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Original article

Role of p53 and HER 2 gene expression and epigenetic alterations as potential risk for developing Gastric cancer

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Abstract

Gastric cancer remains one of the second leading causes of cancer-related mortality worldwide. The tumor suppressor gene p53 plays a critical role in regulating cell cycle arrest, apoptosis, and genomic stability. This study was investigates that the relationship between p53, HER2 gene expression and epigenetic alterations, particularly DNA methylation, in assessing the risk of gastric cancer development. A comparative analysis was conducted between 50 gastric cancer patients and 50 healthy controls to evaluate p53 and HER2 expression levels. Our findings demonstrate that reduced p53, HER2 expression, coupled with aberrant promoter hypermethylation, is significantly associated with increased gastric cancer risk. These results suggest that epigenetic regulation of p53 and HER2 may serve as a valuable biomarker for early detection and risk stratification.

Key Words: *p53 , HER2 and DNA methylation*

Introduction

Gastric cancer is the second leading cause of cancer mortality in the world. Malignant neoplasm arising from stomach are various types, of that predominantly seen are groups of gastric adenocarcinoma, 90% of all gastric cancers they originate from gastric mucosal glands and the management, especially in advanced stages, has evolved relatively little[1]. Surgical resection remains the mainstay of treatment and can cure patients with early-stage cancer. The survival rate of patients with advanced resectable gastric or gastroesophageal junction (GEJ) cancers, however, remains poor despite new treatment strategies, such as perioperative chemotherapy1 or adjuvant chemoradiation. New therapies are urgently needed. A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with the signalling cascades involved in cell differentiation, proliferation, and survival[2].

Biological prognostic factors are often derived from the genetic process, which is thought to represent a crucial step to gastric cancer includes HER2, Ecadherin, EGFR, DNA copy number changes, microsatellite instability, and changes in expression of several factors including thymidilate synthase, beta-catenin, mucin antigen, p53, COX-2,

matrix metalloproteinases, and vascular endothelial growth factor receptor(VEGF)4. Some of these potential prognostic factors can also be predictive of response to therapy as they are a molecular target either to chemotherapeutics or to biologic/targeted therapies4, such as trastuzumab in HER2- positive tumors[3].

Gastric cancer is a multifactorial disease influenced by genetic, environmental, and epigenetic factors. Among tumor suppressor genes, *p53* is one of the most frequently altered genes in human cancers. It functions as the "guardian of the genome" by maintaining DNA integrity. While mutations in *p53* are well-documented, increasing evidence highlights the importance of epigenetic modifications, such as DNA methylation and histone modification, in regulating gene expression without altering the DNA sequence. Promoter hypermethylation of tumor suppressor genes can lead to transcriptional silencing, contributing to carcinogenesis. *p53* and *HER2* amplification and overexpression have been found to promote tumorigenesis and to be involved in pathogenesis of several human cancers5 like colon, bladder, ovarian, endometrial, lung, uterine cervix , head and neck , esophageal and gastric carcinomas.

The gene for the HER2 protein (ErbB-2, c-erbB2 or Her2/neu) is a protooncogene located on the chromosome 17. The *HER2* gene encodes a 185-kDa transmembrane tyrosine kinase receptor protein, which is a member of the HER family.

HER2 over expression is increasingly recognized as a frequent molecular abnormality, driven as in breast cancer by gene amplification. There is mounting evidence of the role of HER2 overexpression in patients with gastric cancer, and it has been solidly correlated to poor outcomes and a more aggressive disease. Additionally, preclinical data show significant antitumor efficacy of anti-HER2 therapies (particularly monoclonal antibodies directed towards the protein) in *in vitro* and *in vivo* models of gastric cancer[4]. With regard to HER 2 overexpression and the availability of targeted monoclonal antibodies, in our study here we have analysed the expression of HER 2in Intestinal and Diffuse type gastric carcinomas by IHC.

Materials and Methods

In the present study was a descriptive study done from January 2025 to March 2026 for a period of one year and three month in Meenakshi Medical College Hospital and Research Institute, Enathur, Kanchipuram, Tamil Nadu. The present study comprises in total hundred cases which includes fifty cases of gastric carcinoma patients group I and Fifty cases of Control subjects.

All cases were analyzed for their expression of P53 and HER 2 gene in this study. Gastrectomy specimens of 50 patients 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 biochemical records.

