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HISTOMORPHOLOGICAL STUDY OF GASTRIC ADENOCARCINOMA AND THE EXPRESSION OF MUC2 AND Ki67

Histopathology

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ABSTRACT

Gastric cancer accounts for the fifth most common cancer in the world. Lauren's and WHO systems of classification of histomorphological types predominate in clinical practice.Studies show altered MUC2 expression patterns can be regarded as molecular markers of malignant transformation in gastric mucosa and cell proliferation can be assessed by Ki67.Only a handful of studies in Indian population in this regard is available. Therefore, we have conducted a study to evaluate the expression of MUC2 and Ki67 in gastric adenocarcinoma. A cross sectional study was conducted in the department of pathology of a tertiary care hospital in East India on fifty six cases of gastric adenocarcinoma.MUC2 and Ki67 expression was interpreted using light microscope and data analysed by Chi-square test and Fisher Exact Test using Statistical Package for the Social Science (SPSS) version 25.0. Out of **56** cases,the diffuse variant occurred more in the **younger** age group (**25-34 years)** and amongst **females(68.8%**). **Mucinous** type showed **100%** and **tubular** type showed **42.3% MUC2** poitivity.**78.6%** of **poorly differentiated** and **85.7% intestinal** type showed **high Ki67 positivity**.We may conclude that mucinous types of gastric adenocarcinoma show higher MUC2 positivity.Also, Diffuse type gastric adenocarcinoma was commoner in younger age group and females.

KEYWORDS

Gastric adenocarcinoma, Intestinal, Diffuse, Mucinous.

INTRODUCTION

Gastric cancer accounts for the fifth most common cancer in the world[1]. In terms of mortality due to gastric cancer, 770,000 deaths (65% were males) were observed worldwide in 2020[2]. In India ,overall cancer incidence was previously reported as 100-121/100000 in male population and 59-70/100000 in females,thus,accounting for about 3.1% to12.9% of all cancer deaths in different parts of India according to ICMR reports [3]. An array of multifactorial,multigenetic and multistep processes lead to gastric cancer. In the age of microscopic pathology, it has been of utmost interest to look at the histomorphology of tumour cells and classify the different types.

Incidence of gastric carcinoma increases with advancing age. Progression occurs from normal gastric mucosa through chronic gastritis, atrophic gastritis with or without intestinal metaplasia to dysphagia and finally to carcinoma [4]. Foods rich in salt and nitrates (dried and salted fish), smoked meat or fish, pickled vegetables, chilli , peppers, soyabeans, host factor(H.pylori infection) – all pose a higher risk. Smoking , pernicious anaemia, previous history of gastric surgery having gastric stumps , Menetrier's disease , peptic ulcer disease[5] are among other causes.Most organs in the body seem to synthesize more than one type of mucin and it has been studied that in gastric cancer,de novo expression of MUC2 (mucin 2) occurs and isolated expression of MUC2(as in intestinal metaplasia) correlates with metastatic spread and poor survival.The immunoreactivity of Ki67 correlates with the degree of tumour cell proliferation and its expression has been considered a poor prognostic marker in many carcinomas. Thus evaluation of Ki67 could be useful in identifying patients with more aggressive disease and contribute to a better therapeutic approach. These observations suggest that alterations in MUC2 expression profile and Ki67 expression can be regarded as molecular markers of malignant transformation of gastric mucosa.

