Photomicrograph of Moderately differentiated colorectal adenocarcinoma (group 1) [H&E, 100X]

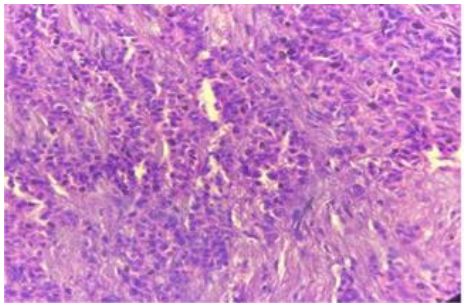

Fig.7: Photomicrograph of poorly differentiated colorectal adenocarcinoma (group 1) [H&E, 100X]

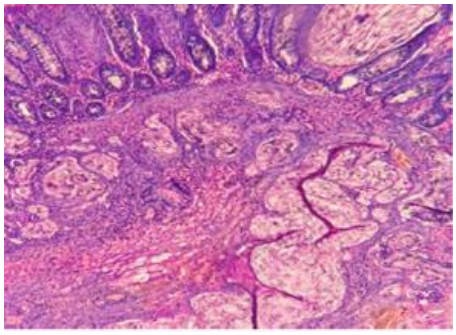

Fig.8: Photomicrograph of Mucinous colorectal adenocarcinoma (group 1) [H&E, 100X]

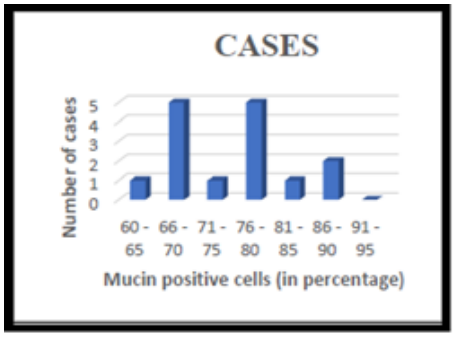

Fig.9: Distribution of mucin-positive cells of cases (group 1) in percentage

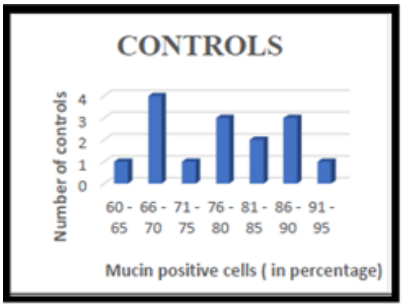

Fig.10: Distribution of mucin-positive cells of controls (group 2) in percentage

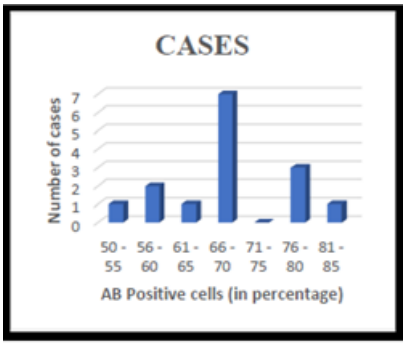

Fig.11: Distribution of AB positive cells of cases (group 1) in percentage

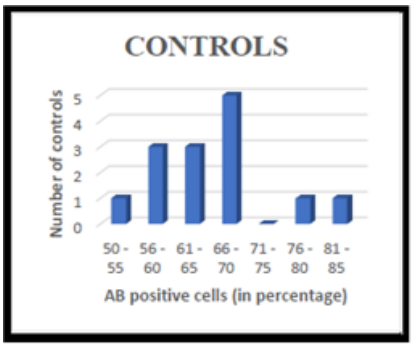

Fig.12: Distribution of AB positive cells of controls (group 2) in percentage

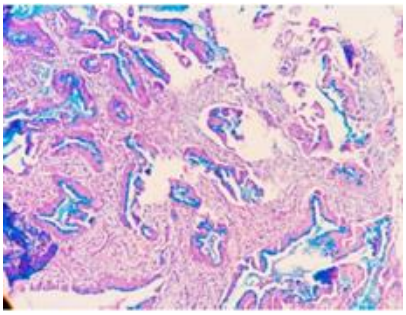

Fig.13: Photomicrograph of Alcian blue positive cells in cases (group 1) [H&E, 100X]

