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Utility of CDX2 Expression in Adenocarcinoma with Special Reference to the Gastrointestinal Tract

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ABSTRACT

Background: Adenocarcinoma of the gastrointestinal tract is attributed to be one of the most common cancer types. The prognosis for this cancer is poor and the overall survival rate observed ranges from 4% to 13%. A more precise diagnosis leads to more effective treatment, therefore, improving the overall outcome. Immunohistochemistry is the most common adjunctive method. CDX2 has recently been found as a sensitive & specific marker for adenocarcinoma of the gastrointestinal tract, especially colorectal adenocarcinoma in recent studies.

Methods: This was a hospital-based cross-sectional study conducted in the Department of Pathology at Subharti Medical College and associated Chhatrapati Shivaji Hospital for 2 years among 60 diagnosed cases of adenocarcinoma. CDX2 immunohistochemical staining was done and expression was seen.

Results: Maximum subjects in this study were from the age group of 51-60 years (25%) and there was a male predominance noted. Adenocarcinoma was located most commonly in the prostate (39.3%) followed by the gastrointestinal tract, which in turn showed most cases of the ascending colon. The final positive outcome of CDX-2 expression was found among 31 of the 60 adenocarcinoma cases. All 18 cases of GIT adenocarcinoma, all 9 cases of Hepatobiliary adenocarcinoma and 4 out of 5 cases of Metastatic adenocarcinoma showed positive expression of CDX-2. No CDX-2 positivity was observed in the adenocarcinoma of the respiratory tract, the male and female reproductive system and pancreas. The sensitivity of CDX-2 expression in adenocarcinoma of the gastrointestinal tract, hepatobiliary system and metastatic adenocarcinoma was calculated as 82.4%, 93.5% and 78.2%, respectively.

Conclusion: In conclusion, our results confirm the clinical utility of antibodies to CDX2 in the identification of adenocarcinomas at different sites, in both primary and metastatic settings.

Key-words: Adenocarcinoma, CDX2, Colorectal cancer (CRC), Gastrointestinal tract, IHC

INTRODUCTION

Adenocarcinoma refers to malignant epithelial neoplasms which grow in a glandular pattern.[1] Adenocarcinomas are the most commonly detected primary carcinomas (≤60% of all metastatic neoplasms of unknown primary), and the seven most common primary sites for these cancers are the colon, breast, ovary, lung, stomach, pancreas, and bile duct. [2] One of the most common cancer types is gastrointestinal tract adenocarcinoma [3].

GI adenocarcinoma is a noteworthy observation. Colorectal cancer (CRC) is one of the most prevalent cancers worldwide, and its incidence is gradually rising. The development of colorectal cancer is significantly influenced by dietary, environmental, and hereditary variables. According to estimates, colorectal cancer accounted for almost 1.8 million new cases and 881,000

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deaths in 2018, or around one out of every ten cancer cases and deaths. In general, colorectal cancer ranks second in terms of mortality but third in terms of incidence [4].

In cases of metastatic adenocarcinoma, determining the tissue of origin can be difficult by histology alone. The use of immunohistochemistry can improve the identification of the exact origin of metastatic adenocarcinoma. A more precise diagnosis leads to more effective treatment, therefore, improving the overall outcome [5].

The most widely utilised adjunctive technique is immunohistochemistry. [6] Cytokeratins, specifically CK7 and CK20, are among the most widely used IHC markers for adenocarcinomas at the moment. The expression pattern of CK7-/CK20+ is a highly distinctive feature of colorectal cancers. Nevertheless, the CK7-/CK20+ expression pattern is not present in all colorectal carcinomas. The creation of novel and more precise indicators of intestinal differentiation is therefore still of interest [7-9].

The nuclear homeobox transcription factor CDX2 is a member of the CDX homeobox genes' caudal-related family. According to reports, CDX2 expression is organ-specific and typically occurs in the nucleus of the alimentary tract's epithelial cells from the proximal duodenum to the distal rectum during embryonic and postnatal life. [10-12]

Hence, the study was done to see the spectrum of adenocarcinoma in various organs and analyse the frequency and significance of CDX-2 expression in Adenocarcinoma GIT and other sites.

MATERIALS AND METHODS

Study Design- A hospital-based cross-sectional study was conducted in the Department of Pathology at Subharti Medical College and associated Chhatrapati Shivaji Hospital. The study spanned two years and included 60 diagnosed cases of adenocarcinoma.

