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Immunohistochemical analysis of cdx2 and its correlation with histopathological parameters of gastric enteric and colorectal epithelial malignancies

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ABSTRACT

Background: Gastrointestinal tract cancers have become the leading causes of death worldwide. In India, the incidence of gastrointestinal carcinomas is increasing due to urbanization, change in food habits and life style. According to National Cancer Registry, gastro-intestinal carcinomas are more common in men than women and more commonly seen in elderly age group. CDX2 is a caudal type Homeo-box gene, encoding a transcription factor that plays an important role in differentiation, proliferation, cell adhesion and migration. CDX2 is often deregulated in cancer and might have oncogenic and tumour suppressor potential.

Objectives: 1) To know the expression of CDX2 in gastric, enteric and colo-rectal epithelial. malignancies. 2) To observe and analyse the staining pattern in various grades and stages of tumour.

Materials and Methods: The resected specimens of gastric, enteric and colo-rectal carcinomas were collected from the Department of Pathology, Mysore Medical College and Research Institute, during the year December 2019 to May 2021. Standard protocol for grossing and histopathological techniques were followed by immunohistochemical staining with CDX2 antibody. Expression of CDX2 marker and its staining pattern in various grades and stages of tumour were recorded and compared with patient's clinicopathological parameters.

Results: A total of 67 cases of Gastrointestinal carcinomas were taken for the study. Positive CDX2 expression was seen in 58 out of 67 cases but the intensity of expression varied. There was significant statistical correlation between the CDX2 expression and histopathological grade (p value <0.05).

Conclusion: The present study showed consistent expression of CDX2 in gastrointestinal carcinomas. The CDX2 expression decreased with increase in grade of the carcinoma.

Therefore, CDX2 can be used as one of the prognostic indicators in intestinal variants gastrointestinal carcinoma.

1. Introduction

The digestive tract is a major site of cancer in humans. Gastric cancer is one of the leading cause of cancer deaths globally. India has a low incidence of gastric cancer compared to the developed countries.[1] Helicobacter pylori infection is considered as the major risk factor for development of gastric carcinoma.

Few studies highlight that CDX2 immunohistochemistry negativity is an independent prognostic factor and indicates worse survival rate.[2] Currently, tumor stage, tumor grade, and microsatellite instability remain the most important prognostic variables that aid in treatment of patients with early-stage cancer. Microarray-derived gene-expression signatures from stem cells and progenitor cells play a significant role but are difficult to translate into clinical tests. Hence, it has proved difficult to identify a

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single prognostic biomarker that is also predictive of benefit from adjuvant chemotherapy.

The treatment for colorectal cancer is multidisciplinary which includes surgery, chemotherapy and radiotherapy. The treatment modality is also based on molecular studies in familial cases. Prognostic biomarkers are key to the risk stratification of patients with gastric, intestinal and colon cancer and the decision to recommend adjuvant chemotherapy, especially in patients with early-stage disease. Few western studies have proved that CDX2-negative tumors are associated with a lower rate of disease-free survival than CDX2-positive tumor. This effect was independent of many known risk factors, including pathological grade and stage.

In Indian literature, very few studies have been done on CDX2 expression and its correlation with clinico-pathological and prognostic significance of the cancers. This heterogeneity of expression is attributed to the different detection techniques and immunohistochemical testing is one of the method to assess CDX2 expression. Our study aims to look at the prevalence of CDX2 immunohistochemistry expression in gastric, intestinal and colon cancers and its correlation with histomorphological parameters.

2. Materials and Methods

Study was done in the Department of Pathology, Mysore Medical College and Research Institute in KR Hospital, Mysore, during the period of December 2019 to May 2021 (18 months). All types gastric, enteric and colorectal epithelial carcinomas were included in the study. In every case standard protocol for surgical grossing of specimens was followed. After conventional processing, paraffin sections of 5μm thickness were stained by haematoxylin and eosin for histopathological study. In addition, 4μm sections was cut from the paraffin block of tumor tissue and was taken on the glass slide coated with Poly-L-Lysine (PLL) for immunohistochemistry to detect CDX2 expression.

The tumors were categorized according to the WHO 2010 classification. Staging was done according to TNM staging. Histological types and grade of the tumors were also determined.

Evaluation of CDX2 expression

The criterion for a positive immune reaction was a brown nuclear expression. A three scaled grading system was chosen for assessing the CDX2 expression.

