Immunohistochemical Expression Of CDX-2 In Gastric Adenocarcinoma And Its Clinico Pathological Correlation

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KEYWORDS

CDX-2, Gastric adenocarcinoma, Immunohistochemistry

ABSTRACT

Introduction: The fifth most frequent cause of cancer-related mortalities globally is gastric cancer. Histologically, adenocarcinoma is the most prevalent form. CDX-2, a transcription factor associated with the caudal related homeobox, is typically found in intestinal metaplasia and gastric cancer (intestinal type). Role of CDX-2 in gastric carcinoma as prognostic variable is not well established.
Aim: The aim of the present study was to analyse CDX-2 expression in different histological types of gastric adenocarcinomas and its relationship with the patients' clinicopathological parameters.
Materials and Methodology: A cross-sectional study was performed using paraffin blocks of gastric adenocarcinoma reported between 2017 and 2024. For light microscopy screening, sections were initially stained with haematoxylin and eosin (H&E) stains. In further sections, primary antibody against CDX-2 was applied. The CDX-2 expression level in gastric adenocarcinoma was evaluated using a scoring system.
Results: Fifty cases with gastric adenocarcinoma were included. Among which only histological type (p-0.027) and histological grade (p-0.000) were statistically significant. Increased CDX-2 expression correlated with higher proportion of intestinal type gastric carcinomas while decreased CDX-2 expression correlated with higher histological grade of tumour.
Conclusion: In conclusion, CDX-2 is a crucial marker for the intestinal type of gastric adenocarcinoma. CDX-2 expression showed statistical significance only with the histological type and grade of the tumour. Although not statistically significant, lower levels of CDX-2 positivity were associated with greater depth of invasion and a higher rate of lymph node metastasis, suggesting that CDX-2 immunoexpression may serve as a significant prognostic marker in gastric adenocarcinoma.

1. Introduction:

The fifth most frequent cause of cancer-related fatalities globally is gastric cancer. In 2020, there were 10,89,103 instances of stomach cancer worldwide, or 14 cases per 1,000,000 people; in India, the prevalence is 4.4 cases per 1,000,000 people. Male incidence rates are double those of females. According to the Indian Council of Medical Research's (ICMR) National Cancer Registry Program, the stomach ranks second for males and fourth for females in Chennai in terms of cancer incidence [1,2].

Biologically and genetically diverse, gastric carcinomas are malignant epithelial neoplasms of the stomach that have a variety of environmental and genetic aetiologies [3,4]. The Lauren classification, which is still in use today, separates gastric cancer into two main histological groups based on growth pattern and microscopic configuration: intestinal and diffuse [4]. Diffuse carcinomas are composed of poorly cohesive cells with little to no gland formation, whereas intestinal type carcinomas generate glands with varying degrees of differentiation [5]. Intestinal metaplasia and gastritis are precursor lesions of gastric carcinomas. Intestinal metaplasia in the stomach and a higher chance of developing gastric cancer, primarily of the intestinal type, are linked to both autoimmune gastritis and Helicobacter pylori (H. pylori)-induced gastritis [3].

The expression of the caudal associated homeobox transcription factor CDX 2 is often limited to the intestinal epithelium in adults. It has a role in intestinal mucosa growth and maintenance [6]. The colon and caecum have the highest quantities of CDX 2 mRNA, while other intestinal tracts have lower levels. The stomach does not express CDX 2 mRNA [7]. While its function as a prognostic marker for colorectal carcinomas is well established, its impact on the prognosis of gastric carcinomas is yet unknown. Approximately 90% of cases of intestinal metaplasia in the gastric mucosa express CDX2 positivity, whereas only 50% of cases of gastric carcinomas do so [8,9]. Better outcomes and a less

aggressive form of gastric carcinoma were observed in patients with CDX-2 positive expression. In order to predict the prognosis of gastric carcinomas, patients with CDX-2 positive expression had a greater survival rate than those with CDX-2 negative expression (p=0.038) [10].