P53 Gene Expression analysis by RTPCR

RNA extraction, cDNA synthesis, and Real-Time PCR were conducted following standard protocols. RNA was extracted using the Origin Diagnostics and Research RNA Extraction Kit, utilizing the guanidine thiocyanate/phenol method with a spin column approach. The purity and concentration of the extracted RNA were assessed using a biospectrometer. Fresh blood was mixed with Buffer RZ in a 3:1 ratio, incubated at 15–30°C for 5 minutes,

followed by chloroform extraction, and centrifuged at 12,000 rpm for 10 minutes at 4°C. The aqueous phase was transferred, mixed with ethanol, and processed through a spin column system before RNA was eluted using RNase-free water.

For cDNA synthesis, 50 µg of RNA was combined with Oligo (dT), random hexamer primers, dNTPs, RT Buffer, and Reverse Transcriptase, incubated at 25°C for 5 minutes and 50°C for 60 minutes, and inactivated at 95°C for 5 minutes. Real-Time PCR was performed in a 20 µL reaction volume containing 2X Real-Time PCR Master Mix, specific primers, cDNA, and nuclease-free water, with thermal cycling conditions including initial denaturation at 95°C for five minutes, followed by 33 cycles of denaturation at 94°C, annealing at 54°C, and extension at 72°C, each lasting 1 minute, and a final extension at 72°C for 10 minutes. Melt curve analysis was conducted to confirm the specificity of the amplified products. Relative gene expression was quantified using the 2^-ΔΔCt method, and results were graphically presented. For p53 gene analysis, specific primers were used: Forward primer 5'-CCTCAGCATCTTATCCAGTGG-3' and Reverse primer 5'-TGGATGGTGGTACAGTCAGAGC-3'. Both primers were 22 base pairs in length, with a GC content of 54.55% and a melting temperature (Tm) of 62.12°C, ensuring reliable amplification and specificity.

HER 2 gene expression by Immuohistochemical method

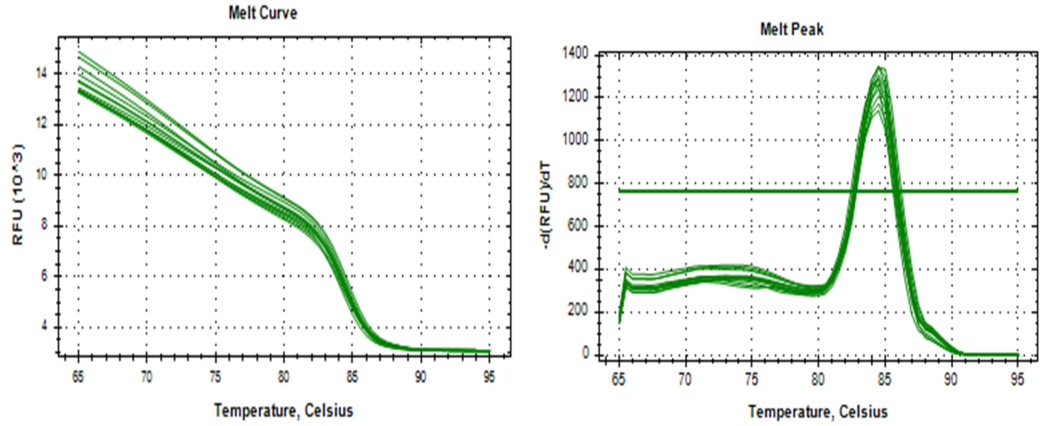
The paraffin blocks of patients who were diagnosed as Gastric carcinomas based on the endoscopy guided biopsy findings, and who underwent gastric resection at Meenakshi Medical College & Hospital , were subjected to H & E for confirmatory report and followed up with IHC. The procedure done was as follows, each section was deparaffinized with xylene , then subsequently washed with absolute and 90% alcohol solutions for about 7 mins and 2 mins respectively. Then they were washed with tap water and distilled water for a period of 5 mins each, followed by incubation after the addition of citrate buffer. The sections were then cooled to room temperature , then after washing in tap water and distilled water for 5 mins and 3 mins respectively, peroxidase block (3% H2O2) was added for 5-8 mins. After 2 changes of distilled water , the sections were washed in TBS wash buffer for a time of 10 – 15 mins. Power block was then added for 10 mins, the excess drained, followed by the addition of primary antibody [HER2 Neu monoclonal antibody BIOGENICS] for 50 minutes, which was then followed by washing in tris buffer for 5 mins twice. Then super enhancer followed by SS label each for a period of 20 mins, again washing twice in tris buffer after both super enhancer and SS label for a period of 5 mins each. Following this freshly prepared DAB chromogen was added , which was prepared by the addition of 1 ml of substrate buffer and 1 drop of DAB chromogen and the sections were washed in distilled water for 2 mins. Now finally the sections were counter stained with Haematoxylin, washed in tap water for 2 mins , air dried, cleared with xylene for 5 mins and mounted with DPX and visualized under the microscope.