Score 0 = no staining or nonspecific staining of tumor cells.

Score 1+ = 0-25% staining of the tumour cells.

Score 2+ = 26-75% staining of tumor cells.

Score 3+ = >75% staining of tumor cells.

The differences in frequency of expression between various subgroups were tested for statistical significance by employing chi square test. p value < 0.05 was considered statistically significant.

3. Results

A total of 67 cases of Gastric, enteric and colo-rectal adenocarcinomas were taken for the study.

In present study, most common site of GI cancer was found to be colon and rectum followed by stomach and small intestine being the least.(Table 1)

Table 1: Distribution of gastro-intestinal cancers based on anatomical site

Site of carcinoma	Number of cases	Percentage
Stomach	15	22.4
Small intestine	4	6
Colon and Rectum	48	71.6
Total	67	100

In the present study, out of 67 cases of GI cancers 27 cases (40%) were well differentiated, majority of them 28 cases (42%) were moderately differentiated and 12 cases (18%) were poorly differentiated cancers.(Table 2)

Table 2: Distribution of gastro-intestinal cancers based on histopathological grade of differentiation

Grade of differentiation	Number of cases	Percentage
Grade I/well differentiated	27	40
Grade II/moderately differentiated	28	42
Grade III/Poorly differentiated	12	18
Total	67	100

Table 3: Distribution of gastro-intestinal cancers based on TNM staging

TNM stage	Number of cases	Percentage
Stage I	16	24
Stage II	28	42
Stage III	21	31
Stage IV	2	3
Total	67	100

In our study, we found that majority of GI cancers 28 (42%) belonged to TNM stage II, followed by 21 cases (31%) belonging to TNM stage III, 16 cases (24%) were categorised under TNM I and only 2 cases (3%) were categorised under TNM stage IV category.(Table 3)

In the present study we found 3+ grading intensity of CDX2 expression pattern in majority 34 cases (51%) of GI cancers, followed by 2+ grading pattern in 16 cases (24%), 11 cases (16%) showed 1+ grading and only 6 cases (9%) of GI cancers showed negative staining pattern.(Table 4)

Table 4: Distribution of gastro-intestinal cancers based on grading of CDX2 expression

Grading of CDX2 expression	Number of cases	Percentage
0 (negative)	6	9%
1+	11	16%
2+	16	24%
3+	34	51%
Total	67	100%

Table 5: Correlation of CDX2 expression pattern with WHO tumour grade

WHO grade	CDX2 expression positive	CDX2 expression negative
Grade I	26	1
Grade II	25	3
Grade III	7	5
Total	58	9

Out of 67 total cases of GI cancers, 58 cases (87%) showed positive expression of CDX2 and 9 cases (13%) showed negative expression. Among positive CDX2 expression cases majority of them showed grade I WHO differentiation followed by grade II differentiation and grade III tumour differentiation were least common. (Table 5)

Out of 9 cases of negative CDX2 expression, majority of them were grade III differentiated, followed by grade II and grade I differentiated tumours were least common among them.

Table 6: Correlation of CDX2 expression pattern with tumour TNM staging

TNM staging	CDX2 expression positive	CDX2 expression negative
Stage I	15	1
Stage II	25	3
Stage III	17	4
Stage IV	1	1
Total	58	9

Out of 58 cases of positive CDX2 expression, majority of them were under stage II TNM staging, followed by state III, stage I and stage IV cancers were least common among them. Among 9 cases of negative CDX2 expression, stage III cancers were more common.(Table 6)

4. Discussion

The positivity of CDX2 expression associated with various GI carcinomas in present study was compared with other studies. In Gastric adenocarcinomas, present study showed majority cases were CDX2 positive with positivity rate being 73%. Similar results were seen in study conducted by Estrada-Munoz et al.[3] with positivity rate being 68%. While in other studies conducted by Halder et al.,[4] and Acenero et al.[5] the positivity rates were 56% and 40% respectively.

In Enteric adenocarcinomas, present study shows positivity rate of 50%. While in other studies by Mizoshita et al.,[6] Overman MJ et al.[7] and Zhang et al.[8] positivity rates were found to be 73%, 70% and 60% respectively.