Methodology

A cross sectional ,observational study was done in Department of Pathology, R.G Kar Medical College and Hospital, Kolkata,West Bengal,India for a period of eighteen months. All primary gastric adenocarcinoma cases reported in endoscopic biopsy as well as resected gastrectomy specimens received in the department of Pathology were included in the study.Samples were taken from fifty six cases diagnosed as gastric adenocarcinoma by systemic random sampling irrespective of age and gender. Non-malignant and benign gastric tumours**,** gastric malignancies other than adenocarcinoma, gastrectomy specimens of the patients who have been treated with chemotherapy or neoadjuvant chemotherapy and gastrectomies done due to causes other than gastric tumour were excluded. Ultra thin sections (3 to 4 microns) were obtained by microtome from formalin fixed paraffin embedded blocks.Sections from selected blocks were made on poly- L- lysine coated slides. After deparaffinisation ,antigen retrieval was done using microwave method. It was then followed by perox blocking.After this, primary antibody was added followed by amplifier and HRP (Poly Horse Radish Peroxidase) label. These were subsequently followed by DAB (Diaminobenzidine) visualisation and Haematoxylin counterstaining. Appropriate controls were run along with each batch. IHC was performed using commercially available monoclonal antibodies for MUC2 and Ki67 : MUC2(MRQ-18) Mouse Monoclonal Antibody, CLL MARQU and Ki-67 –GM001 Mouse Monoclonal Antibody, PATHNSITU.Antibody used was the anti-mouse monoclonal antibody from supernatant diluted in TRIS buffered saline pH 7.3-7.7 with protein base and preserved with sodium azide(<0.1%). The secondary antibody kit used was: PathnSitu PolyExcel Peroxide Quencher:50 ml, PathnSitu PolyExcel Poly HRP :50 ml, PathnSitu PolyExcel Target Binder :50 ml, PathnSitu PolyExcel Stunn DAB Buffer:60 ml, PathnSitu PolyExcel Stunn DAB Chromogen: 5 ml. The sections were then counterstained with Harris Hematoxylin and mounted.Positive controls used were;colon for MUC2 and lymph node for Ki67.Analysis was performed by using IBM SPSS 25 version. Chi square test and Fisher's exact test were used wherever applicable to test the significant difference between proportions. P value of 0.05 was taken as the cut- off point to determine the statistically significant results.

Scoring Of Ki67 in IHC

The samples were analyzed following recommendations by the International Ki-67 in Breast Cancer Working Group[6] ; positive Ki-67 staining was defined as only a positive nuclear staining and the average score was recorded by assessing complete sections including hot spots (if present). In full sections, at least three high-power fields (×40 objective) were selected regardless of the staining intensity [6]. For proper grouping of results, cases were then categorized into: gastric cancers with a high Ki-67 score (>20%) and gastric cancers with a low Ki-67 score (≤20%) [7,8].

Scoring of MUC2 in IHC[9]

MUC2 expression is concentrated in the perinuclear zone and cytoplasm.

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 cells were stained.Staining of less than 5% of neoplastic cells was considered as negative.

RESULT AND ANALYSIS

In our study, out of fifty six cases,53.6% were in the age group of 25-50

years and 46.4% were in age group of 51-76 years. *Diffuse type*(Lauren's)[10] *gastric adenocarcinoma (60%) occurred mostly in the younger age group of 25-50years* out of which *the study population in age group of 25-34 years alone showed 66.7% of diffuse adenocarcinoma.71.4% were males and 28.6% were females, showing that males were more affected by gastric cancer. Among female patients diffuse type was more common (68.8%).*

Table 1: Clinico-pathological Parameters In The Study Population

Chacracteristics		Frequency	Percentage
GENDER	Male	40	71.4%
	Female	16	28.6%
AGE (YRS)	25-50	30	53.6%
	51-76	26	46.4%
GRADE	Well	14	25%
	Moderate	16	28.2%
	Poor	26	46.4%
	Intestinal	30	53.6%
TYPE (LAUREN'S)	Diffuse	26	46.4%
	Tubular	26	46.4%
	Mucinous	5	8.9%
TYPE (WHO,2019)	Signet Ring	9	16.1%
	Poorly cohesive (non-signet ring)	16	28.6%
LVSI	Present	30	53.6%
	Absent	26	46.4%
Characteristics		Frequency	Percentage
LYMPH NODE	Positive	35	62.5%
	Negative	21	37.5%
MUC2	Positive	16	28.6%
	Negative	40	71.4%
Ki67	High	14	25%
	Low	42	75%