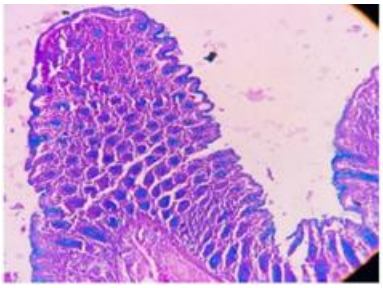

Fig.14: Photomicrograph of Alcian blue positive cells in controls (group 2) [H&E, 100X]

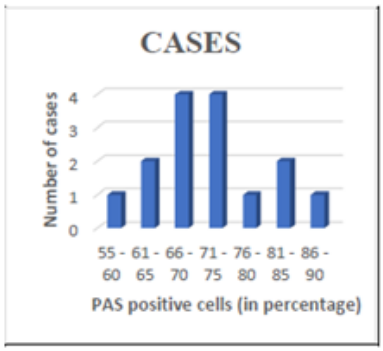

Fig.15: Distribution of PAS positive cells of cases (group 1) in percentage

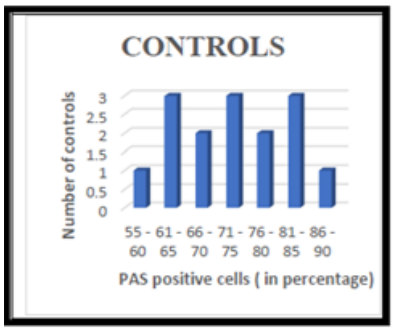

Fig.16: Distribution of PAS positive cells of controls (group 2) in percentage

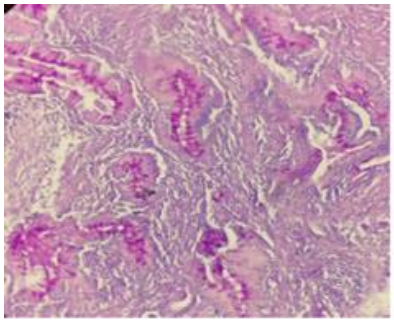

Fig.17: Photomicrograph of PAS-positive cells in cases (group 1) [H&E, 100X]

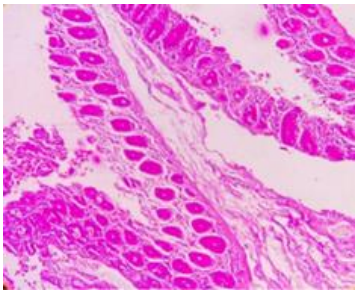

Fig.18: Photomicrograph of PAS-positive cells in controls (group 2) [H&E, 100X]

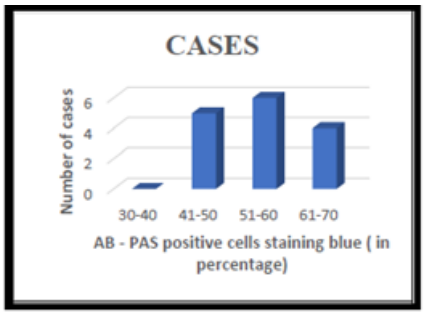

Fig.19: Distribution of AB-PAS positive cells staining blue of cases (group 1) in percentage

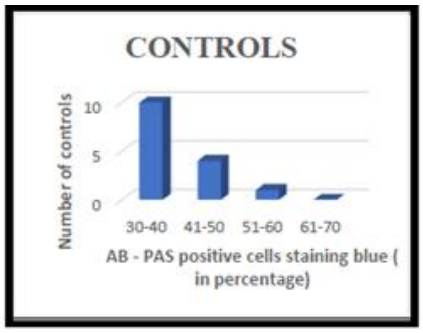

Fig.20: Distribution of AB-PAS positive cells staining blue of controls (group 2) in percentage

