CDX2 EXPRESSION IN GASTROINTESTINAL TUMOURS

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ABSTRACT

BACKGROUND
CDX2 is a nuclear homeobox transcription factor that belongs to the caudal-related family of CDX homeobox genes. The gene encoding CDX2 is a nonclustered hexapeptide located on chromosome 13q12-13. Homeobox genes play an essential role in the control of normal embryonic development. CDX2 is crucial for axial patterning of the alimentary tract during embryonic development and is involved in the processes of intestinal cell proliferation, differentiation, adhesion and apoptosis.[1] It is considered specific for enterocytes and has been used for the diagnosis of primary and metastatic colorectal adenocarcinoma.

MATERIALS AND METHODS
This is a prospective study done at a tertiary hospital over a 2 year period from 2014 to 2016. 115 surgically resected and biopsy specimens of intestinal lesions of this period were included in the study.

RESULTS
Demographic data, clinical details and histopathology of these subjects were studied. Routinely processed formalin fixed paraffin embedded serial section of 3µm was subjected to immunohistochemistry with CDX2.

CONCLUSION
CDX2 could be used in conjunction with other intestinal tumour markers to enhance the prognostic utility in these tumours. Hence, CDX2 should always be used as a part of a broader immune histochemical panel.

KEYWORDS
CDX2, Gastrointestinal Tumours.

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BACKGROUND

CDX2 is a nuclear homeobox transcription factor that belongs to the caudal-related family of CDX homeobox genes. CDX2 is crucial for axial patterning of the alimentary tract during embryonic development and is involved in the processes of intestinal cell proliferation, differentiation, adhesion and apoptosis.[1,2] It is considered specific for enterocytes and has been used for the diagnosis of primary and metastatic colorectal adenocarcinoma.[3] By binding to enhancer sequences in the promoter regions of target genes, CDX2 triggers transcription of genes that encode intestine-specific proteins.[4]

Aim and Objective- To study the expression of CDX2 in gastrointestinal tumours and to evaluate its utility as a prognostic marker for gastrointestinal tumours.

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MATERIALS AND METHODS

This is a prospective cohort study conducted in a tertiary hospital over a period of 2 years from 2014 to 2016. 115 surgical specimens obtained at biopsy or surgical resection of gastrointestinal malignancies based on the clinical symptoms were analysed individually. The data collection was performed by chart review. The following are the-

Inclusion Criteria

1. Any age group or gender.
2. Histopathologically-diagnosed gastrointestinal malignancies.

Exclusion Criteria

1. Neoplasms other than gastrointestinal.
2. Specimens with inadequate immunohistochemical staining for analysis purposes.

Patient data including age, gender, ethnicity, clinical symptomatology and specific diagnosis were collected. Specimen details included the site of the specimen and histological differentiation. The specimens were indentified and processed using formalin-fixed, paraffin-embedded serial sections and subjected to immunohistochemistry. All the individual specimens were further analysed for presence

or absence of CDX2 expression, this was then correlated with the differentiation of the tumour correlating with histomorphological findings to identify a positive or negative correlation. The results were compared with the literature.

IHC Results

- Positive - Brown stain in nuclei are considered positive.
- Tumour cells with positive nuclear staining was considered positive.
- Control - Intestinal epithelium.

RESULTS AND OBSERVATION

Gender	Number of Cases	Percentage
Male	69	60%
Female	46	40%
Table 1. Distribution According to Gender		

Out of 115 cases, 69 (60%) were males and 46 (40%) were females.