Tubular variant(WHO classification,2019)[11]of gastric adenocarcinoma *showed 42.3% MUC2 positivity. The mucinous variant showed a 100% positivity with MUC2 and the results as per table no.2 and figure no.1 were statistically significant with a p value of < 0.05.* In our study,the signet ring variant and poorly cohesive types did not show MUC2 positivity. *Around 50% of intestinal and 3.8% of diffuse type of gastric adenocarcinoma showed MUC2 positivity with a statistically significant p value of <.001.This study showed MUC2 positivity in 62.5% cases of well differentiated gastric cancer and MUC2 positivity in 6.3% cases of poorly differentiated gastric carcinoma.The well and moderately differentiated cases of gastric cancer together showed around 93.8% MUC2 positivity. Around 85.7% cases with lymphovascular invasion* (LVSI) *showed significant result with p value <0.05 and a high Ki67. Higher positive expression of Ki67 was seen in 7.1% of the well differentiated carcinomas,12.5 % among the moderately differentiated and in 78.6% of the poorly differentiated carcinomas as per table no. 3 and figure no.* 2.

Table 2

WHO Histological Type	MUC2 +VE	MUC2-VE	Total
Tubular	11	15	26
Row percentage	42.3%	57.7%	100%
Column percentage	68.8%	100%	83.9%
Mucinous	5	0	5
Row percentage	100%	0%	100%
Column percentage	31.3%	0%	16.1%
Total	16	15	31
Row percentage	51.6%	48.4%	100%
Column percentage	100%	100%	100%

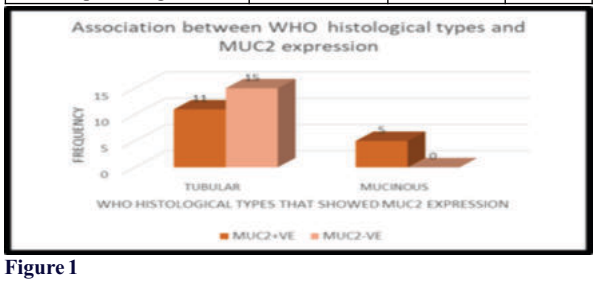

Figure 1

Table 2 & Figure 1:Show association between who histological types (tubular and mucinous) and muc2 expression

Table 3 & Figure2 : Show Histological Grade Of Tumour And Ki67 Expression

Table 3

Histological Grade	Ki67(High)	Ki67(Low)	Total
Well Differentiated	1	13	14
Row percentage	7.1%	92.9%	100%
Column percentage	7.1%	31%	25%
Moderately Differentiated	2	14	16
Row percentage	12.5%	87.5%	100%
Column percentage	14.3%	33.3%	28.6%
Poorly Differentiated	11	15	26
Row percentage	42.3%	57.7%	100%
Column percentage	78.6%	35.7%	46.4%
Total	14	42	56
Row percentage	25%	75%	100%
Column percentage	100%	100%	100%

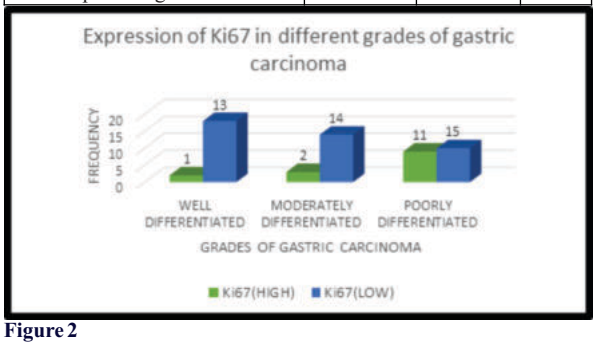

Figure 2

DISCUSSION

*In our study, the mucinous type and intestinal type showed a higher MUC expression.*According to another study done by *Zhang ,et al.*[12] on ninety-four patients of gastric adenocarcinoma MUC2 had a positive expression rate of 84%, with the *highest expression level in mucinous carcinomas (100%) and lowest expression level in signet-ring cell carcinoma. Our study also showed that positive MUC 2 expression was more in well and moderately differentiated gastric adenocarcinoma than in the poorly differentiated* type which was also in concordance with the study by *Zhang ,et al.*[12].

