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EXPRESSION OF HER2NEU, PD-L1 AND KI-67 IN GASTRIC ADENOCARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

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Contributions

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Dr Vijayashree R - Concept and design of study, analysis and interpretation of data; supervising the performance of the experimental methods involved in this study, drafting the article, revising it critically for important intellectual content; and final approval of the version to be published.

Dr Pooja E Moorthy - Concept and design of study, analysis and interpretation of data; supervising the performance of the experimental methods involved in this study, drafting the article, revising it critically for important intellectual content; and final approval of the version to be published.

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ABSTRACT:

Introduction

Gastric adenocarcinoma is a leading cause of mortality in the world. The patient is usually in the advanced stage with distant metastasis at the time of diagnosis. The purpose of this study is to evaluate Ki-67 index, which is the rate of proliferation of the tumor, Her2neu, which is responsible for the rapid increase and survival of the tumor cells and PD-L1, which is in charge of central immune tolerance.

Aims and objectives

To evaluate the expression of Ki-67 LI, Her2neu and PD-L1 in gastric adenocarcinomas.

To determine the value of each marker as a single prognostic indicator.

To establish correlation between these markers, clinicopathological parameters and clinical outcome.

Materials and methods

We obtained 42 cases of gastric adenocarcinoma from archival blocks since 2018 and included newly diagnosed cases until February 2022. Hematoxylin and eosin slides were assessed to determine the grade of tumor. Immunohistochemistry of Her2neu, PD-L1 and Ki-67 was performed based on the protocol manual provided by the institute. Appropriate control slides were used along with the tumor tissue, following which, scoring of the IHC slides was done. Clinical details and survival status of the patients was collected.

Results

The most common age group affected was the older age group, with a male preponderance and most of them were grade 3 tumors. There was no correlation between Ki-67, PD-L1, Her2 with the gender, age, grade, but there was significant correlation between the expression of these markers with distant metastasis and mortality status. All three markers were associated with a poor prognosis.

Conclusion

In order to minimize mortality due to gastric carcinoma, combined targeted therapy with anti-Her2neu and anti-PD-L1 in carcinomas that express these markers can be considered to improve the over-all survival of the patient.

Keywords: Immunohistochemistry, Her2Neu, PDL-1, Ki-67, Gastric adenocarcinoma, Targeted therapy

1. INTRODUCTION

Gastric adeno-carcinoma remains to be the second most common cause of cancer related deaths. Despite advances in treatment protocol and early detection of the tumor, it is consistently associated with a high mortality rate. Almost all of gastric cancers are adenocarcinomas and they can be anatomically classified broadly into gastric and gastro-esophageal adeno-carcinomas and histologically into diffuse type and intestinal type of cancers. (1) The risk factors associated are many, but the most important association is H. Pylori infection, which has been established over the years. In addition to H. Pylori, the host and the bacterial virulence as well as other environmental and lifestyle factors like smoking, consuming food that are smoked, with a high salt content, alcoholism, genetic predisposition also plays a role in carcinogenesis and development of adeno-carcinoma. (2)

In order to minimize the number of deaths that occur due to gastric cancers, several studies are being done to recognize the different patterns of the tumor, their site, grade, invasion, metastasis along with other socioeconomic, environmental and hereditary factors that influence the prognosis and the clinical outcome of patients with gastric adenocarcinoma. All over the world, gastric adenocarcinomas have a male preponderance and affects a slightly older population. The tumors present in the proximal and distal part of the stomach behave differently in terms of aggressiveness and invasion. As such, no screening program has become a protocol or a part of early detection of gastric cancers.

Most gastric/gastroesophageal cancers are detected only after the development of symptoms and these are symptoms of gastric outlet obstruction such as, early satiety, vomiting and weight loss. Diagnostic imaging followed by an endoscopic biopsy of the tumor is essential to know the type of tumor, followed by surgical resection – either a partial or complete gastrectomy, based on the location of the tumor is done. PET CT imaging to evaluate the metastatic spread to the lymph node and adjacent organs. (2)

Majority of the patients are at an advanced stage at the time of diagnosis. With the tumor at a more advanced stage at the time of diagnosis, surgery could be out of question for the patient due to their deteriorating general condition. Only palliative care can aid the patient, in order to improve the quality of life and to a limited extent, prolong the life expectancy.

Numerous treatment modalities and targeted therapies are underway for the management of gastric cancers. In our study, the markers of interest are Ki-67, Her2neu and PD-L1. These markers have already been studied individually and their role in contributing towards the genesis of gastric adenocarcinoma has also been established. Based on the expression of these markers, targeted treatment regimen can be prescribed to improve the over-all survival of patients with advanced gastric cancers and even in patients with distant metastases.

Subjectand Methods

Approval for the observational study was obtained from the Institutional Human Ethics Committee (IHEC).

Tissue Sample Collection

We conducted a study which included 42 cases of gastric adenocarcinoma. The tissue blocks were collected, the clinical details of the patients, including presenting complaints, and other pathological parameters of relevance were sought from the Medical Records Department after obtaining permission through proper channel. IHC staining Haematoxylin and Eosin was being done in the representative area of the tumour

The anti-bodies and their clones:

Ki-67: Clone - MIB1- Tonsil, Breast carcinoma

Her2neu: Clone - EP3. Positive control - Breast carcinoma

PD-L1: Clone – B7H1P. Positive control - Lung squamous cell carcinoma, Placenta

Scoring of Ki-67, Her2neu and PDL1 after Immunohistochemistry.

