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Original Research Article

Expression of MUC5AC in normal gastric mucosa, intestinal metaplasia and gastric carcinoma by immunohistochemistry

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ABSTRACT

Background: Carcinomas of the stomach are a heterogeneous group of lesions in terms of architecture, pattern of growth, cell differentiation, and histogenesis. Altered MUC5AC expression patterns have been reported previously in intestinal metaplasia as well as in gastric cancer. The aim of the study was to analyse the expression pattern of MUC5AC in normal, pre-neoplastic and neoplastic gastric epithelium.
Methods: Formalin fixed paraffin embedded sections of sixty cases which include twenty cases of each normal gastric mucosa, intestinal metaplasia and gastric carcinoma were taken up for the study and subjected to immunohistochemistry using MUC5AC.
Results: The intensity of MUC5AC immunostaining in normal gastric mucosa, intestinal metaplasia and gastric carcinoma was evaluated. Immunoreactivity was graded as 0 (negative), ± (trace positive), + (positive) or ++ (strongly positive). Statistical analysis was performed with Chi-Square test and significant differences were noted between these 3 groups (p value <0.05).
Conclusions: Authors concluded that MUC5AC expression rates might be good parameters in progression of intestinal metaplasia to gastric carcinoma and might be a good prognostic marker for gastric carcinoma as it is very well implicated in understanding of gastric carcinogenesis.

Keywords: Gastric cancer, Immunohistochemistry, Intestinal metaplasia, MUC5AC

INTRODUCTION

Gastric mucins are critical cytoprotective proteins synthesized by gastric epithelial cells. Mucins are heavily glycosylated glycoproteins that are the major components of the mucous viscous gel covering the surface of epithelial tissues.[1] Mucins have been implicated in the pathogenesis of benign and malignant diseases of secretory epithelial cells. A total of 20 human mucins have now been recognized.[2] According to their structure and function, mucins can be divided into secreted mucins and transmembrane mucins. Secreted mucins include MUC5AC. The mucin proteins are encoded by various MUC genes.[3] The genes for MUC5AC are found in a cluster on chromosome 11p15.5 and synthesis of the proteins is regulated by biologically active molecules, including cytokines, bacterial products and growth factors.[4] Changes in the expression levels and/or distribution profiles of MUC5AC occur in cancers of the lung, gastrointestinal tract, pancreas, hepatobiliary system and reproductive system.[5] Numerous alterations of gastric mucins have been described in inflammatory, metaplastic

and malignant diseases of the stomach.[6] The transition from intestinal metaplasia to adenocarcinoma is characterized by further qualitative and quantitative alterations of mucin related antigens.[7,8] Alterations of mucin-type glycoproteins may contribute to changes in cancer cell growth regulation, immune recognition and cellular adhesion, which in turn may influence the invasive and metastatic capabilities of cancer.[9]

In gastric cancer, alterations in mucin polypeptide expression have been reported with a loss of expression of MUC5AC, increased mucin heterogeneity and glycosylation changes, including exposure of simple mucin-type carbohydrates.[10-12] These observations suggest that mucin alterations can be regarded as "molecular" markers of malignant transformation of gastric mucosa.[13] The majority of gastric carcinomas, particularly the "intestinal" type, which is the most common in populations at high risk is preceded by a precancerous intestinal metaplasia.[6] Altered mucin expression patterns have been reported previously in intestinal metaplasia, including under expression of MUC1, MUC5AC, and MUC6.[10,14] In gastric cancer, various alterations in mucin polypeptide expression have been reported i.e. loss of expression of MUC5AC.[10,11] Keeping all these alterations in mind, in this present study the authors have characterized the pattern of MUC5AC expression in normal gastric mucosa, intestinal metaplasia and gastric carcinoma.

METHODS

The study was conducted at a tertiary care hospital. Sixty cases were selected, 20 cases each of normal gastric mucosa, intestinal metaplasia and gastric carcinoma. All cases were analyzed for their expression of mucin MUC5AC (Bio Gen X, USA) by immunohistochemistry.