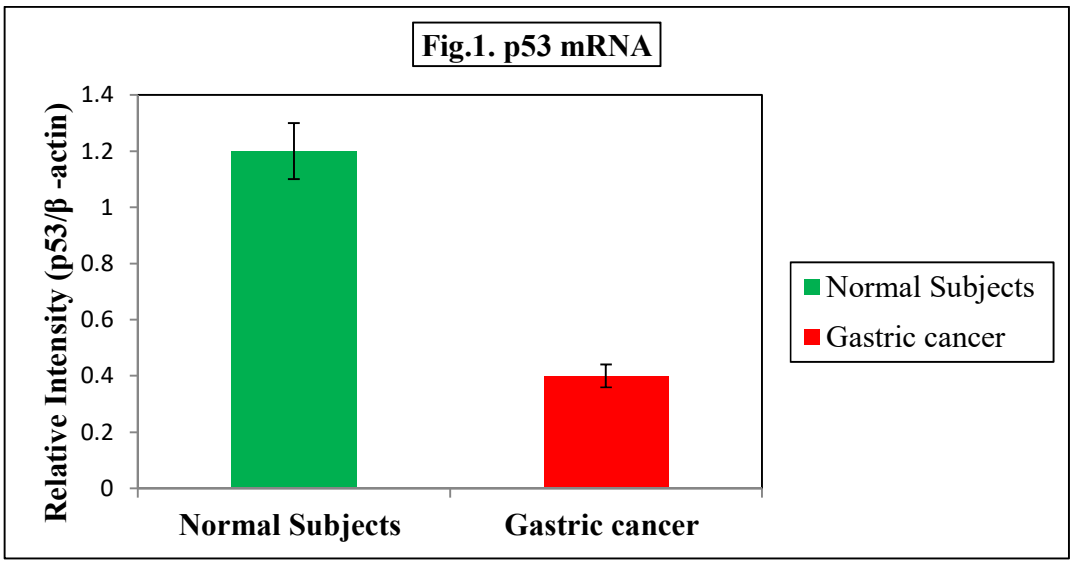
Results

P53 Gene Expression analysis by RTPCR

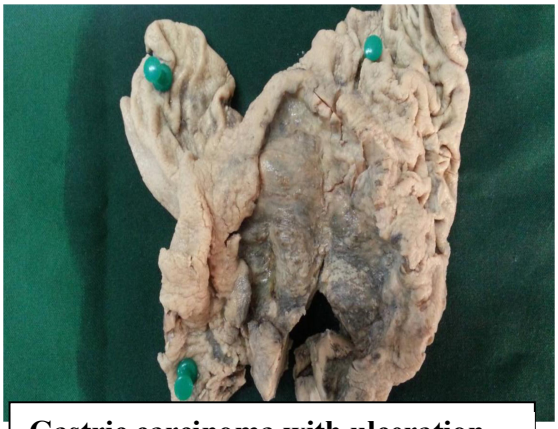
A total of 40 individuals had positive expression of the p53 gene, according to research findings, which corresponds to 80% of the patients.

The effect of p53 gene expression in serum is seen in Fig 1. In gastric cancer, we found a substantial decrease in p53 mRNA concentration and protein expression was in the current study.