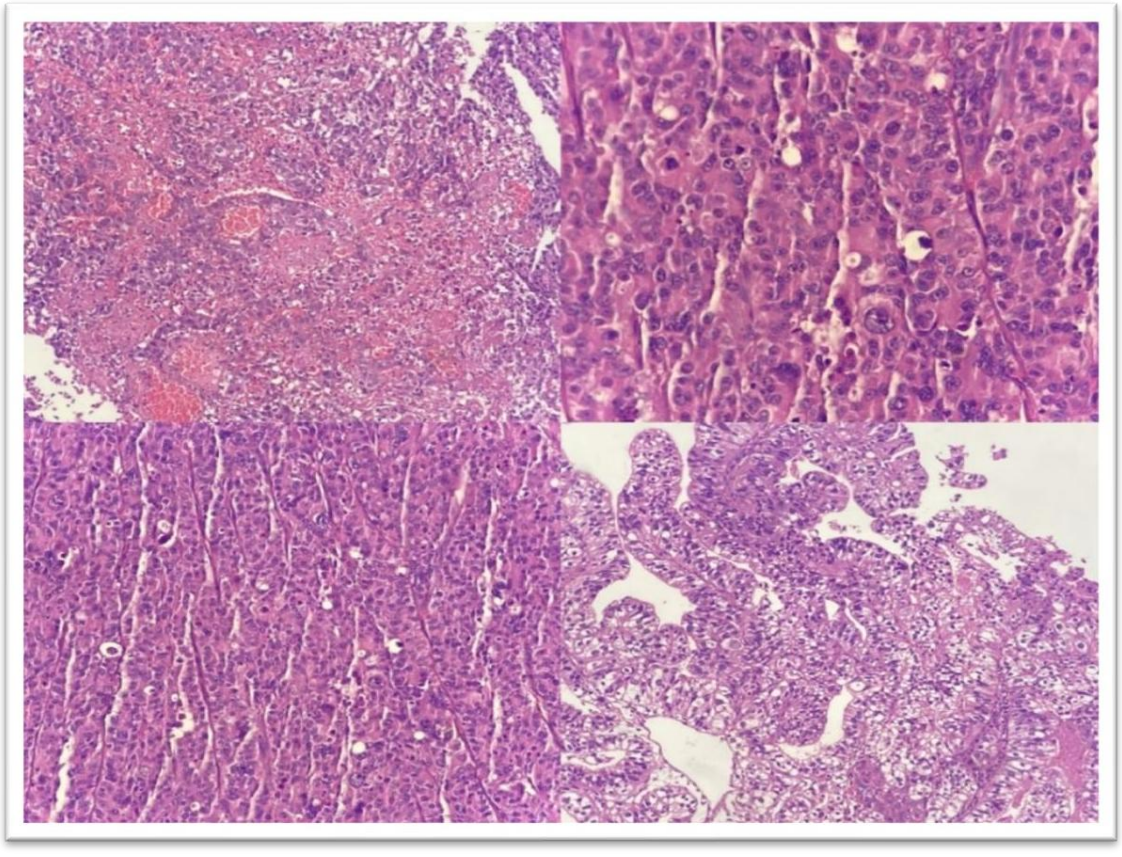
Ki-67 was scored as <25%, 25 to 50%, 50 to 75% and >75% of tumour cells. It stains the nucleus of the tumour cells 100 tumour cells were counted for each case of gastric adenocarcinoma.

Her2neu was scored based on the cytoplasmic staining. For biopsy specimen, the staining intensity and whether the stain is taken up completely or only in the basolateral part was identified and >/= 5 cohesive cells had to be positive. For resection specimen >/= 10 cohesive cells had to be positive. The scoring was done as 0, +1, +2, +3, based on the intensity and the completeness of the membrane staining.

PD-L1 was scored based on the nuclear staining of the tumour cells. A combined positive score was taken upon counting the tumour cells along with the lymphocytes, macrophages which was divided by the number of tumour cells and then multiplied by 100. A combined positive score of >1 was taken as a positive staining and a combined positive score of <1 was taken as a negative staining. (Figures 1-5)

