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Pathology

MUCIN HISTOCHEMISTRY IN COLORECTAL CARCINOMA

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ABSTRACT Many parameters of colorectal carcinoma have been investigated. An important parameter is mucin production. However, data regarding mucin production is confusing. This study was conducted to note the characteristics of colorectal carcinoma in regard to mucin production using histochemistry. A cross sectional observational study was conducted in 50 cases of colorectal carcinoma. Sections were stained with PAS, Alcian blue- PAS, Alcian blue-Acid fuchsin stains. It was observed that significant depletion of total mucin occurs in colorectal cancers. Neutral mucin is replaced by acidic mucin in cancer. Sulphomucins are depleted while sialomucins predominate in carcinoma.

KEYWORDS : Mucin, Acid, Neutral, Colorectal

INTRODUCTION

In India colorectal carcinoma is the fourth most commonly diagnosed cancer in males and third among females.[1] Many parameters for colorectal cancers have been investigated including histopathological type, grade, vertical extension with serosal involvement, resection margin, size etc. One important parameter for prognosis and management is mucin production. Evidence regarding mucin production and its role in cancer is confusing.[2]

This study was conducted to note the clinicopathological characteristics of colorectal carcinoma in regard to mucin production using simple histochemistry which is easily available in under resourced laboratories. Mucins are complex carbohydrates secreted by different types of epithelial cells and glandular tissues. Connective tissue mucosubstances are called ' mucopolysaccharides' whereas those secreted by epithelia are referred to as mucins.[3]

Mucosubstances are classified into two categories a) neutral mucins b) acidic mucins. Acidic mucins are classified into weakly acidic and strongly acidic.[3,4,5] Weakly acidic mucins contain terminal carboxyl group and are called as carboxylated mucins or sialomucins. Strongly acidic mucins contain sulphate groups and are called as sulphomucins.

AIMS AND OBJECTIVES

To know the mucin distribution pattern in colorectal carcinoma in comparison to normal colonic mucosa and also among different grades.

MATERIALS AND METHOD

An observational study was done in a retrospective way on resected specimens of colorectal carcinoma. Paraffin blocks from normal looking colonic mucosa at least 5cm away from the tumor were used as normal controls.

Fixation was done with 10% formol saline with 2% calcium acetate and a pinch of phosphotungstic acid to help preserve mucins. Sections were stained with :

a) PAS (Periodic acid Schiff) reagent which stains all carbohydrates including mucopolysaccharides magenta
b) Alcian blue (AB): At pH 2.5, it is weakly acidic and stains both carboxylated and sulphomucins.
c) Combined AB-PAS can stain different types of mucin such as neutral mucins: magenta
   Carboxylated mucins: blue
   Sulphated mucins: purple
d) Combined Acid fuchsin(AF)- Alcian blue(AB) stain carboxylated mucins blue and sulphated mucins purple

All the results obtained were tabulated into different grades ranging from 1+ to 4+.[6,7,8]

1) 4+ very strong positive
2) 3+ strong positive
3) 2+ moderate reaction
4) 1+ weak reaction
5) 0 negative reaction

INCLUSION CRITERIA: Blocks from cases of colorectal carcinoma received in the department.

EXCLUSION CRITERIA: inadequately resected specimens
No clinical history
Malignant tumor other than adenocarcinoma
Improperly fixed specimens

SAMPLE SIZE: 50 cases of colorectal carcinoma and tissue from same specimen at least 5cm away from tumor and appearing normal

SAMPLE DESIGN: cross sectional, observational

INVESTIGATION: Sections were stained with Haematoxylin and eosin stain, Alcian blue-PAS, Alcian blue -acid fuchsin. Mucin expression was compared in controls and cases and also among different grades.

STATISTICAL ANALYSIS: Analysis was done using the SPSS 20.0. Nominal categorical data between the groups was compared using chisquare test. For all statistical analysis a p- value less than .05 was taken to have significant association.