Hence, the present study was proposed to analyse CDX2 expression in different histological types of gastric adenocarcinomas and their relationship with the patients' clinicopathological parameters. Fewer than five studies, to the best of current knowledge, have investigated CDX-2 expression in stomach cancer within the South Indian population.

2. Materials And Methods

The current study was conducted between October 2023 and September 2024, spanning a full year. It is an observational study conducted at a hospital using a cross-sectional study design. Fifty patients who had total or subtotal gastrectomy between 2017 and 2024 were included in the study.

3. Inclusion And Exclusion Criteria

All gastrectomy cases both total and subtotal gastrectomy, reported as gastric adenocarcinoma were included. Non-neoplastic lesions of the stomach, Gastric tumours other than adenocarcinoma and cases with extensive tumour necrosis were excluded.

4. Staining Techniques

All gastrectomy specimens were received in 10% buffered formalin. Proper fixation, grossing, tissue processing, and section cutting were done per standard protocol. For histopathological examination (HPE), the sections were stained by haematoxylin and eosin (H&E). For old cases, patient details were collected from the case sheets. Tissue paraffin blocks and H&E-stained slides were obtained from the department archive. For CDX-2 IHC staining, additional sections were cut from the paraffin block of tissue and were taken on a glass slide coated with adhesive aminopropyltriethoxysilane (APTES). Primary antihuman antibody against CDX-2 (rabbit monoclonal antibody from path-in-situ) was used. Nuclear expression was considered positive for CDX-2 in tumour cells.

5. Scoring Method

The positively stained tumour cells were scored at ×400 magnification. Semi-quantitative estimation of CDX-2 expression was done. The percentage of tumour cells with distinct nuclear immunopositivity was used to score CDX-2 expression. Colon adenocarcinoma was taken as positive control. The CDX-2 scoring results were calculated as follows:

- Score 0: 0%-5% positive tumour cells.
- Score 1: >5%- 35% positive tumour cells.
- Score 2: >35%-65% positive tumour cells.
- Score 3: >65% positive tumour cells.

Samples with score 0 were considered negative [11].

6. Statistical Analysis

Data was entered into Microsoft excel datasheet and was analysed using SPSS Statistics version 23.0. Pearson's Chi-square test was done to study the correlation of different parameters. A p-value less than 0.05 was considered statistically significant.

7. Result

With a mean age of 59.6 years (range from 39 to 79 years), the 50 cases of stomach adenocarcinomas in the study included 40 males and 10 females. Most of the patients (42 cases, 84.0%) did not follow a vegetarian diet. Most patients were smokers (38 cases, 76.0%).

The Lauren classification was used to histologically categorise the stomach carcinomas into intestinal,

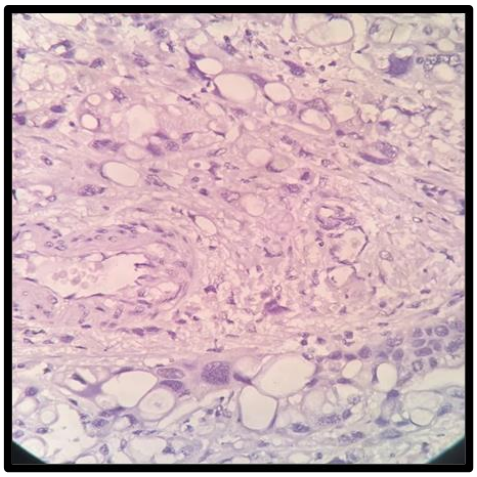
diffuse, or mixed kinds. There were thirty-two intestinal types, fifteen diffuse types, and three mixed types among them.

Based on the percentage of glandular differentiation, the forms were further histologically categorised into well, moderate, and poorly differentiated forms. Twenty-four poorly-differentiated cases (48%), seven well-differentiated cases (14%), and nineteen moderately-differentiated cases (38%).