Figures

Figure 1 Images show poorly differentiated tumour in sheets



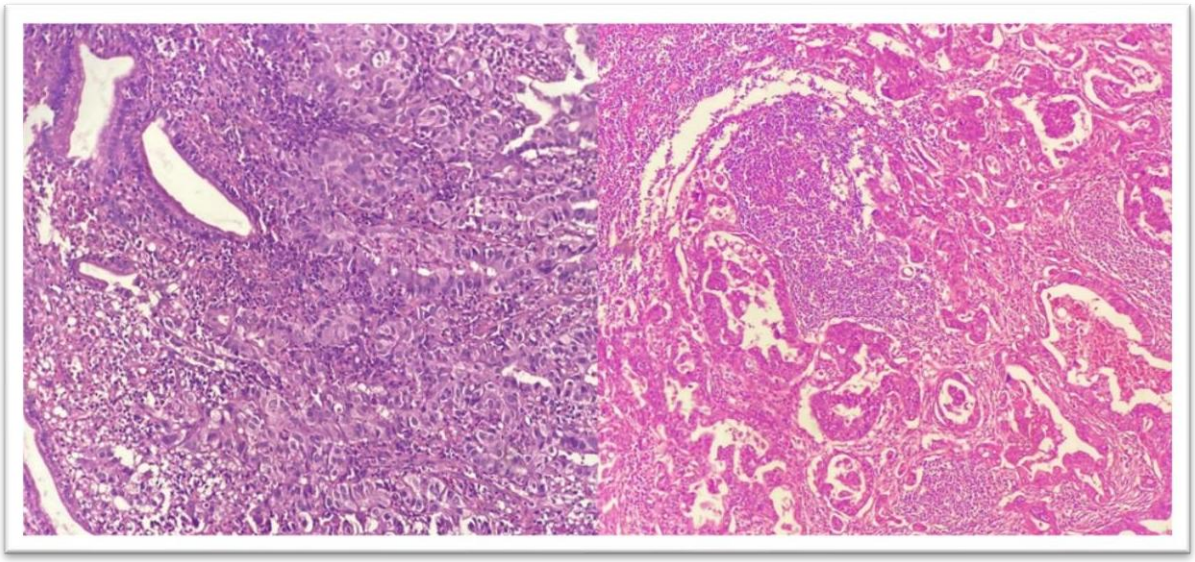


Figure 2 Image a shows some glandular differentiation of the tumour with the adjacent areas showing sheets of tumour cells. Image b shows lymph node metastasis. We can see the tumour cells with some lymphoid population surrounding them.

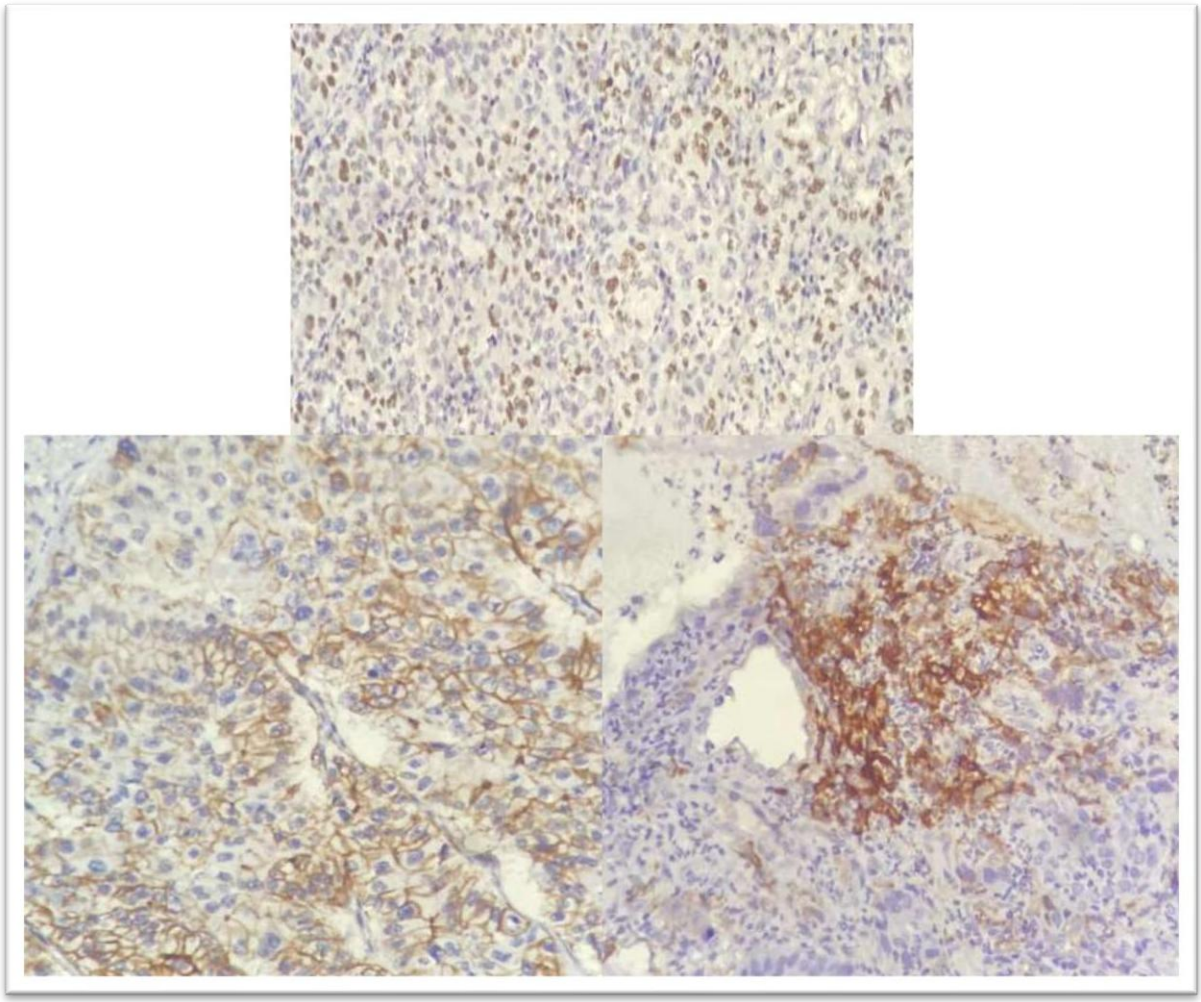


Figure 3 Image a shows Ki-67 LI of 25 to 50% of tumour cells. Image b shows Her2 staining 3+. Image c shows PD-L1 staining with a CPS >1