Inclusion and Exclusion Criteria

Inclusion Criteria- The study's inclusion criteria were all paraffin-embedded tissue sections from adenocarcinoma cases that had been diagnosed.

Exclusion Criteria- Endoscopic biopsies with inadequate representative tissue, autolyzed biopsies, resections after pre-surgical neoadjuvant therapy, and tissue blocks containing extensive necrosis and hemorrhage were excluded.

Tumor Classification- Tumor sections were graded and subtyped according to the organ-specific criteria of the World Health Organization (WHO) Classification of Tumors. Well-differentiated and moderately differentiated tumors were grouped as low-grade tumors, whereas poorly differentiated or undifferentiated tumors were classified as high-grade tumors.

Immunohistochemistry (IHC) Procedure

Every tissue segment underwent CDX2 immuno-histochemistry (IHC) staining.

Semi-Quantitative Grading of CDX2 Staining

0: Cell staining is very rare or there is no positivity.
1+: The staining of less than 10% of cells
2+: 10–50% stained cells
3+: Over half of the cells were stained

Staining Intensity Grading

0. No staining is present.
1+: Very weak and dubious staining
2+: Staining that is definitive, mild, or moderate
3+: Strong, distinct staining

A colon biopsy slides with 3+ CDX2 positivity (both proportion and intensity) was used as a positive control.

Statistical Analysis- Data entry was done in Microsoft Excel, and statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 24.0. Appropriate statistical tests were applied to determine significance levels.

RESULTS

Maximum subjects in this study were from the age group of 51-60 years (25%) followed by >70 as well as 41-50 years (23.21%). Overall Adenocarcinoma was associated with elderly age. Of the total 60 cases of adenocarcinoma, 66.7% (40 cases) were males, and 33.3% (20 cases) were females. There was a male predominance noted. Adenocarcinoma was located most commonly in the prostate (39.3%) followed by gastrointestinal tract adenocarcinomas (18, 30.00%). Hepatobiliary System, Metastatic Adenocarcinoma,

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Respiratory System, Female Reproductive System and others (Pancreatic Adenocarcinoma) had 9 (15.00%), 5 (8.37%), 3 (5.00%) and 1 (1.67%) case respectively. (Table 1).

Table 1: Site distribution of Adenocarcinoma cases

Sites	Number of Cases	Percentage (%)
Male Reproductive System	22	39.3
Gastrointestinal Tract	18	30.0
Hepatobiliary System	9	15.0
Metastatic	5	8.37
Respiratory System	3	5.0
Female Reproductive System	2	3.33
Others	1	1.67

Within the gastrointestinal tract, the highest number of cases was observed in the ascending colon (6 cases, 33.34%), followed by the rectum (4 cases, 22.23%). 2 cases (11.11%) were observed in the stomach, transverse colon and sigmoid colon, respectively. Both, anal canal and esophagus had 1 case each (5.55%) (Table 2).

Table 2: Site Distribution within GIT Adenocarcinoma

Site within GIT	Number of Cases	Percentage (%)
Ascending Colon	6	33.34
Rectum	4	22.23
Stomach	2	11.11
Transverse Colon	2	11.11
Sigmoid Colon	2	11.11
Anal Canal	1	5.55
Esophagus	1	5.55

The 18 gastrointestinal tract adenocarcinomas were further classified into well- differentiated, moderately differentiated, mucin-secreting, signet ring cell type of adenocarcinoma which had 8 (44.45%), 5 (27.78%), 4 (22.22%), and 1 (5.55%) case, respectively. There was no case of poorly differentiated Adenocarcinoma (Table 3).