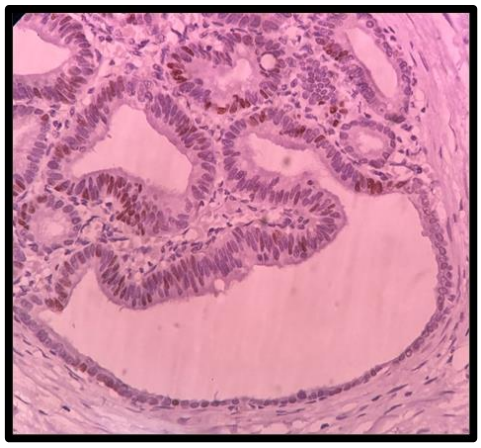
In total 16 cases showed extension up to serosal layer out of which 8 were intestinal type (50%) and 8 were of diffuse type (50%). Lymph vascular invasion (LVI) was seen in 8 cases of diffuse type and 18 cases of intestinal type and 2 cases of mixed type. While perineural infiltration (PNI) was seen in 7 cases of diffuse type and 18 cases of intestinal type and 2 cases of mixed type.

Only five cases of diffuse type exhibited CDX-2 positivity of score 1, while twenty-four of the thirty-two intestinal type patients displayed CDX-2 immunopositivity [Table/Fig-2-4]. A statistically significant ($p < 0.027$) negative immunoreactivity for CDX-2 was seen in the remaining 10 cases of diffuse type carcinomas [Table/Fig-1].

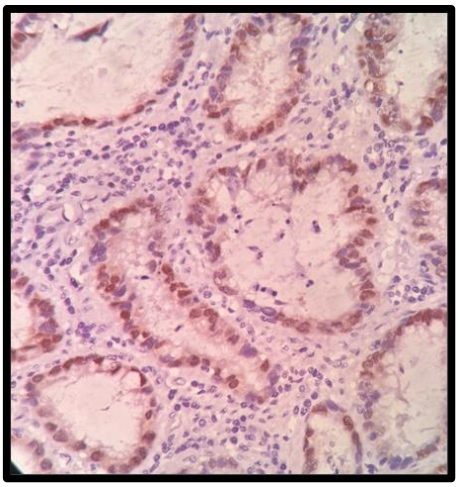
Lymph node involvement was associated with grade of CDX-2 immunoreactivity, LVI, and PNI. Of the 38 cases with lymph node involvement, 16 were immunonegative for CDX2, while only 2 cases with CDX2 score 3 were positive for nodal metastasis; however, this was not statistically significant ($p = 0.469$). The majority of the cases that were immunonegative for CDX2 had LVI ($p = 0.06$) and PNI ($p = 0.335$), though this was not statistically significant.



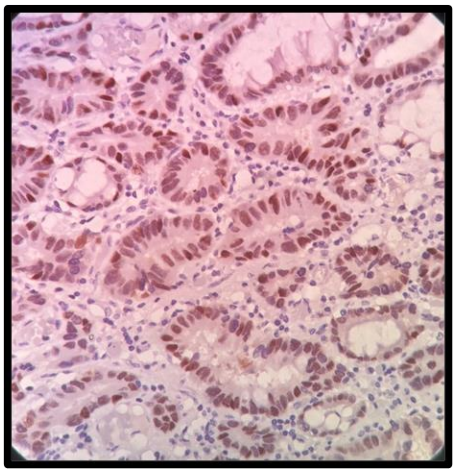
[Table/Fig-1]: CDX-2 showing ≤5% nuclear staining in tumour cells- Score 0. (IHC x 400)



[Table/Fig-2]: CDX-2 showing 6-35% nuclear staining in tumour cells- Score 1. (IHC x 400)



[Table/Fig-3]: CDX-2 showing 36-65% nuclear staining in tumour cells- Score 2. (IHC x 400)



[Table/Fig-4]: CDX-2 showing >65% nuclear staining in tumour cells- Score 3. (IHC x 400)

8. Discussion

One of the leading causes of cancer-related mortality in the world is gastric carcinoma. More often, these cancers are being detected and prognosticated using newer criteria and markers. In those under 30, gastric carcinomas are uncommon, and as people age, their prevalence gradually rises [4].