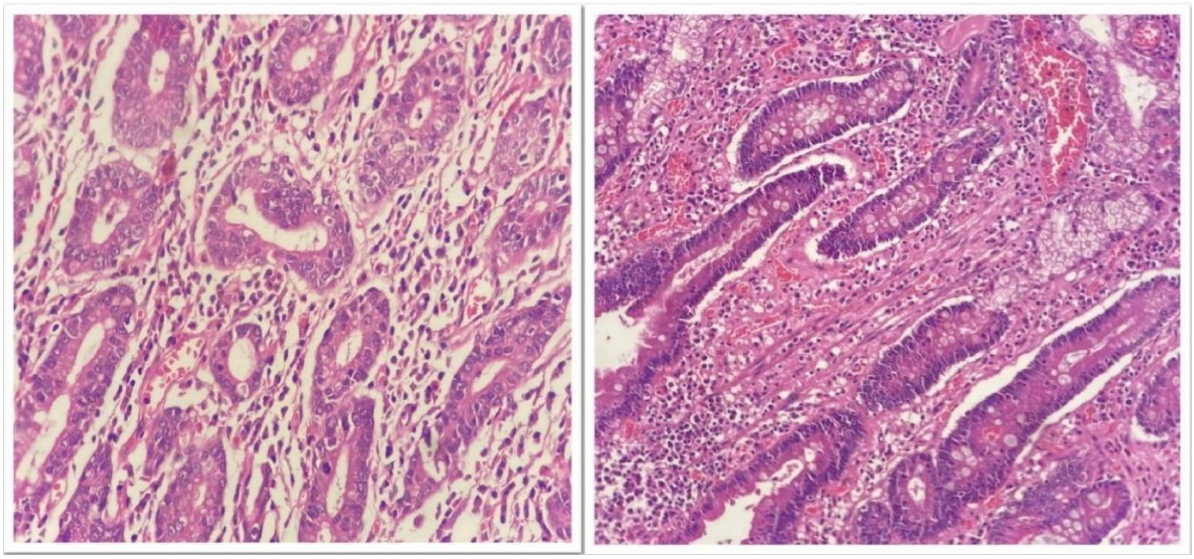


Figure 4 Image a shows well-differentiated glands. Image b shows intestinal metaplasia.

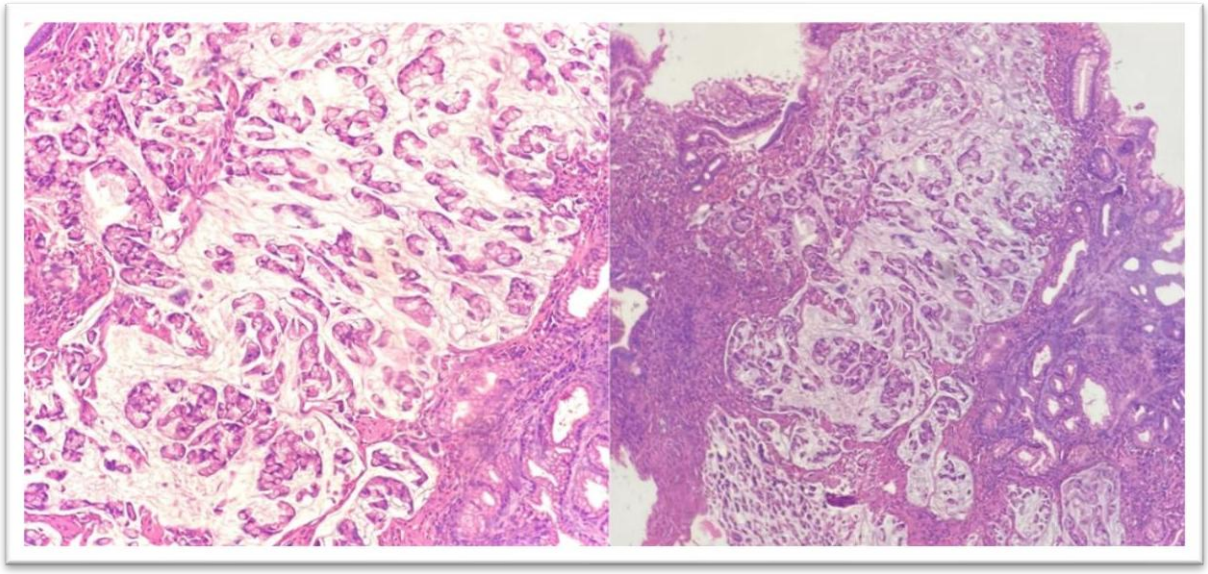


Figure 5 Images show signet-ring cells floating in pools of mucin

2. RESULTS

Statistical analysis

The frequencies and percentage analysis of various parameters was done using SPSS software version 23. Data visualization was done using SPSS software version 25. (Table 1-8)

Table 1: Distribution of the Participants in Terms of H. Pylori Status (n = 42)

H. Pylori Status	Frequency	Percentage	95% CI
Positive	29	69.0%	52.8% - 81.9%
Negative	13	31.0%	18.1% - 47.2%

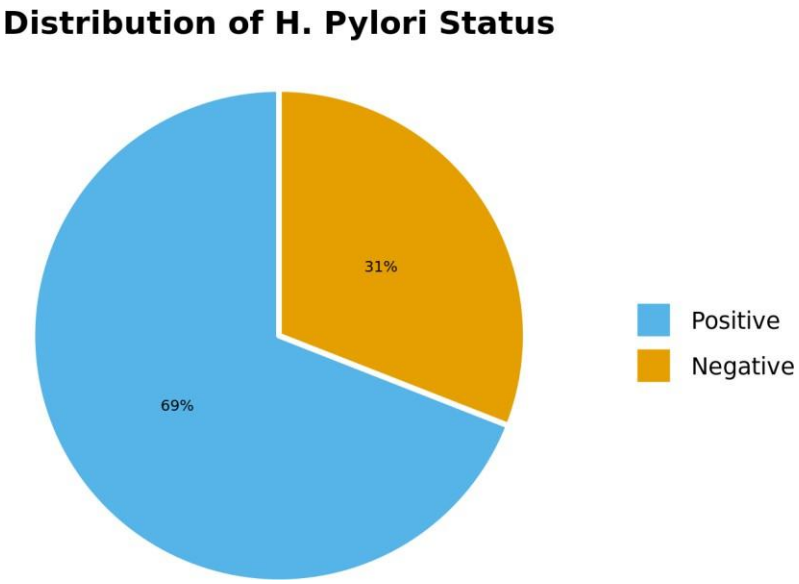


Table 2: Distribution of the Participants in Terms of Her2 (n = 42)