Age Group	Number of Cases	Percentage
21-30 yrs.	6	5.2%
31-40 yrs.	17	14.7%
41-50 yrs.	18	15.7%
51-60 yrs.	22	19%
61-70 yrs.	42	36.5%
71-80 yrs.	10	8.7%
Table 2. Distribution According to Age		

Histological Type	Number of Cases	Percentage
Colorectal	38	33%
Oesophageal	29	25.2%
Gallbladder adenocarcinoma	6	5.2%
Gastric	37	32%
Gastrointestinal neuroendocrine tumour	1	0.8%
Pancreatic carcinomas	4	3.5%
Table 3. Histological Distribution of Cases		

Type of Carcinoma	Number of Cases	CDX2 Grading
Oesophageal	29	
Barrett's oesophagitis	4	3+
Squamous cell carcinomas	20	0
Adenosquamous carcinomas	3	2+
Adenocarcinomas	2	3+
Gastric (Laurens classification)	37	
Intestinal metaplasia	4	2+
Intestinal type	13	3+
Diffuse type	20	0
Colorectal	38	
Well differentiated	15	4+
Moderately differentiated	18	4+
Poorly differentiated	5	0
Pancreatic adenocarcinoma	4	2+
Gallbladder adenocarcinoma	6	2+
Neuroendocrine tumours	1	0
Table 4. Distribution of Cases and their Nuclear Staining		

Type of Carcinoma	CDX2 Positive Cases
Oesophageal	9/29
Gastric	17/37
Colorectal	33/38
Gallbladder	4/6
Neuroendocrine	0/1
Pancreatic adenocarcinoma	4/4
Table 5. CDX2 Expression in Different Carcinomas	

IHC STAINING

IHC staining was graded 0 to 4+ based on nuclear staining for CDX2.

Nuclear staining was considered positive and scored on the following basis*.

Grade	CDX2 Staining
0	No detectable staining
1+	<25% positive cells
2+	25-49% positive cells
3+	50-74% positive cells
4+	>75% positive cells
Table 6. IHC Staining	

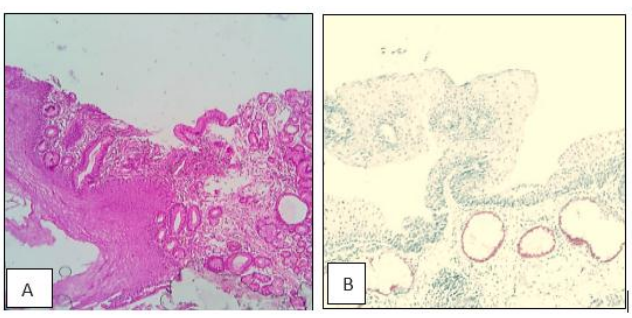

Figure 1. A and B Barrett's Oesophagus - CDX2 Expression (3+)

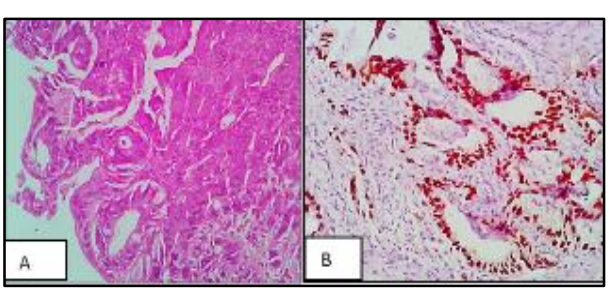

Figure 2. (A and B). Gastric Carcinoma - Intestinal Type - CDX2 Expression (3+)

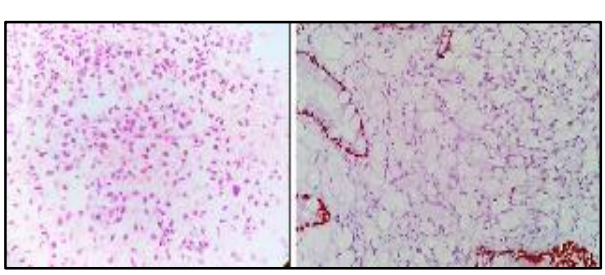

Figure 3. Gastric Carcinoma- Diffuse Type - CDX2 Expression (0) Grade

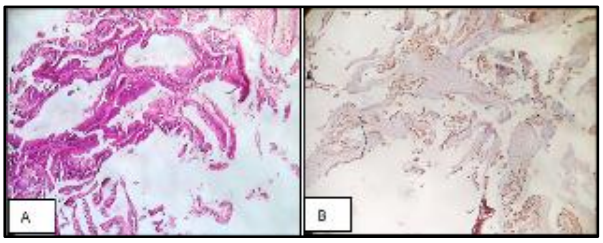

Figure 4 (A and B). Colorectal Carcinoma - Well-Differentiated Papillary Adenocarcinoma CDX2 Expression (4+)

