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RESEARCH ARTICLE

EXPRESSION OF MUC2 IN NORMAL GASTRIC MUCOSA, INTESTINAL METAPLASIA AND GASTRIC CARCINOMA BY IMMUNOHISTOCHEMISTRY.

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Abstract

Aim: Carcinomas of the stomach are a heterogeneous group of lesions in terms of architecture, pattern of growth, cell differentiation, and histogenesis. Altered MUC 2 expression patterns have been reported previously in intestinal metaplasia as well as in gastric cancer which include increased mucin heterogeneity, glycosylation changes and de novo expression of MUC2 in intestinal metaplasia as well as gastric carcinoma suggesting that MUC2 alterations can be regarded as "molecular" markers of malignant transformation of gastric mucosa. The aim of the study is to analyze the expression pattern of Mucin MUC2 in Normal, Pre-Neoplastic & Neoplastic Gastric Epithelium.

Materials and 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 MUC2.

Results : The intensity of MUC2 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).

Conclusion: Mucin expression in intestinal metaplasia and gastric carcinoma is fairly complex. However, we conclude that MUC2 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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Introduction:-

Gastric mucins are critical cytoprotective proteins synthesized by gastric epithelial cells[1]. According to their structure and function, mucins can be divided into secreted mucins and transmembrane mucins. Secreted mucins can be gel-forming or non-gel-forming, and include MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9 and MUC19. Transmembrane mucins include MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20 and MUC21 [2].

The genes for mucin MUC2 is found in a cluster on chromosome 11p15.5 and codes for a typical secretory mucin which is predominantly found in colorectal goblet cells. The normal gastric mucosa does not express MUC2 [3,4,5] . Altered mucin expression patterns have been reported previously in intestinal metaplasia as well as in gastric cancer which include increased mucin heterogeneity, glycosylation changes and de novo expression of MUC2 in gastric carcinoma [6,7,8]. These observations suggest that MUC2 alterations can be regarded as "molecular" markers of malignant transformation of gastric mucosa. Keeping all these alterations in mind, in this present study we have characterized the pattern of MUC2 expression in normal gastric mucosa, intestinal metaplasia and gastric carcinoma

Materials and Methods:-

The study was conducted at Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu. 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 MUC 2(BIOGENEX USA) by IHC.

Gastrectomy specimens of 20 patients (15 males, 5 females) with gastric carcinomas diagnosed in the department of pathology in a tertiary care hospital 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. Histological classifications, according to the Lauren's macroscopic classification[9], according to the Borrmann's classification[10] and the World Health Organization (WHO) [11]were done.

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 received in the department of pathology.

The use of histologically normal resection margins from patients undergoing gastric resection may not be the ideal normal control. Previous studies have compared the immunohisto-chemical staining using mucin antibodies in normal surgical resection margins of cancer specimens to the staining observed in normal biopsy specimens from patients with normal stomach determined by endoscopy. They found that the quality of staining for all antibodies was no different in these two groups, with the exception that the intensity of staining was slightly less in the endoscopic biopsies compared with the surgical resection margins. These data support the use of resection margins as normal tissue.

Scoring system:-

MUC2 expression is concentrated in perinuclear zone.
While the intensity of the staining was assessed as follows:

1. 0 (negative),
2. ± (trace positive),
3. + (positive): 5-50% of cells were stained
4. ++ (strongly positive) >50% of cells were stained

The immunostaining result was considered positive if at least 5% of the cells were stained. When less than 5% of neoplastic cells were stained, the immunostaining result was considered negative.

Statistical analyses:-

Statistical analyses of all results were done by using Chi square test at level of significance $p \leq 0.05$ was done.

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

Results:-

The present study is a descriptive (retrospective + prospective) study done from January 2010 to June 2012 in Meenakshi Medical College and Research Institute, Kanchipuram. Tamil Nadu.

In the present study age of the cases ranged from 23 to 78 years with mean age being 51.6 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. Out of these 20 cases of intestinal metaplasia 18 cases were taken either from subtotal or partial gastrectomy specimens and 2 cases were taken from endoscopic biopsies (Table:1). In this present study we 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.