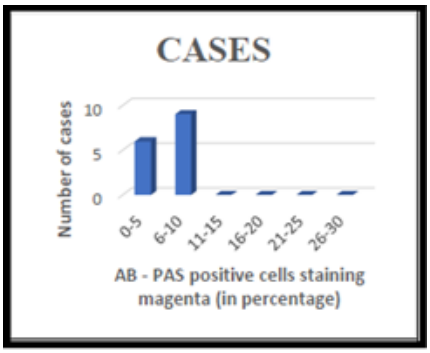

Fig.21: Distribution of AB-PAS positive cells staining magenta of cases (group 1) in percentage

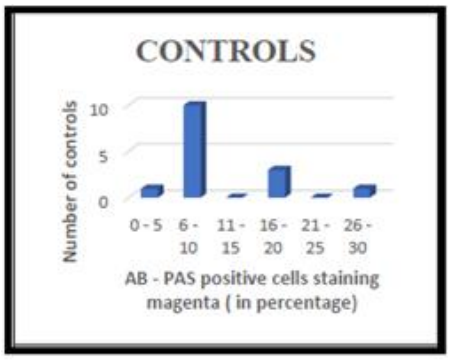

Fig.22: Distribution of AB-PAS positive cells staining magenta of controls (group 2) in percentage

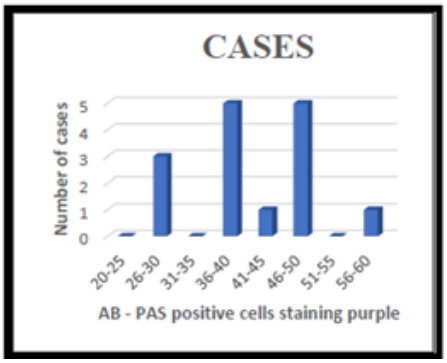

Fig.23: Distribution of AB-PAS positive cells staining purple of cases (group 1) in percentage

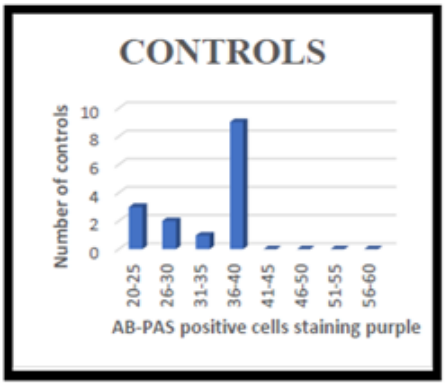

Fig.24: Distribution of AB-PAS positive cells staining purple of controls (group 2) in percentage

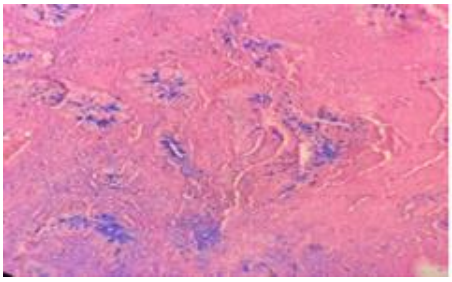

Fig. 25: Photomicrograph of AB-PAS positive cells staining blue, magenta and purple in cases (group 1) [H&E, 100X]

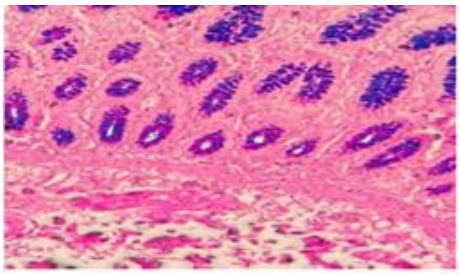

Fig. 26: Photomicrograph of AB-PAS positive cells staining blue, magenta and purple in controls (group 2) [H&E, 100X

Discussion

The study was conducted in the Department of Pathology, Hindu Rao Hospital, Delhi. A total of 15 cases (group 1) of colorectal carcinomas and 15 controls (group 2) of normal colonic mucosa were included in the study.