Table 3: Histopathological Distribution of GIT Adenocarcinoma

Histopathological Type	Number of Cases	Percentage (%)
Well-Differentiated	8	44.45
Moderately Differentiated	5	27.78
Mucin-Secreting	4	22.22
Signet Ring Cell Type	1	5.55

Within the Hepatobiliary System, there were 7 cases (77.78%) of Gall Bladder Adenocarcinoma, and 1 case (11.11%) each of ampullary and liver adenocarcinoma. The 9 cases of Hepatobiliary Adenocarcinoma were further classified into Moderately- differentiated, Mucin-secreting and Poorly Differentiated Adenocarcinoma and among these categories, there were 5 cases (55.56%), 3 cases (33.33%) and 1 case (11.11%), respectively. All the 22 cases included in the study were Prostate Adenocarcinomas which were classified based on the Gleason Score. 10 cases (45.45%) had Gleason Score 7 (3+4) Grade Group 2, while there were 8 cases (36.37%) and 4 cases (18.18%) with a Gleason Score 7 (4+3) Grade Group 3 and Gleason Score 8 (4+4) Grade Group 4, respectively. Among metastatic adenocarcinoma, 2 cases (40%) observed were from the omentum and 1 case (20%) each from Bone Marrow, Lymph Node, and Liver. These cases were all metastatic adenocarcinomas of unknown primary.

All 60 cases were subjected to CDX-2 Immunohistochemistry and it was noted that 31 cases (51.7%) were CDX-2 positive, whereas 29 cases (48.3%) were CDX-2 negative (Table 4).

Table 4: CDX-2 Expression among study subjects

CDX-2 Expression	Number of Cases	Percentage (%)
Positive	31	51.7
Negative	29	48.3

All 18 cases of GIT Adenocarcinoma showed CDX-2 positivity. Out of the 8 cases of well-differentiated adenocarcinoma, 2 cases and 6 cases were graded as 2+ and 3+, respectively for a percentage of CDX-2 positive tumor cells, whereas the grading of intensity was 2+ and

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3+, in 4 cases each. Among 5 mucin-secreting adenocarcinomas, 1 and 4 cases respectively were 2+ and 3+, for a percentage of CDX-2 positive tumor cells, while 1 and 4 cases each were graded as 2+ and 3+ for intensity of CDX-2 staining. 1 case of Moderately differentiated adenocarcinoma showed 2+ grading in both categories, while the rest of the 3 cases were 3+ in both categories. There was one case of signet ring adenocarcinoma, which showed a grading of 3+ in both categories (Table 5).

Table 5: CDX-2 Expression in Gastrointestinal Tract

Histopathological Diagnosis	No. of Cases	Grading of Percentage of CDX-2 Positive Tumor Cells	Grading of Intensity of CDX-2 Staining	Final Impression
Well-Differentiated Adenocarcinoma	8	0	2	6
Mucin Secreting Adenocarcinoma	5	0	1	4
Moderately Differentiated Adenocarcinoma	4	0	1	3
Signet Ring Type of Adenocarcinoma	1	0	0	1
Total	18	0	4	14

All 9 cases of Hepatobiliary adenocarcinoma and 4 out of 5 cases of Metastatic adenocarcinoma showed positive expression of CDX-2. No CDX-2 positivity was observed in the adenocarcinoma of the respiratory tract, the male and female reproductive system and pancreas. We applied the diagnostic efficacy test, which gave the sensitivity, specificity and diagnostic efficacy of CDX-2 in GIT as 82.4%, 74.9% and 77.5%, respectively (Table 6). In the hepatobiliary system, the sensitivity, specificity and diagnostic accuracy of CDX-2 were found as 93.5%, 84.8% and 88.7%, respectively. In metastatic adenocarcinomas, Sensitivity, specificity and Diagnostic accuracy of CDX-2 were calculated as 78.2%, 70.60% and 72.10%, respectively.

Table 6: Diagnostic efficacy of CDX-2 Expression in Gastrointestinal Tract

Parameter	Sensitivity (%)	Specificity (%)	Diagnostic Accuracy (%)
GIT Adenocarcinoma	82.4	74.9	77.5
Hepatobiliary System	93.5	84.8	88.7
Metastatic Adenocarcinoma	78.2	70.6	72.1

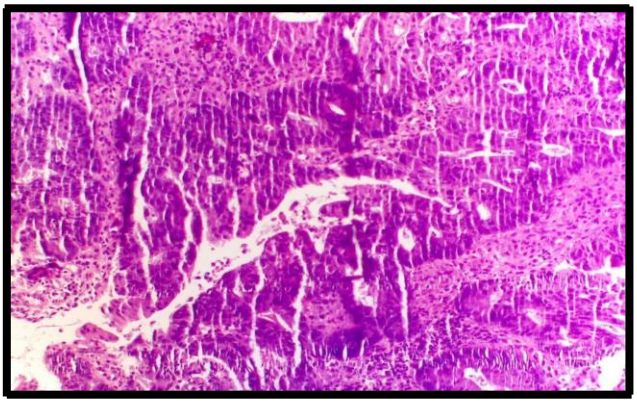

Fig. 1: Adenocarcinoma- Ascending Colon (H&E X 100)