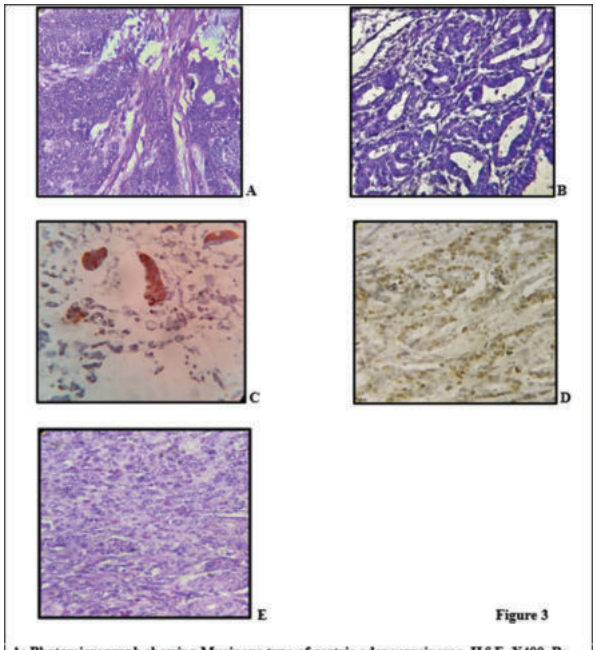

Figure 3

A: Photomicrograph showing Mucinous type of gastric adenocarcinoma, H&E, X400. B: Photomicrograph showing intestinal type, well differentiated gastric adenocarcinoma, H&E, X400. C:Photomicrograph showing positive MUC2 immunostain (≥ 5%) in gastric adenocarcinoma(mucinous),X400. D:Photomicrograph showing positive high Ki67 immunostain (>20%) in gastric adenocarcinoma,X400. E:Photomicrograph showing poorly differentiated, Poorly cohesive type of gastric adenocarcinoma,H&E,X400.

The study of 36 cases of gastric carcinoma conducted by *Babu, et al.*[13] also showed MUC2 positivity and the study also suggested that *MUC2, a marker of intestinal metaplasia, may be used for an early detection of this lesion in cases of H .pylori infection in pre-neoplastic human gastric epithelium.In our study,high grade cancers show a higher Ki67 expression*. These results were in accordance with the study done by *Pramanik ,et al*. in 2020. Also, *Ki67 expression is increased in cases with lymphovascular invasion*.

CONCLUSION

Though overall gastric cancer was still more common among males, from this study we may conclude that diffuse type of gastric adenocarcinoma is commonly seen in younger age group and females. MUC2 is a marker of malignant transformation in stomach and in our study intestinal and mucinous variants show a higher MUC2 expression with nearly all the mucinous variants showing MUC2 positivity.MUC2 being a marker for intestinal metaplasia may also be helpful in *H.pylori* infected cases for an early detection.High grade of tumour ,lymph node metastasis and lymphovascular proliferation show a high ki67 expression level. High Ki67 is thus a sign of poor prognosis associated with a good chance of clinical response to chemotherapy and can be an interesting biomarker of prognosis. However, owing to smaller sample size from a relatively small geographical area, the results may be confirmed in a wider set –up.Also, we were unable to follow up the cases due to Covid19 pandemic. Multicentric studies involving larger number of cases over a larger geographic area and duration will be helpful in verifying our findings.

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