Table 1:- Distribution of intestinal metaplasia with gastric lesions

Gastric Lesions	No. of Cases	Percentage
Benign	7	35
Malignant	13	65

Distribution of Intestinal Metaplasia with Gastric Carcinoma:-
Intestinal metaplasia was seen in association with 13 cases of gastric carcinoma. We further sub divided these 13 cases of gastric carcinoma according to type of intestinal metaplasia into complete or incomplete type. We observed 8 (62%) cases of complete type and 5 (38%) cases of incomplete type intestinal metaplasia associated with gastric carcinoma cases.

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 [9] 15(75%) cases were of intestinal type and 5(25%) cases were of diffuse type in this study. In this study according to Borrmann's classification[10], 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). In this study, according to WHO classification[11] 10 (50%) cases of tubular/papillary type, 6 (30%) cases of signet ring type and 2 (10%) cases of each mucinous and undifferentiated type were noted.

Expression Pattern of MUC 2:-
MUC2 was not detected in normal gastric mucosa(Fig 1a &1b).

Expression Pattern of MUC 2 in Complete Intestinal Metaplasia:-
Among the 7 cases of complete intestinal metaplasia, MUC2 was expressed strongly and 2 cases showed moderate immunoreactivity and weak staining was seen in 1 cases(Fig 2a&2b).

Expression Pattern of MUC2 in Incomplete Intestinal Metaplasia:-
MUC2 was strongly expressed in 5 cases of incomplete intestina. While 2 cases showed moderate and 3 cases showed weak positivity.

Expression Pattern of MUC2 in Gastric Carcinoma:-
MUC2 expression was seen in 18 cases(Fig 3a&3b). Nine cases showed strong MUC2 immunoreactivity while 5 cases showed moderate, 4 cases were weakly reactive for MUC2. While negative immune reactivity for MUC2 was observed in 2 cases. Expression of MUC2 are summarized in table 2.

Table 2:-Expression of MUC2 in Normal, Intestinal metaplasia and Gastric Carcinoma

Histopathology Diagnosis	MUC 2			
	++	+	±	-
Normal	0	0	0	20
Intestinal metaplasia	12	4	4	0
Gastric Carcinoma	9	5	4	2

Fig 1a:-Normal histology of gastric mucosa.