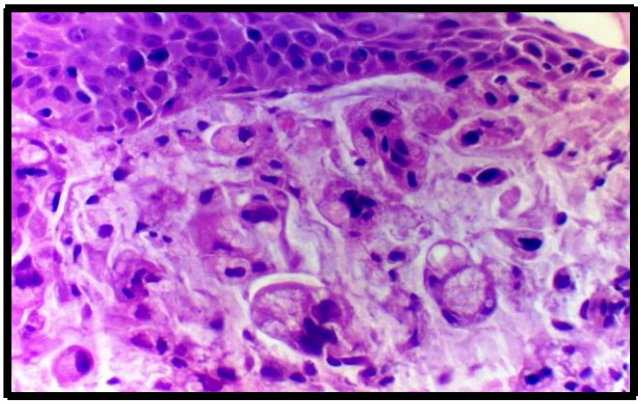

Fig. 2: Adenocarcinoma Esophagus (H&E X400)

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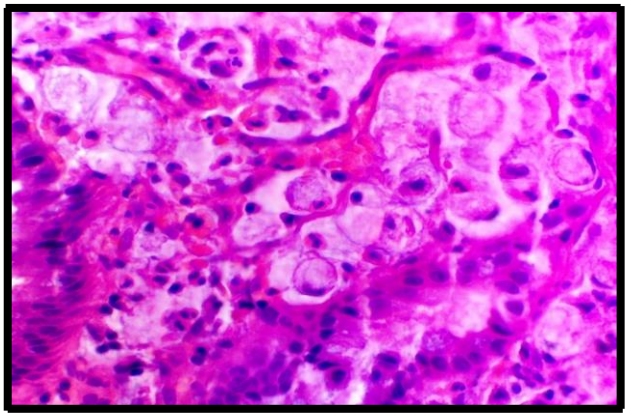

Fig. 3: Mucin secreting adenocarcinoma- Rectum (H&E, X400)

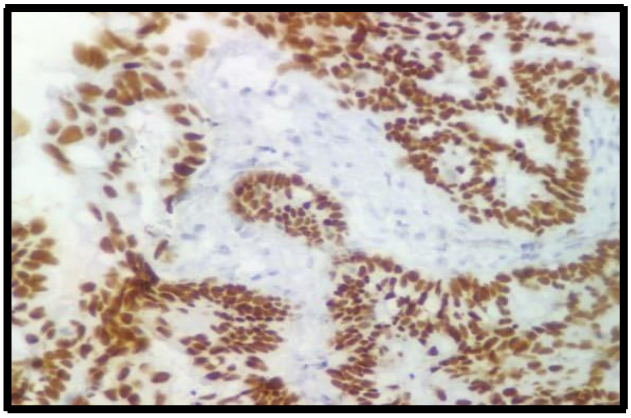

Fig. 4: Strong Nuclear Positivity of CDX-2 in Well Differentiated Adenocarcinoma Transverse Colon (H&E, X400)

DISCUSSION

The nuclear homeobox transcription factor CDX2 is a member of the caudal-related CDX homeobox gene family. The non-clustered hexapeptide gene that codes for CDX2 is found on chromosome 13q12-13. Homeobox genes are crucial for regulating healthy embryonic development. In addition to being involved in intestinal cell proliferation, differentiation, adhesion, and apoptosis, CDX2 is essential for the axial patterning of the alimentary tract throughout embryonic development. At the level of gene transcription, CDX2 works inside the cell to promote differentiation and prevent proliferation. By triggering the transcription of intestinal-specific proteins such as MUC2, sucrase, isomaltose, and carbonic anhydrase I, it promotes the differentiation of intestinal epithelium. By upregulating WAF1/p21, a CDK inhibitor that stops the cell cycle when DNA damage occurs, CDX2 prevents epithelial growth. According to Das *et al.* reports, CDX2 expression is organ-specific and typically occurs in the nucleus of the alimentary tract's epithelial cells from the proximal duodenum to the distal rectum during embryonic and postnatal life.[6]

In Western nations, colorectal cancer ranks second in terms of both incidence and fatality. The progressive accumulation of genetic changes affecting numerous tumour suppressor genes and proto-oncogenes, including APC, K-ras, p53, and BCL2, is linked to colorectal malignancies. No gene specific to the bowel has been linked to colorectal cancer thus far, but these genes are equally important for malignancies that do not originate in the gut.