The age of the cases (group 1) included in the present study ranged from 15 to 75 years, with a mean of 47 years. In our study maximum number of cases were in the age group of 46 to 55 years (40%) followed by 36 to 45 years (20%). The age of the controls (group 2) included in the study ranged from 15 to 75 years, with a mean age of 45 years. In our study maximum number of controls were in the age group of 36 to 45 years followed by 46 to 55 years. The difference in age between cases and controls was statistically insignificant (p-value - 0.5, Mann – Whitney U test). In a study conducted by Patil PS et al [9] at Tata Memorial hospital

from August 2013 to August 2014 on 800 patients diagnosed with colorectal cancer, the mean age was 47.2 years ranging from 11 years to 85 years. In another retrospective observational study from central India conducted by Sudarshan V et al[10] in the Department of Pathology JNM Medical College Raipur on 233 patients over eight years (January 2003 to December 2010), the median age at diagnosis was 43 years with 39% of the patients diagnosed at the age of 40 or younger.

In the present study out of 15 cases (group 1), eight (53%) were males and seven (47%) were females. Out of 15 controls (group 2), eight (53%) were males and seven (47%) were females. In the study conducted by Patil PS et al9 on 800 patients diagnosed with colorectal carcinoma, 65% were males and 35% were females. The statistical analysis of gender differences was not provided in the study. In the study conducted by Borgohain M et al[11] on 50 diagnosed cases of colorectal carcinoma, 33% were males and 17% were females, hence the ratio between males: females was 1.94:1. The statistical analysis of gender differences is not mentioned in the study. In our study, no statistically significant difference was observed in the gender differences in cases and controls. This could be due to the lesser number of cases and controls included in our study.

In the present study out of 15 cases, six (40%) were clinically diagnosed as Carcinoma colon, four (27%) as carcinoma rectum, and five (33%) as rectal growth. Most of the cases (60%) were detected in the rectum. Our findings were consistent with the study conducted by Borgohain M et al[11] in which out of 50 cases, 28 (56%) cases were detected in the rectum. Our findings were also consistent with another retrospective study conducted by Qing et al[12] on 690 consecutive patients with colorectal cancer in Cleveland Clinic Florida, U.S.A., and 870 consecutive patients with colorectal cancer in Nan Fang Hospital affiliated to the First Military Medical University, China over the past 11 years from 1990 to 2000. In this study, the maximum number of cases (717 cases, 48%) were detected in the rectum.

In the present study, out of 15 cases (group1), seven cases (46.7%) were well-differentiated, four (26.6%) were moderately differentiated and three (20%) were poorly differentiated adenocarcinoma. One case (6.7%) showed features of mucinous adenocarcinoma. In a study conducted by Borgohain M et al11 on 50 cases of colorectal carcinoma, 60% were moderately differentiated, 22% were well-differentiated (22%) and 18% were poorly differentiated.

Mucins are complex carbohydrates secreted by different types of epithelial cells and glandular tissues of the gastrointestinal tract. There has been growing recognition in recent years that the demonstration of these substances is difficult, complex, and affected by the types of mucins present.[13]

Mucins play a crucial role in physical protection as well as in regulating the concentration and passage of water, ions, and other immune mediators such as antimicrobial peptides (AMPs) and immunoglobulin-A (IgA) within the gut.[14] Uniquely, the stomach and colon contain a dual-layer of mucus that is composed of polymeric sheets of these highly glycosylated mucins.[15]

Mucin content was studied in both cases (group1) and controls (group 2). Histochemical stains AB, PAS, and AB – PAS were used to demonstrate acidic and neutral mucin content in both the groups. The percentage of mucin-positive cells in the cases was observed. In our study mucin was detected in all the adenocarcinoma