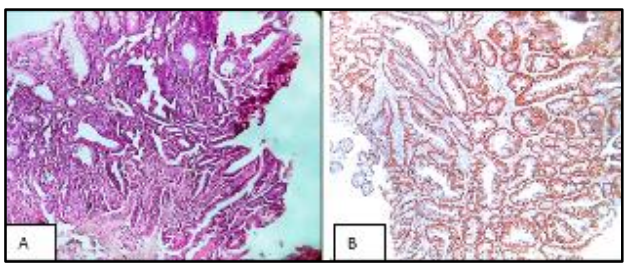

Figure 5 (A and B). Colorectal Carcinoma - Moderately-Differentiated Papillary Adenocarcinoma - CDX2 Expression (4+)

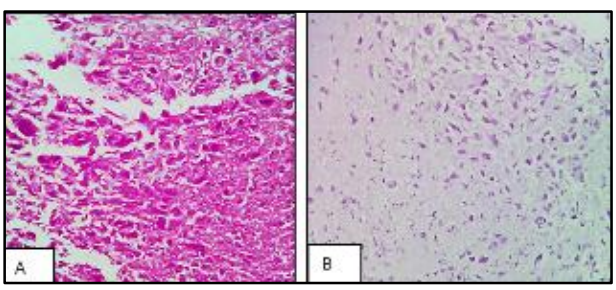

Figure 6 (A and B). Colorectal Carcinoma- Poorly-Differentiated Papillary Adenocarcinoma - CDX2 Expression (0 grade)

DISCUSSION

CDX2 being a transcription factor shows a nuclear immunostaining pattern. Nuclear expression of transcription factors has several distinct advantages over cytoplasmic "differentiation" markers.[5] Firstly, transcription factors generally yield an "all or none" signal with the vast majority of positive cases containing positive signal in more than 90% of the target cell population.[6] Secondly, the nuclear localisation of the signal is much less likely to be confused with biotin or other sources of false-positive cytoplasmic signals. Third, there is no association between the levels of expression of nuclear transcription factors and the state of differentiation of the tumour.[6,7]

Findings in the Present Study- Clinically, most of the patients in this study presented with the complaints of difficulty in swallowing, pain abdomen, altered bowel habits, per rectal bleeding, weight loss, etc. with variable time duration.

CDX2 Expression in Oesophageal Tumours

Benign neoplastic lesions of the oesophagus are very rare and not encountered in our present study.

Barrett's oesophagus is a complication of chronic GERD, the greatest concern in Barrett's oesophagus is that it confers an increased risk of oesophageal adenocarcinoma. CDX2 is not expressed in normal oesophageal and gastric epithelial cells, but is expressed in intestinal metaplasia of oesophagus.[8,9,10,11,12]

In the present study, 4 cases of Barrett's oesophagus were evaluated for CDX2 expression and the nuclear staining was observed as 3+.

3+ staining was also observed in the 2 adenocarcinoma cases. Adenosquamous carcinoma also presented 2+ nuclear staining. All cases of squamous cell carcinoma revealed negative CDX2 expression. CDX2 expression in present study correlated with Lord et al study.[13]

Lord et al investigated the expression of CDX2 and PITX1 in Barrett's oesophagus and associated adenocarcinomas. Negative CDX2 staining was observed in normal squamous oesophageal lining while strong 3+ nuclear staining was seen in all cases of Barrett's intestinal metaplasia, dysplasia and associated carcinoma.[10]

CDX2 Expression in Gastric Adenocarcinomas

Adenocarcinoma is the most common malignancy of the stomach. Gastric tumours with an intestinal morphology tend to form bulky tumours composed of glandular structures, while cancers with a diffuse infiltrative growth pattern are more often composed of signet ring cells.