It is commonly known that CDX-2 is a prognostic factor for colorectal tumours. Its function as a prognostic indicator for other carcinomas, such as gastric carcinoma, is unclear, albeit [4]. In their investigation, Saha et al. discovered a male to female sex ratio of 2.7:1 and a median age of 55 years [12]. However, Janssen et al.'s investigation revealed no gender disparity in the prevalence of diffuse gastric cancer. On the other hand, men were more than twice as likely as women to have intestine type carcinomas [13]. The male to female ratio in this study was 4:1, and the mean age was 59.64 years.

Gastric cancers are closely linked to environmental variables. The two most significant risk factors, other from H. pylori infection, are tobacco use and dietary choices. In their research, Machida Montani et al. discovered a substantial correlation between smoking and noncardiac stomach carcinomas and H. pylori infection [14]. Additionally, Praud et al. discovered that smoking increased the risk of cardia gastric cancer by a little amount when compared to noncardia gastric cancer [15]. About 76.0% of the cases in this study smoked, and 84.0% of the cases had a nonvegetarian diet.

A small number of studies claim that a positive prognosis is associated with CDX2 expression [11]. In the current study, 60% of patients are ulcero-infiltrative, irrespective of their gross appearance only 40% cases have been CDX-2 negative.

In their research on gastric carcinomas, Henson et al. [17] and Wu et al. [16] discovered that the diffuse

type of gastric carcinoma increased steadily with age. However, Saha et al. [12] hypothesised that the intestinal subtype was substantially more prevalent in older adults than in younger adults. The vast majority of the intestinal cases in this investigation were poorly differentiated.

In their investigation, Cambruzzi et al. discovered that lesions categorised as T3 and N1 were more common [18]. This conclusion is comparable to present study, which found that T3 lesions predominated in 70% of instances of advanced gastric cancer.

According to Liu et al., the survival rate is shorter in cases where lymphatic invasion was found, making the presence of LVI a significant prognostic indicator for stomach malignancies without lymph node metastases.[19] According to present study, LVI and PNI were discovered in 16% and 14% of diffuse type carcinomas, respectively, but LVI and PNI were detected in 36% and 46% of intestinal type, respectively. In all, 76% of patients exhibit lymph node involvement, with intestinal type cases accounting for 46% and diffuse type cases for 24%. In the present study also it was found that 16 cases with a CDX-2 score of 0, associated with lymph node involvement whereas only 2 cases with CDX-2 score of 3 were associated with lymph node involvement, but this was not statistically significant($p>0.05$).

In their research, Kim et al. [20] and Halder et al. [4] discovered a correlation between a higher percentage of intestinal type malignancies and elevated CDX2 expression. However, there was no statistically significant correlation between CDX2 expression and the histological type of tumour, according to Pranjali et al. [11]. The majority of intestinal type cases in this investigation tested positive for CDX2. Only five diffuse-type instances had focal CDX2 positivity. Strong CDX2 expression has been found to be positively correlated with intestinal type gastric adenocarcinoma ($p = 0.027$).

This study was conducted in a small cohort. Hence, subsequent studies with large cohort that examine the relationship between CDX2 expression and cancer-specific 5-year survival rates are necessary for a conclusive evaluation of CDX2's prognostic significance in gastric carcinoma.

9. Conclusion

In conclusion, increased CDX-2 expression showed a statistically significant correlation with the intestinal histological type and lower tumour grade. Although not statistically significant, lower CDX-2 expression was associated with greater depth of invasion as well as higher rate of lymph node metastasis, suggesting that CDX-2 immunoexpression may serve as a valuable prognostic marker in gastric adenocarcinoma.

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