Gastrectomy specimens of 20 patients (15 males, 5 females) with gastric carcinomas diagnosed were selected for this study. The data on the age, sex and other clinical details of the patients were obtained by reviewing clinical charts and pathological records. Hematoxylin-eosin slides of the cases were evaluated, and findings were noted in the prescribed data sheet. This study was performed after written informed consent obtained from the patients.

Normal tissue and tissue with intestinal metaplasia were obtained from specimens immediately adjacent to carcinomas (transitional mucosa) or histologically normal mucosa obtained from the resection margins of the surgical specimen or endoscopic biopsies. The histologically normal resection margins were taken as normal tissue.[15]

Statistical analysis

Statistical analyses of all results were done by using Chi square test level of ≤0.05 considered as statistically significant.

Ethical concern

Ethical clearance was obtained from the Ethical committee meeting conducted at Meenakshi Medical College and Research Institute, Kanchipuram, Tamil Nadu, India.

Table 1: Scoring system used in the study.

Score	0	±	+	++
Results	Negative	Trace positive	Positive (5-50% cells stained)	Strongly positive (>50% cells stained)

RESULTS

The present study was a descriptive (retrospective and prospective) study done from January 2010 to June 2012 in Meenakshi Medical College and Research Institute, Kanchipuram, Tamil Nadu, India. In the present study, age of the cases ranged from 23 to 78 years. Majority of the intestinal metaplasia and gastric carcinoma cases were seen between 40 to 70 years of age. Mean age for intestinal metaplasia and gastric carcinoma was 44.3 years and 58.9 years respectively.

Distribution of intestinal metaplasia

In the present study, total 20 cases of intestinal metaplasia were taken. Among these 20 cases 13 (65%) cases were seen along with gastric adenocarcinoma while 7 (35%) cases were either associated with chronic gastritis or peptic ulcer. In this present study, authors further sub-divided intestinal metaplasia into complete type (type I) and incomplete type (type II). Ten cases of complete type and 10 cases of incomplete type of intestinal metaplasia were noted.

Distribution of intestinal metaplasia with gastric carcinoma

Intestinal metaplasia was seen in association with 13 cases of gastric carcinoma. with 8 (62%) cases of complete type and 5 (38%) cases of incomplete type.

Classification of gastric carcinoma

In present study, gastric carcinomas were classified according to Lauren's, Borrmann's and WHO classifications. According to Lauren's classification, 15 (75%) cases were of intestinal type and 5 (25%) cases were of diffuse type. According to Borrmann's classification, 2 (10%) cases were of polypoid type (type 1), 3 (15%) cases were of fungiform type (type 2), 10 (50%) cases were of ulcerated type (type 3) and 5 (25%) cases were of diffuse type (type 4) were noted and according to WHO classification 10 (50%) cases of tubular/papillary type, 6 (30%) cases of signet ring type

and 2 (10%) cases of each mucinous and undifferentiated type.

Expression pattern of MUC5AC in normal gastric mucosa

MUC5AC was highly expressed in 17 cases of normal gastric mucosa, 2 cases showed weak positivity and one case was negative for MUC5AC (Figure 1 A and B).

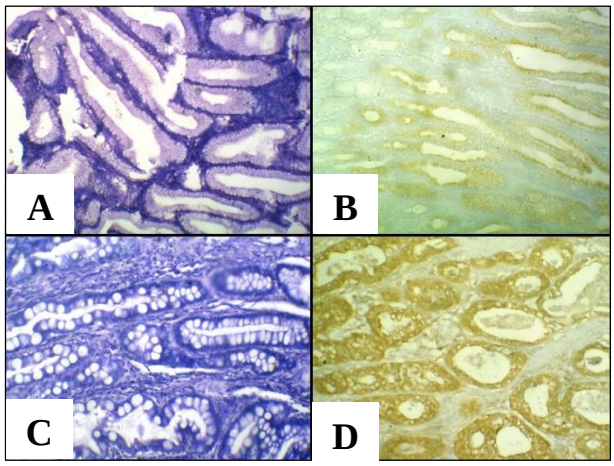

Figure 1: MUC5AC expression in stomach. A) Normal histology of gastric mucosa (40x), B) Immunoreactivity in normal gastric mucosa with MUC5AC on IHC (10x), C) Intestinal metaplasia (40x), (D) Immunoreactivity in intestinal metaplasia with MUC5AC on IHC immunoreactivity in intestinal metaplasia (10x).