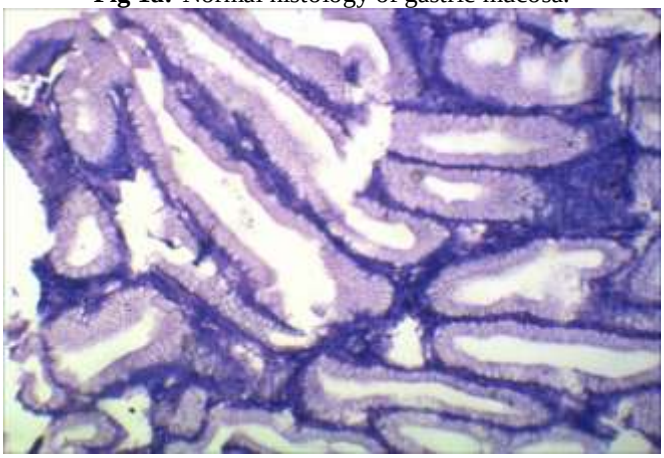


Fig 1b:- MUC2 IHC of normal gastric mucosa

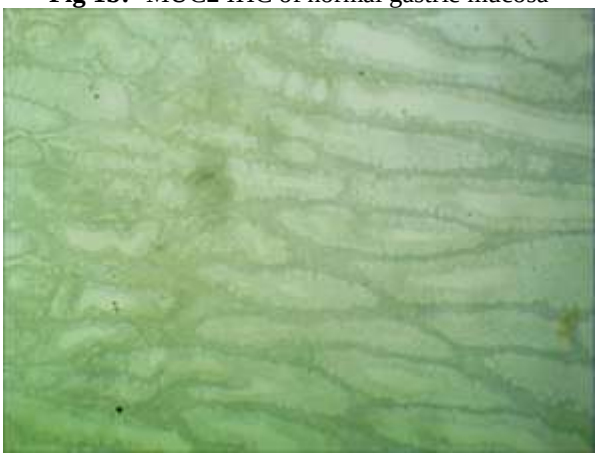


Fig 2a:- Intestinal metaplasia of gastric mucosa**.**

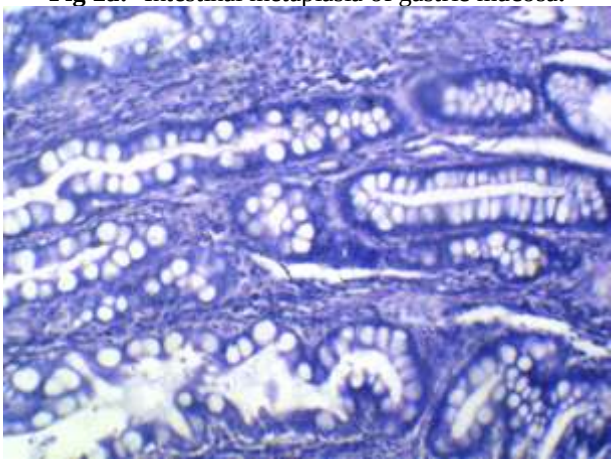


Fig 2b:- Muc 2a IHC in case of intestinal metaplasia

Fig 3a:- histology of gastric carcinoma

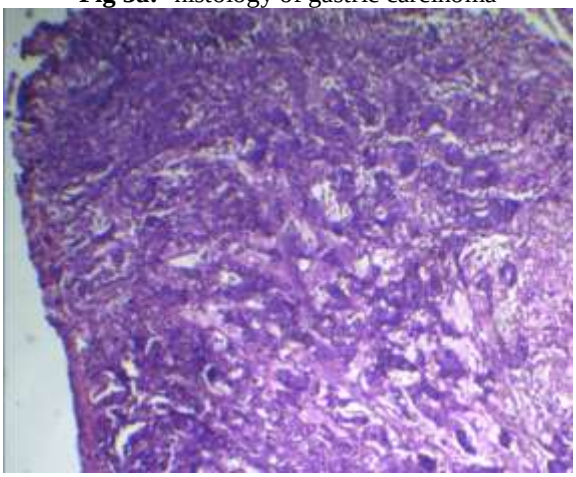
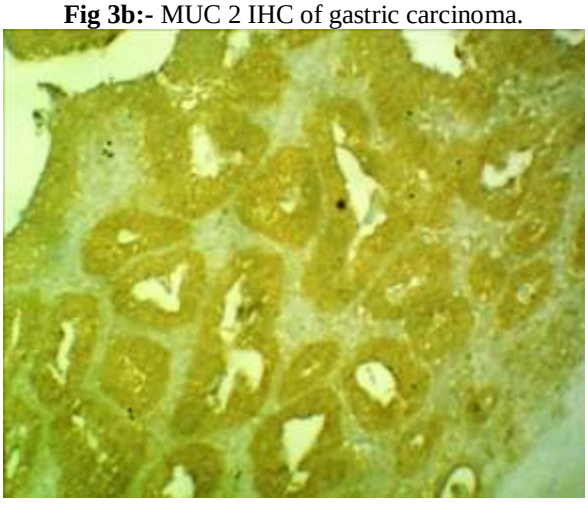


Fig 3b:- MUC 2 IHC of gastric carcinoma.



Discussion:-

In the present study total 60 cases were studied comprising 20 cases of each normal gastric mucosa, intestinal metaplasia and gastric carcinoma.In our study the age of the cases ranged from 23 to 78 years with mean age being 54.6 years. Majority of the intestinal metaplasia and gastric carcinoma cases were seen between 40 to 70 years of age. In present study for our convenience we have broadly divided intestinal metaplasia in two types (A) Complete

type and (B) Incomplete type. We observed 10 cases of complete type and 10 cases of incomplete type. Reis CA, David L et al[5] in their study noted 20 cases of complete type and 26 cases of incomplete type intestinal metaplasia.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. The comparison of intestinal metaplasia in our study and other studies is given in table 3.