In the present study, CDX2 expression was evaluated in 37 cases of gastric carcinomas in which 3 cases were of intestinal metaplasia, 13 were intestinal type and 21 were diffused type gastric cancers. In this study, in cases of intestinal metaplasia, CDX2 expression was reported and nuclear staining grade was +2. Increased CDX2 expression is more frequently associated with intestinal type of gastric cancer compared to diffuse types. CDX2 expression in present study correlated with Liu et al study.[14,15,16]

Liu et al inferred through his study of 70 gastric adenocarcinoma of which 45 cases were of intestinal type and 19 cases of diffuse type and other six cases of mucinous adenocarcinoma that decrease of CDX2 expression in gastric dysplasia suggests that CDX2 plays a similar anticarcinogenic role in intestinal metaplasia as it does in colon, intestinal metaplasia or dysplasia with low expression of CDX2 may serve as predictive for gastric cancer.[10,15,16]

	Total Cases	IM	Intestinal Type	Diffuse Type	CDX2 Expression in IM	Intestinal Type	Diffuse Type
Present study	37	3	13	21	3/3	12/13	0/21
Liu Q et al	70	-	45	25	30/45	30/45	13/25

Table 7. CDX2 Expression in Gastric Adenocarcinomas

CDX2 Expression in Gallbladder Adenocarcinomas

In the present study which includes 6 cases of adenocarcinomas of gallbladder, positive CDX2 immunohistochemistry nuclear positivity (grade 2+) was observed in all cases. No cases of metaplasia and dysplasia were observed in the study period.[17]

Sakamoto et al investigated the expression of CDX2 in human gallbladders with cholelithiasis and reported CDX2 expression in 92% of gallbladder intestinal metaplasias.[18]

	Total Cases	CDX2 Expression
Present study	6	4/6
Sakomato et al	103 (12 intestinal metaplasias)	11/12 (92%)

Table 8. CDX2 Expression in Gallbladder Adenocarcinomas

CDX2 Expression in Colorectal Carcinomas

Recent immunohistochemical studies have reported that CDX2 is a specific and sensitive marker for adenocarcinoma of the gastrointestinal tract, particularly colorectal adenocarcinoma.

In the present study, 38 cases of colorectal carcinomas were evaluated, of which 15 cases were well-differentiated, 18 cases were moderately-differentiated and 5 cases were poorly-differentiated. All these cases were evaluated for CDX2 expression. Well-differentiated and moderately-differentiated colorectal cancers showed strong CDX2 nuclear staining of 4+, which accounted for 87% of the total cases, which correlated with the previous studies.

Moskaluk et al[4] examined CDX2 expression in tissue microarrays containing 745.

Cancers from many anatomic sites and observed strong positive staining in 90% of colonic adenocarcinomas.

	Percentage of CDX2 Expression in the Total Cases
Present study	87%
Moskaluk et al[4]	90%

Table 9. CDX2 Expression in Colorectal Carcinomas

In identification of high-risk stage II and stage III colon cancers was done to check the use of CDX2 as a prognostic biomarker.[19] TNM staging was done to categorise the stage II and stage III colon cancers to process for CDX2 immunohistochemistry. It has been noticed in the study done by Piero Dalerba.[19]

In the present study, CDX2 expression was also evaluated in stage II and stage III colorectal carcinomas to check for the prognostication in 23 cases. The APR and colectomy specimens were staged with TNM staging system. CDX2 expression was separately analysed to evaluate the variation in each. Tumours that showed widespread nuclear expression were scored as CDX2 positive. Tumours in which either completely lacked expression or showed faint nuclear expression were scored CDX2 negative.

CONCLUSION

CDX2 is a useful IHC marker for intestinal metaplasia in gastrointestinal carcinoma. In this study, CDX2 expression appears to be more specific to colorectal carcinomas. CDX2 is a useful IHC marker for intestinal metaplasia in gastrointestinal carcinoma.

Based on the current literature, we conclude that all the cases in the present study with TNM stage II and III showing CDX2 negativity might benefit from chemotherapy after surgical resection.

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