Her2	Frequency	Percentage	95% CI
2+	11	26.2%	14.4% - 42.3%
3+	7	16.7%	7.5% - 32.0%
Negative	24	57.1%	41.1% - 71.9%

Table 3: Distribution of the Participants in Terms of PD-L1 (n = 42)

PD-L1	Frequency	Percentage	95% CI
CPS <1	21	50.0%	35.5% - 64.5%
CPS >1	21	50.0%	35.5% - 64.5%

50.0% of the participants had PD-L1: CPS <1. 50.0% of the participants had PD-L1: CPS >1.

Table 4: Distribution of the Participants in Terms of Ki-67 (n = 42)

Ki-67	Frequency	Percentage	95% CI
<25%	16	38.1%	24.0% - 54.3%
25 to 50%	19	45.2%	30.2% - 61.2%
50 to 75%	4	9.5%	3.1% - 23.5%
>75%	3	7.1%	1.9% - 20.6%

Table 5: Association Between Ki-67 and Grade (n = 42)

Grade	Ki-67					Fisher's Exact Test	
	<25%	25 to 50%	50 to 75%	>75%	Total	$\chi 2$	P Value
Grade 1 (Well-differentiated)	4 (25.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	5 (11.9%)	9.881	0.081

Grade	Ki-67					Fisher's Exact Test	
	<25%	25 to 50%	50 to 75%	>75%	Total	χ2	P Value
Grade 2 (Moderately differentiated)	3 (18.8%)	10 (52.6%)	1 (25.0%)	1 (33.3%)	15 (35.7%)		
Grade 3 (Poorly differentiated)	9 (56.2%)	9 (47.4%)	3 (75.0%)	1 (33.3%)	22 (52.4%)		
Total	16 (100.0%)	19 (100.0%)	4 (100.0%)	3 (100.0%)	42 (100.0%)		

Fisher's exact test was used to explore the association between 'Ki-67' and 'Grade' as more than 20% of the total number of cells had an expected count of less than 5.

Ki-67:

<25%: 25.0% of the participants had Grade 1, 18.8% had Grade 2. 56.2% had Grade 3.
25 to 50%: 0% of the participants had Grade 1, 52.6% had Grade 2, 47.4% had Grade 3.
50 to 75%: 0.0% of the participants had Grade 1. 25% had Grade 2, 75.0% had Grade 3.
>75%: 33.3% of the participants had Grade 1, 33.3% had Grade 2, 33.3% had Grade 3.

Table 6: Association Between Her2 and Grade (n = 42)

Grade	Her2				Fisher's Exact Test	
	2+	3+	Negative	Total	χ2	P Value
Grade 1	0 (0.0%)	2 (28.6%)	3 (12.5%)	5 (11.9%)	6.931	0.115
Grade 2	3 (27.3%)	4 (57.1%)	8 (33.3%)	15 (35.7%)		
Grade 3	8 (72.7%)	1 (14.3%)	13 (54.2%)	22 (52.4%)		
Total	11 (100.0%)	7 (100.0%)	24 (100.0%)	42 (100.0%)		
PD-L1	**Her2**				**Fisher's Exact Test**	
	2+	**3+**	**Negative**	**Total**	**χ2**	**P Value**
CPS <1	7 (63.6%)	0 (0.0%)	14 (58.3%)	21 (50.0%)	8.485	0.015
CPS >1	4 (36.4%)	7 (100.0%)	10 (41.7%)	21 (50.0%)		
Total	11 (100.0%)	7 (100.0%)	24 (100.0%)	42 (100.0%)		

Her2: 2+: 0.0% of the participants had Grade 1. 27.3% had Grade 2]. 72.7 had Grade 3.
Her2: 3+: 28.6% of the participants had Grade 1. 57.1% had Grade 2]. 14.3% had Grade 3.
Her2: Negative: 12.5% of the participants had Grade 1. 33.3% had Grade 2. 54.2% had Grade 3.

Table 7: Association Between Her2 and PD-L1 (n = 42)

PD-L1	Her2				Fisher's Exact Test	
	2+	3+	Negative	Total	χ2	P Value
CPS <1	7 (63.6%)	0 (0.0%)	14 (58.3%)	21 (50.0%)	8.485	0.015
CPS >1	4 (36.4%)	7 (100.0%)	10 (41.7%)	21 (50.0%)		

PD-L1	Her2				Fisher's Exact Test	
	2+	3+	Negative	Total	$\chi 2$	P Value
Total	11 (100.0%)	7 (100.0%)	24 (100.0%)	42 (100.0%)		

Her2: 2+: 63.6% of the participants had PD-L1: CPS <1. 36.4% had PD-L1: CPS >1.
Her2: 3+: 100.0% of the participants had PD-L1: CPS >1.
Her2: Negative: 58.3% of the participants had PD-L1: CPS <1. 41.7% had PD-L1: CPS >1.
Participants in the group Her2: 2+ had the largest proportion of PD-L1: CPS <1. Participants in the group Her2: 3+ had the largest proportion of PD-L1: CPS >1.