cases (100% of the cases). Out of 15 cases (group 1), five (33.3% of the cases) showed mucin positivity in the range of 66 –70% and 76 – 80% of tumour cells each followed by two cases (13.3% each of the cases) which showed 86 – 90% mucin positive cells. One case (6.7% each of the cases) showed mucin-positive cells in the range of 60 – 65%,71 – 75% and 81 – 85% of tumour cells each. The mean (±SD) percentage of mucin-positive cells in cases was 76.6% ± (6.7%). In our study mucin was detected in all the controls (100% of the controls). Out of 15 controls (group 2), four (26.6%) showed 66 – 70% mucin positive cells followed by three controls (20% each of the controls) which showed 76 – 80% and 86 – 90% mucin positive cells each. One case (6.7% each of the cases) showed mucin-positive cells in the range of 91 – 95%, 71 – 75% and 60 – 65% of tumour cells each. The mean (±SD) percentage of mucin-positive cells of the controls was 79.6% ± (9.3%). The difference in the percentage of mucin-positive cells in cases and controls was statistically insignificant (p-value – 0.4, Mann – Whitney U test).

Our study was consistent with the study conducted by Nikhumbh RD et al[16] on 30 cases of colorectal cancer and 30 controls. Histochemical stains such as PAS, PAS – D, and Alcian blue were used to demonstrate mucin in cases and controls. Out of 30 cases, all of them (100%) showed mucin positivity and out of 30 controls, all of them (100%) showed mucin positivity. However, a study conducted by Borgohain M et al[11] on 50 cases of colorectal carcinoma showed mucin positivity only in 35 cases (70%) with 15 cases (30%) showing no mucin positivity. In our study, the difference in the percentage of mucin-positive cells in cases and controls was statistically insignificant (p-value – 0.4, Mann – Whitney U test).

To demonstrate the type of mucins, special stains such as AB, PAS, and Combined AB – PAS is commonly used. PAS technique is particularly sensitive to the detection of neutral mucins. PAS also stains some of the acidic mucins. Alcian blue stains acidic mucins only. The combined AB – PAS technique is widely used for the detection and characterization of mucosubstances in the tissue sections. The Alcian blue–PAS technique is a simple procedure and appears to differentiate sharply between acidic mucins and neutral mucins. In the combined Alcian blue (pH 2.5) – PAS technique blue colour depicts the presence of acidic mucin. The magenta colour depicts the presence of neutral mucins and the purple colour denotes the mixture of acidic and neutral mucins.

Alcian blue (pH 2.5) stains acidic mucins only. The percentage of alcian blue (AB) positive cells in the cases were observed. In our study out of 15 cases (group 1), seven (46.6%) showed AB positive cells in the range of 66 – 70% followed by three cases (20%) that showed 76 – 80% AB positive cells. Two cases (13.3%) showed 56 – 60% AB positive cells and one case (6.7% each of the cases) showed 81 – 85%, 61 – 65%, and 50 – 55% AB positive cells each. The mean (±SD) percentage of AB positive cells in cases was70% ± (9.0%). In our study out of 15 controls (group 2), five controls (33.3%) showed AB positive cells in the range of 66 – 70% followed by four controls (26.6%) which showed 56 – 60% AB positive cells. One control (6.7% each of the cases) showed 81 – 85%, 76 – 80% and 50 – 55% AB positive cells. The mean (±SD) percentage of AB-positive cells in controls was 67% ± (7.9%). The difference in the percentage of AB-positive cells in cases and controls was statistically insignificant (p-value – 0.2,

Mann – Whitney U test). This can be due to the presence of a mixture of acidic and neutral mucin in a few cells.

In a study conducted by Zakout YM et al[17] on 50 cases of colorectal cancer and 50 controls with all of them stained with alcian blue, a mild or moderate increase in acidic mucin was detected in 18 (36%) cases compared to six (12%) controls. Excessive increase was detected in 12 (24%) cases compared to 0 (0%) controls. Their findings indicated that acid mucin increases in colorectal carcinoma and that was found to be statistically significant (p-value < 0.0001).