In Colorectal adenocarcinomas, our study shows a CDX2 positivity rate of 93% which is similar to study conducted by Werling et al.[9] and Neumann et al.[10] showing CDX2 positivity rates of 98% and 97% respectively. While in studies conducted by El-Rafaey et al.[11] the positivity rate was 81%.

In present study, the intensity of CDX2 expression in gastrointestinal carcinoma was evaluated by applying the scoring system, similar scoring system was applied by the studies mentioned above. (Table 7)

In gastric adenocarcinomas, present study shows majority cases with 2+ grading which is similar to the study by Harras HF et al.[12] While in study conducted by Halder et al.[4] majority of cases shows 3+ CDX2 grading followed by grade 2+.(Table 8)

In Enteric adenocarcinoma, present study showed majority cases with 1+ CDX2 expression. While in study conducted by Zhang et al.[8] majority cases showed 3+ grading of CDX2 expression.

In colorectal adenocarcinoma, present study showed 31 cases with 3+ grading of CDX2 expression which is similar to study conducted by Nayak et al.[13] While in study conducted by Mesina et al.[14] majority cases showed 2+ grading of CDX2 expression.

However, there were not many studies found, which correlated CDX2 expression pattern with various histopathological parameters like histological grade and stage of the carcinoma. In our study, parameters like histological grade and stage were compared with CDX2 expression patterns in Gastric, enteric and colorectal carcinomas.

In gastric carcinomas, out of 15 cases 2 were negative for CDX2 staining. Out of the remaining 13 cases, it was found that there was no significant correlation between CDX2 expression and histopathological grade and stage of gastric adenocarcinomas.

In Enteric carcinomas, out of 4 cases 1 was negative for CDX2 staining. Out of remaining 3 cases, it was found that there was no significant correlation between CDX2 expression and histopathological grade and stage of enteric adenocarcinomas.

In colorectal carcinomas, out of 48 cases 3 cases were negative for CDX2 expression. Out of remaining 45 cases, there was significant negative correlation between the grading of CDX2 expression and the differentiation of colorectal adenocarcinomas with higher grade cancers showing lower grading of CDX2 (p-value=<0.05) which was similar to study conducted by J Brunn et al.[15]

Table 7: Comparison of CDX2 expression pattern in various gastrointestinal carcinomas

S. No.	Study	No. of cases	CDX2 Positive	CDX2 Negative	Percentage positivity
Gastric					
1	Halder et al	50	28	22	56
2	Estrada-Munoz et al	92	63	29	68
3	Acenero et al	57	23	34	40
4	Present study	15	11	4	73
Enteric					
1	Overman MJ et al	54	38	16	70
2	Zhang et al	30	18	12	60
3	Mizoshita et al	86	63	23	73
4	Present Study	4	2	2	50
Colorectal					
1	Werling et al	75	74	1	98
2	Neumann et al	503	489	14	97
3	El-Refaey et al	43	35	8	81
4	Tahir et al	125	112	13	90
5	Present study	48	45	3	93

Table 8: Comparing CDX2 grading in gastrointestinal carcinomas

S. No.	Study	No. of cases	CDX2 grading			
			0+	1+	2+	3+
Gastric						
1	Halder et al	52	21	1	12	15
2	Harras HF et al	50	14	10	17	9
3	Present study	15	2	4	6	3
Enteric						
1	Zhang et al	30	12	-	4	14
2	Present study	4	1	2	1	0
Colorectal						
1	Mesina et al	82	13	2	37	30
2	Nayak et al	38	1	7	12	18
4	Present study	48	3	5	9	31

While there was no significant correlation between grading of CDX2 expression and differentiation of colorectal carcinomas.

5. Conclusion

CDX2 protein plays a vital role in development and differentiation of epithelial cells in Gastro-intestinal tract. There was decreased expression of CDX2 in higher grades and stages of tumour, also we found reduced expression in upper GI adenocarcinomas compared to colorectum. Therefore, CDX2 can be used to differentiate upper and lower GI malignancies. Hence, detection of CDX2 expression can be important in determining the prognosis and treatment outcomes since higher grade tumours have poor outcome.

For future importance of CDX2 as a biomarker for gastrointestinal malignancy with clinical relevance, extensive research is necessary to assess the major functions of CDX2 in tumour progression and metastasis. In view of available data, CDX2 expression in GI cancer is likely to become an essential prognostic indicator and also a diagnostic tool.

6. Source of Funding

None.

7. Conflict of Interest

None.

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