Table 8: Association Between PD-L1 and Grade (n = 42)

Grade	PD-L1			Fisher's Exact Test	
	CPS <1	CPS >1	Total	$\chi 2$	P Value
Grade 1 (Well-differentiated)	3 (14.3%)	2 (9.5%)	5 (11.9%)	2.594	0.262
Grade 2 (Moderately differentiated)	5 (23.8%)	10 (47.6%)	15 (35.7%)		
Grade 3 (Poorly differentiated)	13 (61.9%)	9 (42.9%)	22 (52.4%)		
Total	21 (100.0%)	21 (100.0%)	42 (100.0%)		

PD-L1: CPS <1: 4.3% of the participants had Grade 1. 23.8% had Grade 2. 61.9% had Grade 3.
PD-L1: CPS >1: 9.5% of the participants had Grade 1. 47.6% had Grade 2. 42.9% had Grade 3.

DISCUSSION

We conducted a study which included 42 cases of gastric adenocarcinoma. Several parameters such as clinical data of the patient, type of tumour and mortality were assessed along with Ki-67, Her2neu and PDL1 status to throw light on the expression of these markers and outcome of patients.

In our study, we correlated the expression of Ki-67, Her2neu and PD-L1 with one another, and also with other clinicopathological parameters such as the age, gender, presenting complaints, Helicobacter Pylori status, tumour invasion, distant metastasis, and mortality. Most relevant correlation made, was between expression of the above mentioned immunohistochemical markers, distant metastasis and mortality. The idea of this study is to contribute towards forming a treatment protocol where combined targeted therapy (immunotherapy with anti-Her2 and anti-PD-L1) can be used to treat patients with gastric adeno-carcinoma based on the expression of these markers, to understand the course of the disease, and to establish the value of these markers as single prognostic indicators for further management and treatment.

The most common age group that was affected are between 50 and 60 years of age, with a male preponderance. In a study conducted by Hyun Kim D et al. two-hundred and eighty-six cases were assessed. The patient features were analysed, and the average age was found to be 60.8 years with a male-female ratio of 1.9:1, which is similar to the demographics in our study. Out of 286 cases, 57 cases were located in the proximal part of the stomach, 105 cases were situated in the mid portion and 124 cases were in the distal part of the stomach(3). The most common site amongst our 42 cases was the antrum followed by the pylorus and the body of stomach.

In general, gastric carcinoma patients present with non-specific symptoms like dyspepsia, abdominal pain, anorexia and loss of weight. (4) Most of our patients had presenting complains of gastric-outlet obstruction such as, abdominal pain, vomiting, dyspepsia, loss of weight and appetite. The grade of the tumours was assessed by looking at the haematoxylin and eosin-stained tissue sections. More than $1/3^{rd}$ cases were poorly differentiated tumours (Grade-3),

40% were moderately differentiated (Grade-2) and only 5% were well-differentiated (Grade-1) adenocarcinomas. Invasion was predominantly to the level of serosa in most cases.
H.Pylori was positive in 69% of cases. Distant metastasis was seen in 54.8% cases. Chemotherapy and radiation status of the patients were also assessed. Her2neu was positive in 42.9% of cases, PDL1 was positive in 50% of cases and Ki-67 was >25% in 26 out of 42 cases. The mortality rate among these 42 cases was 57.1%.

Association of Ki-67 with all parameters:
We scored the Ki-67 staining into 4 categories: <25%, 25 to 50%, 50 to 75% and >75%. In order to score the staining, 100 tumour cells were counted and the proportion of cells that have taken up the nuclear stain was considered as percentage of Ki-67 positive cells. In a study by Taban S et al, a different method of scoring Ki-67 was adopted, where the percentage of the positively stained cells was under two broad categories: >/= 45% and <45%. The expression of Ki-67 was hence classified as either low or high, and there was no intermediate category. (5)
A meta-analysis which consisted of 7078 patients from 53 different studies on gastric adenocarcinoma from various parts of the world was conducted by Xiong D et al. According to this study, higher Ki-67 was seen in tumours of a larger size, invasive, had distant metastasis and most of these tumours were also poorly differentiated. This study was also able to estimate that, the p53 pathway is also influenced by MKI67 (6) In our study, there was significant correlation between the rate of metastasis and Ki67. 75% and 100% of tumours had metastasis with a proliferating index of 50 to 75% and >75% respectively, although the size of the tumour and p53 expression was not taken into consideration. Invasion of the tumour across all the layers of the stomach was seen and had no correlation with Ki-67 in our study.
In our study, a Ki-67 proliferation of 25% to 50% and 50% to 75% were all grade 2 and 3 tumours. For tumours with >75% Ki-67 index, equal distribution across grade 1, 2 and 3 was seen. There is minimal correlation between the grade and proliferating index.
Boger C et al. re-evaluated the significance of Ki-67 that the intra-tumoral heterogeneity, and how different populations of tumour cells can co-exist in the same patient. When gastric biopsy specimen is assessed for morphology or when immunohistochemistry is used in a small biopsy of a large tumour, sampling error can miss various morphological patterns and vastly alter the grade and invasiveness of the tumour. The small tumour tissue can fail to be representative of the large tumour. They also observed that tissue micro-array can underestimate the proliferating index. (7) Lee H et al had established that a low Ki-67 is associated with poor prognosis and they have utilised TMA (tissue microarray) which can lead to the sampling error (8), which was pointed out by Boger C et al. Because of such limitations, they performed their study with tissue blocks and found that there was no significant relation between Ki-67 index, clinicopathological parameters and patient survival. Hence, they concluded that Ki-67 index cannot be used as a prognostic indicator in gastric adenocarcinomas. (7)
Ki-67 and Her2 status along with p53 was evaluated by Ahmed A et al, in a meta-analysis consisting of 5,600 cases from 29 studies. Moderate to high Ki-67 positively correlated with Her2 staining and this association adds value and allows for Ki-67 to be considered even as an individual parameter to predict prognosis. 83% of Her2 positive cases also showed a high proliferating index, and was also associated with diffuse p53 staining in these cases. (9)
Correlation between Ki-67 and Her2neu was made in our study, which showed that most tumours with a 3+ Her2 staining had a proliferating index of >75%.
Concurrent expression of Ki-67 and Her2neu warrants a poor prognosis, as it is associated with a higher tumour stage, lympho-vascular invasion and an aggressive tumour nature as established by El-Gendi S et al. in their study with 20 cases of gastric adenocarcinoma. (10)