PAS stains neutral mucins and some of the acidic mucins. The percentage of PAS-positive cells in the cases was observed. In our study out of 15 cases, four (26.6% each of the cases) showed PAS-positive cells in the range of 66 – 70% and 71 – 75% each followed by two cases (13.35% each of the cases) which showed 81 – 85% and 61 – 65% PAS-positive cells each. One case (6.7% each of the cases) showed 86 – 90%, 76 – 80% and 55 – 60% each. The mean (±SD) percentage of PAS-positive cells in cases was 73.6% ± (8.9%). In our study out of 15 controls, three (20% each of the controls) showed 81 – 85%, 71 – 75% and 61 – 65% PAS-positive cells each followed by two controls (13.3% each of the cases) which showed 76 – 80% and 66 – 70% PAS-positive cells each. One control (6.7% each of the cases) showed 86 – 90% and 55 – 60% each. The mean (±SD) percentage of PAS-positive cells in controls was 75% ± (9.0%). The difference in the percentage of PAS-positive cells in cases and controls was statistically insignificant (p-value – 0.7, Mann-Whitney U test). This can be due to the presence of a mixture of acidic and neutral mucin in a few cells.

The study conducted by G Roopashree et al[18] on 25 cases of carcinoma colon and 25 control specimens of normal colon demonstrated the mucin content by staining the cases and controls with PAS-D. Out of 25 cases, 18 cases (72%) showed moderate reaction and seven cases (28%) showed a weak reaction. Out of 25 controls, 22 (88%) showed strong positive reactions and three (12%) showed weak reactions with PAS -D. The statistical analysis of the difference in PAS-positive cells between cases and controls is not provided in their study.

In another study conducted by Nikhumbh R et al[16] on 30 specimens of carcinoma colon and 30 specimens of the normal colon were stained by PAS. In their study, normal colon gave moderate to strong reactivity and carcinoma colon gave mild to moderate reactivity with PAS. The statistical analysis of the difference in PAS-positive cells between cases and controls is not provided in their study.

To depict the distribution of mucins, AB – PAS stained was used in our study. The cells are stained with various colours such as blue, magenta, and purple denoting acidic mucin, neutral mucin, and a mixture of acidic and neutral mucins respectively. The percentage of AB-PAS positive cells staining blue of the cases was observed. In our study out of 15 cases, six (40%) showed AB-PAS positive cells staining blue in the range of 51 – 60% followed by five cases (33.3%) which showed 41 – 50% blue staining cells and four cases (26.7%) showed 61 – 70% blue staining cells. The mean (±SD) percentage of AB-PAS positive cells staining blue in cases was 59% ± (8.0%). Out of 15 controls, 10 of them (66.7%) showed 30 – 40% blue staining cells followed by 4 controls (26.6%) which showed 41 – 50 % blue staining cells and one control (6.7%) showed 51 – 60% of blue staining cells. The mean (±SD) percentage of AB-PAS positive cells staining blue in controls was 43.6% ± (9.3%). The difference in the percentage of cells staining blue color

with AB-PAS in cases and controls was statistically significant (p-value – 0.0002, Mann-Whitney U test).

The percentage of AB-PAS positive cells staining magenta in the cases was observed. In our study out of 15 cases, nine cases (60%) showed 6 – 10% magenta stained cells followed by six cases (40%) staining 0 – 5% cells staining magenta. The mean (±SD) percentage of AB-PAS positive cells staining magenta in cases was 6.6 % ± (4.4%). Out of 15 controls, 10 of them (66.6%) showed 6 – 10% magenta staining cells followed by three controls (20%) showing 16 – 20% magenta staining cells and one control (6.7% each of the controls) showing 26 – 30% and 0 – 5% of magenta staining cells. The mean (±SD) percentage of AB-PAS positive cells staining magenta in controls was 12.6% ± (5.9%). The difference in the percentage of cells staining magenta color with AB - PAS in cases and controls was statistically significant (p-value – 0.005, Mann Whitney U test).

The percentage of AB-PAS positive cells staining purple in the cases was observed. In our study out of 15 cases, nine of them (60%) showed 36 – 40% purple staining cells followed by three cases (20%) showing 20 –25% purple staining cells. Two (13.3%) cases showed 26 – 30% of purple staining cells and one case (6.7%) showed 31 – 35% purple staining cells. The mean (±SD) percentage of AB-PAS positive cells staining purple in cases was 34.3% ± (8.2%). Out of 15 controls, five controls (33.3%) showed 36 – 40% and 46 – 50% purple stained cells each followed by three controls (20%) staining 26 – 30% cells purple. One control each (6.7%) stained 41 – 45% and 56 – 60% cells purple. The mean (±SD) percentage of AB-PAS positive cells staining purple was 43.6% ± (8.1%). The difference in the percentage of cells staining purple color with AB - PAS in cases and controls was statistically significant (p-value – 0.005).