These findings show that CDX2 plays a critical homeotic role in the gastrointestinal tract's development by directing endoderm towards the midgut rather than the foregut. In contrast to its crucial involvement during gut formation, Cdx2's function in the adult intestine is still unclear. Surprisingly, Cdx2 expression drops to the tumour grade in human colorectal malignancies. Furthermore, oncogenic mechanisms in colon cancer cells downregulate the gene. We hypothesised that CDX2 has a tumour suppressor function as a result of these findings. [13]

In our study, all 18 cases of GIT Adenocarcinoma showed CDX-2 positivity and were graded as 2+ or 3+, for percentage and intensity of CDX-2 positive tumor cells, there were a total of 9 cases of hepatobiliary adenocarcinoma, and all the cases stained positive for CDX-2, and showed a grade of 2+ or 3+ for a percentage of CDX-2 positive tumor.

Tahir et al, reviewed 125 diagnosed colorectal adenocarcinomas, where 112 cases (89.6%) were CDX-2 positive while 13 (10.4%) were CDX-2 negative. The CDX-2 expression was further stratified according to site and type of colorectal carcinoma and was found significant for both ($p<0.05$).[14]

The expression of CDX2 in neoplastic tissues was mostly, but not entirely, restricted to gastrointestinal tract adenocarcinomas. Similar to our findings, a large study by Werling *et al.* [15] found that CDX2 expression was most frequently found in GI tract adenocarcinomas. They also found that all colorectal adenocarcinomas expressed CDX2, with 74 out of 75 cases showing high-level expression (score of 3+ in 64 cases and 2+ in 10 cases); however, no carcinomas primary to the breast, lung,

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salivary gland, urothelium, pleura, kidney, or liver demonstrated significant expression of CDX2. Heterogeneous expression of CDX2 was seen in adenocarcinomas that are primary to the stomach, oesophagus, and pancreaticobiliary system, also known as extraintestinal GI adenocarcinomas [15].

In a study conducted by Moskaluk *et al.* extensive nuclear staining (>50% of cells) for CdX2 was most commonly seen in gastrointestinal adenocarcinomas (54 of 60, 90%), which is to the results of our study [16].

Table 7: Comparison of CDX-2 positivity in GIT Adenocarcinoma among various studies

Study	Number of GIT Adenocarcinoma Cases / Total Adenocarcinoma Cases	Percentage of GIT Adenocarcinoma Cases Showing CDX-2 Positivity
Tahir *et al.* [14]	112/125	89.6%
Werling *et al.* [15]	74/75	98.6%
Moskaluk *et al.* [16]	54/60	90%
Kuntz *et al.* [7]	1320/1104	83.6%
Current Study	18/18	100%

CONCLUSIONS

In conclusion, our results confirm the clinical utility of antibodies to CDX2 in the identification of adenocarcinomas at different sites, in both primary and metastatic settings. As CDX2 was identified almost in all cases of gastrointestinal carcinoma and of hepatobiliary origin, our study showed that in the context of the differential diagnosis of adenocarcinomas (especially the mucinous type), CDX-2 should be added to the existing panel of immunohistochemical markers to aid in the differential diagnosis of metastatic colorectal or hepatobiliary carcinoma versus primary mucinous carcinoma of other sites.

CONTRIBUTION OF AUTHORS

Research concept- Shubhangi Gupta, Shivani Tomar
Research design- Shubhangi Gupta, Shivani Tomar
Supervision- Rani Bansal, Anjali Khare
Materials- Shubhangi Gupta, Shivani Tomar
Data collection- Shubhangi Gupta, Shivani Tomar
Data analysis and Interpretation- Rani Bansal, Anjali Khare
Literature search- Shubhangi Gupta, Shivani Tomar
Writing article- Shubhangi Gupta, Shivani Tomar
Critical review- Rani Bansal, Anjali Khare
Article editing- Shubhangi Gupta, Shivani Tomar
Final approval- Rani Bansal, Anjali Khare

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