Correlation between Ki-67 and PD-L1 showed that all tumours with a higher proliferation index of 50 to 75% and >75% had a PD-L1 CPS>1.
Based on several authors and their analyses, be it a meta-analysis or an original study, high Ki-67 LI has to do with a low over-all survival and an aggressive tumour behaviour, which correlates positively with our study of 42 cases with clinicopathological parameters.

Association of Her2neu with all parameters
Her2 neu expression in breast cancers are already established to have a poor outcome but a positive Her2 staining in gastric adenocarcinomas still remains to be fully understood.
Most of grade 3 carcinomas were Her2: 2+ and more than half of grade 2 tumours were Her2: 3+. Stronger staining was seen with better tumour differentiation. This correlates with a study done by Wei Z et al, where stronger Her2 staining was seen in tumours that were well and moderately differentiated, and these tumours had lympho-vascular invasion, also similar to the results in our study.(11)
The expression of Her2neu was seen to be varied in gastric cancers and gastro-oesophageal cancers according to a study conducted by Boku N. In this study, based on the positive IHC staining of Her2 2+/3+, chemotherapy was given. The ToGA trial was conducted which tested the effectiveness and safety of Trastuzumab, which established that combination of chemotherapy and Trastuzumab showed longer over-all survival rather than treatment with an isolated chemotherapy regimen. They were also able to prove that higher Her2 expression had a better response to treatment with Trastuzumab as compared to a lower Her2 staining. Continuing Trastuzumab as mono-therapy also seemed to extend disease free survival. (12)
A meta-analysis conducted by Lei Y et al. compared 41 studies which correlated Her2 status and gastric carcinomas. They found that Her2 staining correlated with poor prognosis, with most tumours in a higher stage at presentation, exhibiting nodal metastases and also were found to be well-differentiated tumours.(13)
Our study also showed serosal invasion in 54.5% cases which had a 2+ Her2 scoring and 14.3% cases which had a 3+ Her2 scoring. We also established that all of Her2: 3+ tumours were metastasizing, which means that, a stronger Her2 staining status can indicate the metastatic potential of a tumour. All of the patients with a 3+ Her2 staining were dead upon follow-up. These results positively correlate with the meta-analysis performed by Lei Y et al.
A study conducted by Beer A et al. compares the expression of PD-L1 and Her2neu in gastro-oesophageal carcinomas. In their study, the expression of PD-L1 was similar in both Her2 positive and negative tumours. No significant correlation or co-expression of these markers were seen. Moreover, Her2 positive status was associated with a better prognosis (14)In our study, tumours with a Her2: 3+ staining showed PD-L1 CPS>1, establishing co-expression of these markers and Her2 positive status had a poor outcome. Most tumours (66.7%) with a negative staining of PD-L1 were also negative for Her2neu.
In situations where co-expression of both markers is seen, combination therapy/immunotherapy targeting both Her2 and PD-L1 should be considered. This has been revealed in a clinical trial, in which they have found advantage in treating patients with anti-Her2 and anti-PD-L1 therapy, where significant tumour shrinkage was seen with combined immunotherapy.(15) This data brings out the importance of testing for both markers in advanced gastric and gastro-oesophageal tumours in order to provide maximum benefit to the patient and to reduce tumour load in advanced cases of gastric adenocarcinoma.

Association of PD-L1 with all parameters:
PD-L1 was scored by counting all the tumour cells along with inflammatory cells and tumour infiltrating lymphocytes, which was then divided by the number of the tumour cells alone and then multiplied by 100. This was the combined positive score. A combined positive score of

>1 was taken as a positive staining for PD-L1. A minimum of 100 tumour cells is mandatory to consider a slide for PD-L1 scoring. (15)

In a study done by Beer A et al. four types of PD-L1 scoring were done:

1. Based on the percentage of membranous, either complete or incomplete positively stained neoplastic cells, which was labelled as the tumour proportion score (TPS)
2. Number of positively stained tumour associated immune cells, for which the intensity of staining was not taken into account
3. Combined positive score (as described above)
4. Interface pattern which is described by Muro K et al. as a group of PD-L1 positive cells at the junction in-between areas where neoplastic cells and normal adjacent stroma is merging.

In this study, gastro-oesophageal tumours were taken and the expression of PD-L1 was assessed in these cases. The tumour associated cells showing a positive PD-L1 staining was associated with a better over-all survival. The interface staining of PD-L1 was also associated with a better outcome. Tumour proportion score was associated with a poor prognosis. Over-all, in their study, PD-L1 staining was mostly focal rather than diffuse, much like what we observed in our study.