In our study, there was a predominance of acidic mucins with traces of neutral mucins in cases (group 1) and a predominance of acidic mucins along with some amount of neutral mucins in controls (group 2). Compared to Cases (group 1) the amount of neutral mucins was more in controls (group 2). In the study conducted by Nikumbh RD et al[16] on 30 cases of carcinoma colon and 30 control specimens of normal colon staining with H and E and special stains such as PAS and AB pH (2.5) PAS was done. Their study showed the predominance of acidic mucins and traces of neutral mucins in carcinoma colon cases. Normal colonic and rectal mucosa showed a predominance of neutral and acidic mucins. In the study conducted by Borgohain M et al[11] on 50 cases of colorectal carcinoma, 35 cases showed the presence of mucin and out of 35 cases, 34 showed the presence of acidic mucin, and one case showed the presence of neutral mucin. Hence the predominant mucin in the study was acidic. In another study conducted by Roopashree et al[18] on 25 cases of carcinoma colon and 25 normal colons, the predominant mucin in carcinoma colon was acidic mucin and a mixture of muco substances was observed with a predominance of neutral and acidic mucins in normal colon.

In the present study, the type of distribution of mucin in adenocarcinoma colorectum was compared with the normal colonic epithelial lining. The alteration of mucin is important due to various reasons stated in the following studies.

The predominance of acidic mucin in carcinoma colon can be supported by the findings of Kim et al[19] who described that there is a highly significant positive relationship between colorectal carcinomas and both

qualitative and quantitative alterations of mucins. The changes in mucin glycoproteins that occur in gastrointestinal cancers may be broadly divided into two general types, aberrant glycosylation and altered expression of mucin polypeptide epitopes. Aberrant glycosylation includes changes in the expression of core region carbohydrates which arise due mainly to incomplete synthesis, and of backbone region and peripheral region carbohydrates which occur mainly due to elongation and modification of existing structures. Thus in colon cancer cells, the carbohydrate side chains of mucin glycoproteins may exhibit multiple cancer-associated carbohydrate antigenic epitopes in either the core region or the peripheral and backbone regions of the carbohydrate side chains. In cancer cells, altered expression of mucin polypeptide antigens occurs either due to sparse and/or incomplete glycosylation, altered transcriptional regulation of tissue-specific mucin genes or dysregulation of mucin genes resulting in the expression of inappropriate or ectopic gene products.[19-23]

The increase in acidic mucins in controls may be explained by some studies which found that some nutritional and psychological factors may induce mucin secretion in the colon. Barcelo A et al[24] reported that some food constituents and their fermentation products provoke mucus secretion in the colon, mainly two algal polysaccharides (alginate and uluvan), and two uronic acids (glucuronic acid and galacturonic acid), and the short-chain fatty acids acetate and butyrate. Furthermore, Casalgluiolo I et al[25] found that acute stress causes mucin release from rat colon.

Alteration of the expression pattern of mucins has been depicted in carcinomas and also their precursor lesions. The predominance of acidic mucins has been demonstrated in the goblet cells of mucosa both around carcinomas and in more distant patches as well, resembling the mucus secretion pattern of the human foetal gut. Such changes, therefore, characterize the early stages of carcinogenesis by providing further evidence of the reversion to an embryonic state. A positive result can lead to a thorough examination of the patient and thus increasing the chances of an early diagnosis of cancer.[13]

Conclusion

Although both the cases and controls showed predominance of acidic mucins, the percentage of cells showing acidic mucins on AB-PAS stain was significantly higher in colorectal adenocarcinoma cases as compared to normal colonic mucosa.

The results of the present study including mucin content and type may be helpful in understanding the pathogenesis of colorectal carcinoma.

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