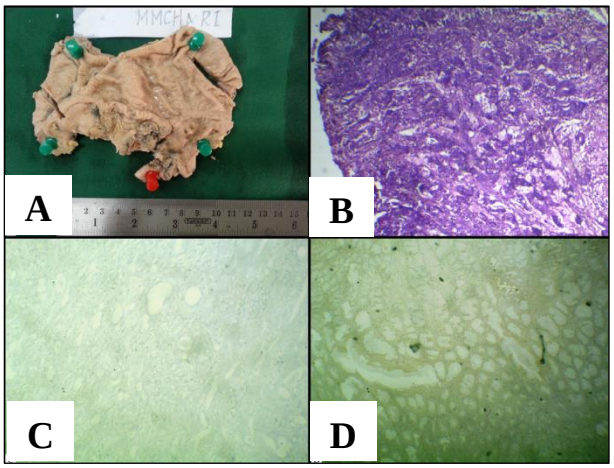

Figure 2: MUC5AC in gastric carcinoma. A) Ulcero-proliferative growth, B) Histopathology of gastric carcinoma (10x), C) MUC5AC negativity (10x) and (D) MUC5AC weak positivity.

Expression pattern of MUC5AC in complete intestinal metaplasia

Seven cases showed no expression of MUC5AC, either in the goblet or in the columnar cells. However, 3 cases showed weak expression of MUC5AC in rare goblet cells.

Expression pattern of MUC5AC in incomplete intestinal metaplasia

There was expression of MUC5AC in 8 cases, both in goblet and in columnar cells. The percentage of stained cells varied from case to case, four cases were moderately positive, 4 cases showed weak positivity and two cases were negative for MUC5AC (Figure 1 C and D).

Expression pattern of MUC5AC in gastric carcinoma

Under expression of MUC5AC was seen in gastric carcinoma. Fifteen cases showed no expression of MUC5AC. However, 2 cases showed moderate positivity and 3 cases showed very weak expression of MUC5AC in superficial foveolar cells (Figure 2).

DISCUSSION

In the study, total 60 cases were studied comprising 20 cases of each normal gastric mucosa, intestinal metaplasia and gastric carcinoma. Authors broadly divided intestinal metaplasia in two types which was complete type and incomplete type. Authors observed 10 cases of complete type and 10 cases of incomplete type. Reis CA et al, in their study noted 20 cases of complete type and 26 cases of incomplete type intestinal metaplasia.[16] In present study, intestinal metaplasia was noted with both benign and malignant cases. Seven (35%) cases were seen with benign conditions like chronic gastritis or peptic ulcers while 13 (65%) cases were observed along with gastric carcinoma (Table 2).

Table 2: Comparison of intestinal metaplasia with benign and malignant gastric lesions.

Study	Benign (%)	Gastric carcinoma (%)
Jass JR et al[17]	42.85	93
Segura DI et al[18]	80.7	96
Silva S et al[19]	24.5	65
Present study	35	65

Classification of gastric carcinoma

The authors classified gastric carcinomas according to Lauren's, Borrmann's and WHO classification. The study conducted at this centre and on using the Lauren's classification the results were compared to various studies and the results were well correlated (Table 3).[20] Also, authors included the Bormann's classification in the study conducted at this centre and the results were compared to various studies and the results were well correlated (Table 4).[24] The study at here was compared to the various studies using WHO classification and the results were well correlated (Table 5).

Table 3: Comparison of gastric carcinoma cases according to Lauren's classification.