Table 3:- Comparison of intestinal metaplasia with benign and malignant gastric lesions

S. No	Study	Benign (%)	Gastric Carcinoma (%)
1	Jass, J. R. & Filipe, M. I (1981)[12]	42.85	93
2	Segura DI & Montero C (1983)[13]	80.7	96
3	Silva, S. & Filipe, M. I (1986)[14]	24.5	65
4	You WC & Blot WJ (1993) [15]	39.8	66.6
5	Present Study	35	65

Classification of Gastric Carcinoma:-

We classified gastric carcinomas according to Lauren's, Borrmann's and WHO classification and following tables (Table:4, Table:5&Table:6) compare our results with the results of other studies.

Table 4:- Comparison of gastric carcinoma cases according to Lauren's classification

Lauren's Classification	Celso A. Reis[16] (2000)		Roessler K et al[17] (2005)		Ozgur Ilhan et al[18] (2010)		Present study (2012)	
	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 5:- Comparison of gastric carcinoma cases according to Borrmann's classification

Borrmann's Classification	Ming S.C[19] (1977)		Roessler K et al[17] (2005)		Ozgur Ilhan et al[18] (2010)		Present study (2012)	
	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 6:- Comparison of gastric carcinoma cases according to WHO classification

WHO Classification	Roessler K et al[17] (2005)		Ozgur Ilhan et al[18] (2010)		Present study (2012)	
	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:-

MUC2 was not detected in normal gastric mucosa. The overall profile of mucin expression in type I (complete) and type II(incomplete) intestinal metaplasia is characterized by de novo expression of the MUC2.The overall profile of mucin expression in gastrc carcinoma is characterized by de novo expression of the MUC2. Table 7 compares the expression of MUC2 in our study with other studies.

Table 7:- Comparison of overall profile of MUC 2 in normal gastric mucosa, intestinal metaplasia and gastric carcinoma

S. No	Study	No. of Cases	MUC 2 percentage			
			0	**1**	**2**	**3**
1	Hong-Kai Zhang et al[20] (2004)	Ca (33)	6	8.4	21.2	63.6
2	Subramani DB. Et al[21](2006)	N (7)	100	0	0	0
		IM (21)	0	0	42.9	57.1
		Ca (36)	0	32.1	29	38.9
3	Ozgur Ilhan et al[18] (2010)	N (nil)	-	-	-	-
		IM (186)	0	22.6	19	58.4
		Ca (257)	9.3	15.1	24.2	51.4
4	Present Study (2012)	N (20)	100	0	0	0
		IM (20)	0	20	20	60
		Ca (20)	10	20	25	45

In agreement with previous studies reporting the distribution of mucins in normal stomach, we found expression of MUC2 was usually not detected in normal gastric mucosa as described in the previous reports Ho SB et al,[7] and Reis Ca et al,[8]

Intestinal metaplasia is one of the lesions identified in the cascade of event that precedes the development of gastric carcinoma. Intestinal metaplasia is accompanied by the induction of de-novo expression MUC2 as well as in gastric cancer.

Conclusion:-

Taking together our data on mucin expression (MUC 2) in normal gastric mucosa ,intestinal metaplasia and gastric cancer , we observed that intestinal metaplasia is accompanied by the induction of de novo expression MUC2. These alterations may favour the development of gastric carcinoma. The present study suggests that MUC2 is a marker of intestinal metaplasia and may be used for the early detection of this lesion in pre-neoplastic human gastric epithelium.

The present study observed de novo expression of MUC 2 in gastric cancer. Recently, various studies have shown that the methylation in the promoter region of the MUC2 gene is determinant for the expression of MUC2 in colon carcinoma cell lines. This mechanism of control of MUC2 gene expression in gastric carcinomas should be addressed in future studies.

Mucin expression in intestinal metaplasia and gastric carcinoma is fairly complex. However, we conclude that MUC 2 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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