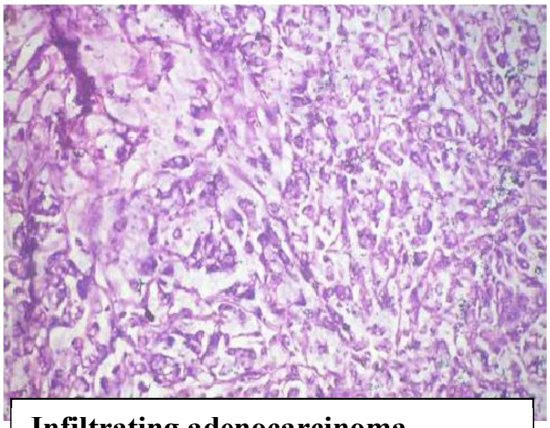




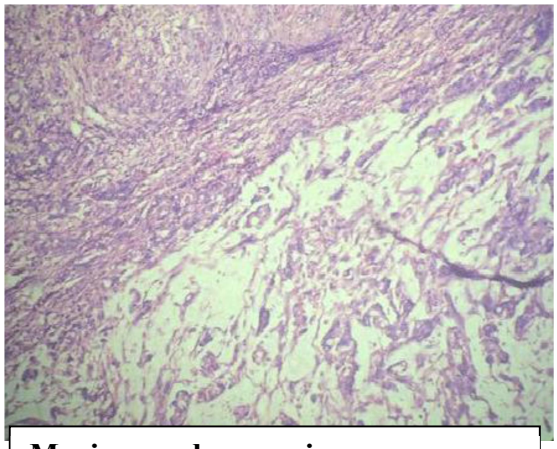
HER 2 gene expression by Immuohistochemical method



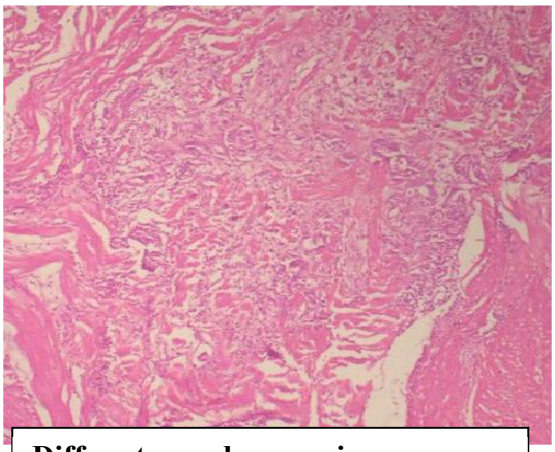
Gastric carcinoma with ulceration



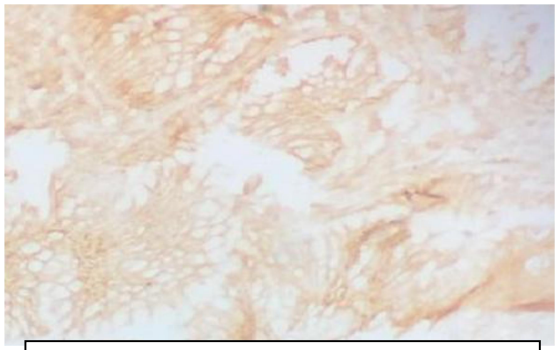
Infiltrating adenocarcinoma – intestinal type (H & E)



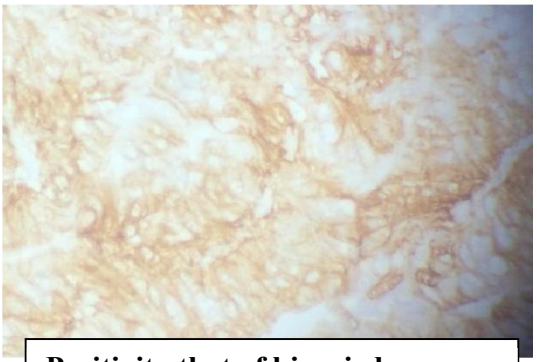
Mucinous adenocarcinoma



Diffuse type adenocarcinoma



3+ HER2 positivity (IHC) (Intestinal type)



Positivity that of biopsied material

Discussion

In many cancers, like Gastric cancer, the *P53* tumor suppressor gene is mutated. p53 is activated by genotoxic stress to facilitate DNA repair and cell cycle arrest, as well as programmed cell death. The activation of p53's tumor-suppressing activities in gastric cancer is often caused by over expression of its negative regulator, MDM2, or by mutations, which account for 30–35% of all gastric cancer cases. Patients with advanced-stage and high-grade tumors also have higher p53 mutation frequencies. The cancer stage refers to the extent or spread of the disease within the body. The staging system varies depending on the cancer type. In general, cancer is categorized into different stages, often numbered from 0 to IV, with higher numbers indicating a more advanced disease.