Lauren's classification	Reis CA et al[21]		Roessler K et al[22]		Ilhan O et al[23]		Present study	
	No.	%	No.	%	No.	%	No.	%
Intestinal	52	63.4	85	44.8	217	84.4	15	75
Diffuse	30	36.6	105	55.2	40	17.6	5	25

Table 4: Comparison of gastric carcinoma cases according to Borrmann's classification.

Borrmann's classification	Ming S.C[25]		Roessler K et al[22]		Ilhan O et al[23]		Present study	
	No.	%	No.	%	No.	%	No.	%
Polypoid (Type 1)	43	25	54	28.4	8	3.1	2	10
Fungiform (Type 2)	10	6	22	11.6	5	1.9	3	15
Ulcerated (Type 3)	73	43	20	10.5	218	84.8	10	50
Diffuse (Type 4)	45	26	94	49.5	26	10.2	5	25

Table 5: Comparison of gastric carcinoma cases according to WHO classification.

WHO classification	Roessler K et al[22]		Ilhan O et al[23]		Present study	
	No.	%	No.	%	No.	%
Tubular/papillary	80	42.1	164	63.8	10	50
Mucinous	4	2.1	21	8.2	2	20
Signet ring	85	44.7	19	7.3	6	30
Undifferentiated	21	11	53	20.6	2	20

Mucin expression

MUC5AC mucin was highly expressed in normal gastric mucosa in foveolar epithelium and mucous neck cells of both antrum and body regions. In complete intestinal metaplasia, there is absence or markedly decreased levels of MUC5AC. In incomplete intestinal metaplasia, there is maintenance of expression of MUC5AC. In gastric carcinoma, there is absent or markedly decreased levels of MUC5AC. The studies conducted across various centres regarding MUC5AC in normal gastric mucosa, intestinal metaplasia and gastric carcinoma is summarized in Table 6.

Table 6: Comparison of overall profile of MUC 5AC in normal gastric mucosa, intestinal metaplasia and gastric carcinoma.

Study	No. of cases	MUC5AC %			
		0	1	2	3
Zhang HK et al[26]	Ca (33)	63.6	17.4	6	3
Subramani DB et al[27]	N (7)	0	0	28.6	71.4
	IM (21)	0	4.8	76.2	19
	Ca (36)	45.1	25.3	19.6	0
Ilhan O et al[23]	N (nil)	-	-	-	-
	IM (186)	48.2	30.6	12.4	8.8
	Ca (257)	57.6	20.4	9.2	12.8
Present study	N (20)	5	10	20	65
	IM (20)	45	35	20	0
	Ca (20)	75	15	10	0

Intestinal metaplasia is one of the lesions identified in the cascade of event that precedes the development of gastric carcinoma. These results support the assumption that the intestinal metaplasia does represent a differentiation of

the mucosa toward intestinal phenotype as observed by Jass JR et al.[17]

The decreased expression of MUC5AC mucin in gastric carcinomas was confirmed in the present study. The expression of MUC5AC in early gastric carcinoma of the present series, regardless of the histological type of the tumors, contrasts with the decreased level expression of MUC5AC immunoreactivity in almost all advanced carcinomas which suggest that all gastric carcinomas retain at least some cells with a gastric phenotype during the first steps of neoplastic development.

CONCLUSION

Taking together this data on mucin expression (MUC5AC) in normal gastric mucosa, intestinal metaplasia and gastric cancer two interpretative hypotheses appear plausible because complete intestinal metaplasia shows markedly decreased levels of MUC5AC and incomplete (both type II and type III) intestinal metaplasia maintains the expression of "gastric mucin", it seems conceivable that complete and incomplete intestinal metaplasia represent, divergent differentiation programs or incomplete type II intestinal metaplasia may represent a first step in the intestinal metaplasia pathway, which may evolve to complete intestinal metaplasia with loss of expression of the "gastric mucin".

Mucin expression in intestinal metaplasia and gastric carcinoma is fairly complex. However, authors concluded that MUC5AC expression rates might be good parameters in progression of intestinal metaplasia to gastric carcinoma and might be a good prognostic marker for gastric carcinoma as it is very well implicated in understanding of gastric carcinogenesis.

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