They have also hypothesised that the different patterns of PD-L1 staining in different studies could be due to the difference in the tissue blocks, some use TMA (tissue microarray) blocks, the use of different anti-bodies, the various ways of grading the positive and negative PD-L1 expression, and the wide range of patient's ethnic background.

A meta-analysis was done by Zhang M et al. which included 136 articles, brought out the clinicopathological and prognostic significance of the marker. PD-L1 expression was associated with a poor prognosis, which was similar to the results in our study. In our study, most patients with a negative PD-L1 staining were alive as opposed to 81% of the patients with a positive PD-L1 staining who were not alive. No significance was established between the grade and the PD-L1 status. There was no correlation between the grade of the tumour and the PD-L1 status in our study as well. However, they found larger tumours to have a better PD-L1 staining, which was not assessed in our study.

The depth of the tumour also had no association with the PD-L1 status in their study. In our study, invasion was predominantly up-to the serosa in most tumours, irrespective of the PD-L1 status and no correlation was demonstrable. They found minimal correlation between lymph node spread and PD-L1 status. Most of the cases with a combined positive score of >1 (85.7%) had distant metastasis in our study. (16)

Hence, PD-L1 positivity can indicate the poor outcome of the patient or the metastatic potential of the tumour.

The same study by Lin C et al. also assessed the relationship between Her2neu and PD-L1 and found that there was no correlation. When they analysed other cohort studies, a negative correlation between PD-L1 and Her2neu was noted. (15)

In a study conducted by Wang L et al. five hundred and fifty cases of gastric adeno-carcinoma were taken. The proportion of PD-L1 positive tumour infiltrating lymphocytes as well as tumour cells was calculated and positive. Positive staining of the non-malignant, benign, gastric mucosal epithelium was not seen. Higher stages of cancers, stage 2, 3 and 4 had a more intense staining for PD-L1. They also discussed about the heterogeneity of the tumour, because of which there could be false-negative results due to the lack of representative tumour tissue that is taken for analysis and that, substitute markers for PD-L1 should be considered and PD-L1 staining has to be standardised further in upcoming experiments. The expression of PD-L1 was predominantly seen in those cases that did not express Human epidermal receptor 2. There was obvious inverse correlation between Her2 and PD-L1 in this study. They also stated a study

done by Oki et al. which established that higher PD-L1 status correlated with a higher Her2 status. In addition to Her2, PD-L1 correlation, they have also included MMR (mis-match repair) protein expression. The Her2 negative cases which showed positive staining for PD-L1 also turned out positive for MMR. Since the cases that express MMR respond well to anti-PD-1 therapy, this was a good addition to their study. (17) In our study, a positive correlation between PD-L1 and Her2 expression was made. Such contrasting results of Her2 and PD-L1 correlation was probably due to the intra-tumoral heterogeneity and difference in the PD-L1 scoring across various studies.

Limitations of the Study

The sample size of our study is only 42 cases of gastric adenocarcinoma, due to the non-availability of clinical details and long-term follow-up of patients. The size of the tumour and the mode of metastasis were also not taken into consideration, due to the lack of data as many of our cases were only biopsy specimen and the actual tumour resection did not take place in our institute. Including mis-match repair proteins could have been a positive addition to our study.

Summary

We included 42 cases of gastric adenocarcinoma in our study. The most common age of presentation was seen in the elderly age group between 50 to 60 years. Most adenocarcinomas were grade 3 and situated in the distal part of the stomach. There was an obvious male preponderance. Invasion of the tumour was to the level of serosa, and distant metastasis was seen in 23 out of 42 cases and most of these cases expressed Ki-67, Her2neu and PD-L1. Ki-67 LI had no correlation with age and gender of the patient, grade of tumour, invasion and positively correlated with distant metastasis, expression of Her2neu and PDL-1. Most tumours that had a higher proliferation index, also had a positive Her2neu and Ki-67 status. Ki-67 index also correlated with mortality.

Most patients with a higher rate of proliferation were not alive on follow-up. Her2neu had no correlation with age and gender of the patient, grade, invasion of the tumour. There was positive correlation between Her2 and location of the tumour (gastro-oesophageal junction) and with the expression of Ki-67 and PD-L1. Positive Her2 status was associated with reduced survival of the patient. PD-L1 had no correlation with the age and gender of the patient, grade of the tumour, invasion. PD-L1 positively correlated with Her2 and Ki-67 status as well as mortality. In our study we have established the relationship of these markers with one another. The value of an individual marker as a prognostic indicator is still in question, but can very well be used in combination with a fair level of certainty. The co-expression of Her2 and PD-L1 can assist the clinician to decide on combined targeted immune-therapy to improve the outcome and over-all survival of the patients. The expression of these markers, however, could point to a more aggressive tumour behaviour with lympho-vascular invasion and distant metastasis. Ki-67 LI, Her2neu and PD-L1 can be considered as poor prognostic indicators according to our study.

3. CONCLUSION

We conclude that, the expression of Ki-67, Her2neu and PD-L1 are seen in more aggressive tumours, which are invasive with distant metastasis and is associated with a poor prognosis. These markers can be used as prognostic indicators in gastric cancers. The expression of Ki-67 is controversial, could mean earlier diagnosis and treatment due to mass forming tendency, but it was also seen that these tumours had a higher incidence of metastasis at the time of presentation. The expression of Her2neu and PD-L1, although seen in tumours of an aggressive

nature, provides an opportunity for us to offer targeted therapy to the patients which can significantly alter and improve the over-all survival.

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