Numerous studies have consistently demonstrated the predictive significance of p53 across different stages of human malignancies. In early-stage malignancies, such as stage I and II, studies have shown that p53 mutations or over expression can serve as predictive markers for disease aggressiveness and poor prognosis. Higher p53 expression levels or the presence of p53 mutations in these stages are often associated with increased tumor invasiveness, higher rates of metastasis, and reduced overall survival rates for patients

Over the past five decades or more, the mortality associated with gastric cancer has decreased markedly in most areas of the world 75-80. Despite the reduced incidence and mortality, gastric cancer remains the second leading cause of cancer death worldwide. Despite a marked decline in fundic and distal tumors, there is a rising incidence of adenocarcinomas of the gastroesophageal junction and gastric cardia.

The chances of a patient surviving 5 years after diagnosis of gastric cancer are still low [5], the survival rate of patients with advanced resectable gastric or gastroesophageal junction (GEJ) cancers, however, remains poor despite new treatment strategies, such as perioperative chemotherapy1 or adjuvant chemoradiation. A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with the signalling cascades involved in cell differentiation, proliferation, and survival. In the present study " Expression of HER 2 in gastric carcinomas", we have analysed the expression of HER2 (Human epidermal growth factor 2) on a total number of 50 cases, of which 30 were resected specimens and 20 that of endoscopic guided biopsied materials .

Gastric carcinomas of different types and from different anatomical regions of the stomach were included in the study. Most of them were adenocarcinomas and the most common region was that of gastric cardia. Sections were studied under haematoxylin and eosin staining, after which the same blocks were studied for HER2 monoclonal antibody staining .They were scored according to the degree of expression of the staining reaction, all those with a score of 3+ were considered as positively expressing the HER2 antigen[6,7].

Gastric carcinomas are a heterogenous group of lesions in terms of architecture, growth pattern, cell differentiation and histogenesis. 10% of the cancers worldwide are that of gastric carcinomas Across the world, there are intriguing regional variations . Gastric cancer has a lower incidence in economically developed Western countries, and reductions in risk are reported in people who migrate from high-incidence areas such as Japan and Korea to low-incidence regions such as the USA. Cancers of the antro-pyloric region are more common in high risk region like

Asia and eastern Europe, where as in low risk regions like north America and northern Europe, tumors of the cardia are more common.

The present study highlights the critical role of epigenetic regulation of *p53* in gastric carcinogenesis. Reduced gene expression may result not only from mutations but also from promoter hypermethylation, leading to transcriptional silencing[8]. Our findings are consistent with previous studies indicating that epigenetic alterations contribute significantly to tumor progression. The high frequency of *p53* methylation in cancer tissues suggests its potential as a diagnostic biomarker.

Conclusion

In the present study we conclude that the reduced *p53,* HER2 expression, coupled with aberrant promoter hypermethylation, is significantly associated with increased gastric cancer risk. These results suggested that epigenetic regulation of *p53* and HER2 may serve as a prognostic and diagnostic biomarker for early detection and risk stratification.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
2. Hwang HJ, Nam SK, Park H, Park Y, Koh J, Na HY, et al. Prediction of *TP53* mutations by p53 immunohistochemistry and their prognostic significance in gastric cancer. J Pathol Transl Med. 2020;54:378–86.
3. Wei K, Jiang L, Wei Y, Wang Y, Qian X, Dai Q, et al. The prognostic significance of p53 expression in gastric cancer: a meta-analysis. J Cancer Res Clin Oncol. 2015;141:735–48.
4. Palanikumar, L. et al. Protein mimetic amyloid inhibitor potently abrogates cancer-associated mutant p53 aggregation and restores tumor suppressor function. *Nat. Commun.* **12**, 3962 (2021).
5. Oue N, Sentani K, Sakamoto N, Yasui W. Clinicopathologic and molecular characteristics of gastric cancer showing gastric and intestinal mucin phenotype. Cancer Sci. 2015;106:951–8.
6. Ahmed A, Al-Tamimi DM. Incorporation of p-53 mutation status and Ki-67 proliferating index in classifying Her2-neu positive gastric adenocarcinoma. Libyan J Med 2018;13(1):1466573.
7. Ahn S, Hong M, Van Vrancken M, Lyou YJ, Kim ST, Park SH, et al. A nCounter CNV assay to detect HER2 amplification: a correlation study with immunohistochemistry and in situ hybridization in advanced gastric cancer. Mol Diagn Ther 2016;20(4):375-83.
8. Simpong DL, Asmah RH, Krampah C, Akakpo PK, Adu P, Asante DB, et al. HER-2 protein overexpression in patients with gastric and oesophageal adenocarcinoma at a tertiary care facility in Ghana. ScientificWorldJournal 2018;2018:1564150.