RESULTS

Total number of cases was 50 with 28 males and 22 females. The mean age was 47.92 and median age was 45 years. Maximum cases were in the age group 41-50. The most common site was rectum(36%), followed by ascending colon(20%), hepatic flexure (12%), sigmoid colon 6%, descending colon 6%, splenic flexure 4% and transverse colon 4%. 46% of the cases were well differentiated and 46% were moderately differentiated, while 8% were poorly differentiated.

74% of the cases were conventional adenocarcinomas and 26% were mucinous adenocarcinomas.

For the mucin stains moderate to strong activity was compared to weak or no activity in each category. PAS with diastase was used to compare total mucin expression in cases and controls. It was observed that normal colonic mucosa showed significantly strong reaction (p value = 0.000000634) and had more total mucin compared to cancers (Table I).

Table I: Distribution pattern of mucin staining as observed in Periodic Acid Schiff stain with diastase digestion (PAS with D)

Colour intensity	+ (1)	++(2)	+++(3)	++++(4)
No of controls (50)	2	24	21	3
No of cases (50)	21	20	9	6

Alcian blue with PAS was used to compare expression of neutral and acidic mucins among cases and controls. Significantly more cases showed acidic mucin moderate to strong positivity while significantly more controls showed neutral mucin positivity (moderate to strong) (Table II)

Table II: Results of combined Alcian blue with PAS at pH 2.5 were analysed in this table. Grade of neutral and acid mucin in adenocarcinomas cases compared to control mucosa had shown predominance of moderate (36%) to strong(44%) reaction for neutral mucin and weak (44%) reaction for acidic mucin in controls whereas there was mild reaction (50%) for neutral mucin, highlighting the depletion of neutral mucin as compared to greater reaction for acid mucin signified by finding predominance of moderate (++ grade) to strong (+++ grade) reaction for acidic mucin i.e. 38% and 32% respectively, in areas of adenocarcinomas.

Colour intensity	Type of mucin	(0)	+ (1)	++(2)	+++(3)	++++(4)
Control (50)	Neutral mucin	0	9	18	22	1
	Acidic mucin	17	22	11	0	0
Cases (50)	Neutral mucin	4	25	19	2	0
	Acidic mucin	5	9	19	16	1

AB-AF stain was used to compare expression of sialo mucin (blue) and sulphomucin (purple) among cases. Significant decrease of sulphomucin was observed in adeno carcinomas compared to significantly greater expression of sialomucins in cancer (Table III). There was more intense positivity for sialomucin and depletion of sulphomucin in poorly differentiated tumors but as the number of cases was few no statistical correlation could be performed.

Table III: Comparison of expression of carboxylated sialomucin and sulphomucin between cases and controls by AB-AF stain as carboxylated sialomucin as Blue and Sulphated mucin as Purple color.

Colour intensity		(0)	+ (1)	++(2)	+++(3)	++++(4)
Control (50)	Sialomucin	15	23	12	0	0
	Sulphomucin	17	15	11	7	0
Cases (50)	Sialomucin	7	16	14	13	0
	Sulphomucin	17	30	3	0	0

DISCUSSION

The age and sex distribution with predominance of males and age group 41-50 years matched with other workers.[9,10,11,12,13,14] All the mucinous carcinomas were high grade in our study. This is comparable to findings by Hadi Ni et al[15] who showed that mucinous adenocarcinomas were mostly high grade. PAS with diastase was used to grade the colour intensity in adenocarcinoma compared to controls.[6,7,8.]

We found weak reaction for mucin in colorectal carcinomas. Previous studies by Roopali D et al [4] and Kwabena et al[16] had shown predominance of neutral mucin in normal colonic mucosa. Our findings corroborate these results. We found increased acid mucin with reduction in neutral mucin in colorectal carcinoma compared to controls. Similar observations were made by others.[3,5,17,18] This study has shown predominance of sialomucin with depletion of sulphomucins in adenocarcinomas like Shah M et al and others[3,5,16]

CONCLUSION

Depletion of total mucin occurs in colorectal cancers . Neutral mucin is replaced by acidic mucins in cancer. Sulphomucins are depleted while sialomucins predominate. Mucin expression in different grades of adenocarcinoma could not be correlated.

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