CD 10 Expression In Colorectal Carcinoma And Premalignant Lesions In A Tertiary Care Hospital

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Abstract: The aim of this work is to study the tumour and stromal expression of CD10 in colorectal adenoma and carcinoma and to study the relationship of CD10 expression in Tumour prognostic factors such as Depth of invasion and Tumour staging. Materials and methods: CD10 expression was studied in tumour cells and stromal cells in 30 colorectal adenomas and 30 colorectal carcinomas using monoclonal antibody to CD10 by immunohistochemistry. Results: Significant progression of CD10 immunohistochemical expression in tumour cells from adenomas to carcinomas was reported in this study (p<0.05). Inverse significant correlation was detected between CD10 expression in tumour cells and depth of tumour invasion as 100% and 80% of T2 and T3 cases respectively showed positive CD10 expression and 100% of T4 cases showed negative Tumour CD10 expression with strong stromal staining.

Keywords: CD 10 EXPRESSION, ADENOMAS, ADENOCARCINOMAS, TUMOUR DEPTH OF INVASION, NODAL STATUS

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I. Introduction:
Colorectal carcinomas account for approximately 9% of all cancers. It is the fourth most common cause of cancer mortality in the world. Colorectal cancer is the 4th most common cause of cancer mortality worldwide and third most commonly diagnosed malignancy among men. It accounts for 9% of newly diagnosed cancer and mainly a disease of developed countries. In India, the annual incidence rates of colon and rectal carcinomas are 4.4 and 4.1 per 100000 respectively. The annual incidence rate for colon cancer in women is 3.9 per 100000. However the incidence of rectal cancer is higher in rural India.

CD 10 is a single pass type II trans membrane matrix metalloproteinase and zinc dependent enzyme involved in carcinogenesis weighing 90-110kDa. It acts through the release of bioactive molecule that stimulate invasion, extracellular matrix degradation, inhibition of apoptosis and stimulating angiogenesis and immune response modulation. Earlier it was used as cell surface marker to identify and differentiate between haematological malignancies. Later CD 10 plays an important role in cancer development and progression in various malignancies.

In colorectal cancer CD 10 has been expressed in tumour cells, tumour associated fibroblast and infiltrating inflammatory cells. Studies have associated this marker with the liver metastasis, venous invasion and progression of tumours to more advanced stages in patients with colorectal neoplasm. Some studies have postulated that CD10 expression may be a useful marker for estimating the biological properties of early colorectal carcinomas.

II. Materials And Methods:
The study was conducted during the period of January 2017 to January 2018. It was carried out in specimens obtained from patients with confirmed histopathological diagnosis of colorectal adenomas and adenocarcinomas. The study was approved by the Ethical committee of Government Stanley Medical College and hospital. It is a Retrospective and comparative study.

STUDY GROUP:
The study sample comprised of 60 cases, 30 of colorectal adenoma and 30 of adenocarcinoma patients. Cases were chosen from the Department of Surgical Gastroenterology, Government Stanley Medical College and hospital. Age, sex, tumour site, histological grade and tumour stage were obtained for all cases. All the 60 cases were screened for CD 10 expression through immunohistochemical assay. Cancer types other than adenocarcinomas, Recurrent and metastatic adenocarcinomas Chemotherapy and/or radiotherapy prior to sampling were all excluded from the study.

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HISTOPATHOLOGICAL EXAMINATION:
The tissues so obtained were processed and sections were cut at 5 microns. Hematoxylin and eosin staining of the sections were done and analysed. Adenomas were classified into low grade and high grade based on dysplasia. In low grade dysplasia stratified nuclei tend to remain in the basal epithelium with minimal nuclear hyperchromasia. In high grade dysplasia the nuclei consistently come to the surface of epithelium, loss of columnar shape with nuclear irregularity and loss of polarity. In Adenocarcinoma there will be loss of architecture, nuclear pleomorphism and invasion. The clinico-pathological characteristics including age, sex, tumour site, histological diagnosis (normal/adenoma/adenocarcinoma), histological grade (adenomas - low grade/high grade dysplasia; adenocarcinoma – low/high) and adenocarcinoma stage were obtained for all the cases.

IMMUNOHISTOCHEMICAL STAINING:
IHC was performed on the selected blocks for CD10. For immunohistochemistry sections were cut at 5 micrometre thickness. Slides coated with home alum were used. Sections were subjected to antigen retrieval using pressure cooker techniques TRIS EDTA (pH 9.2) buffer solution and then treated by HRP (horseradish peroxidase) polymer technique. Finally DAB was used as a chromogen and hematoxylin as a counterstain. Positive control was obtained from renal cell carcinoma which exhibited strong intensity of CD 10 immunostaining.

EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF CD10:
Tumour CD10 was considered positive if more than 10% of tumour cells express fine to coarse cytoplasmic granules. Stromal CD10 was graded according to a 4 point scale based on percentage of positively stained area:

- 0 - <10% positive tumour cells
- +1 - 10-25% positive tumour cells
- +2 - 25% - 50% positive tumour cells
- +3 - >50% positive tumour cells

III. Observation And Results:

Fig1: Gross picture of infiltrative growth in left side colon.

Fig2: Strong cytoplasmic staining of CD10 in Renal cell carcinoma-control
Fig 3: Faint cytoplasmic CD10 Expression in low grade adenoma

Fig 4: Moderate cytoplasmic and membrane CD10 staining in high grade adenoma

Fig 5: Strong cytoplasmic and membrane staining in T2 Depth of invasion with minimal stromal staining.

Fig 6: Moderate cytoplasmic and membrane CD10 Staining in T3 Depth of invasion with moderate stromal cell staining.
IV. Results

Gross, Depth of Tumour invasion, Node, Tumour CD10, Stromal CD10 are Primary explanatory variable.

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis.

<table>
<thead>
<tr>
<th>Group</th>
<th>TUMOURCD10 Mean±STD</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>59.79 ± 39.55</td>
<td>41.41</td>
<td>26.48508</td>
<td>56.33492</td>
</tr>
<tr>
<td>Adenoma</td>
<td>18.38 ± 10.16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean TUMOURCD10 was 59.79±39.55 in subjects with Carcinoma and mean TUMOURCD10 was 18.38 ± 10.16 in subjects with Adenoma. The mean difference across the group is (41.41). It is statistically significant (P Value 0.001) (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>STROMALCD10 Mean±STD</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>61.07 ± 19.88</td>
<td>43.80</td>
<td>35.90196</td>
<td>51.68937</td>
</tr>
<tr>
<td>Adenoma</td>
<td>17.28 ± 8.442</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean STROMALCD10 was 61.07±19.88 in subjects with Carcinoma and mean STROMALCD10 was 17.28 ± 8.442 in subjects with Adenoma. The mean difference across the group is (43.80). It is statistically significant (P Value 0.001)

<table>
<thead>
<tr>
<th>Depth of Tumor</th>
<th>Tumour CD10 Median (IQR)</th>
<th>P value (Kruskal Wallis Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>98 (IQR 98 to 99)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>70 (IQR 65 to 75)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>2 (IQR 0 to 5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Among the study participants, Tumour CD10 Median was 98 (IQR 98 to 99) of T2, Tumour CD10 Median was 70 (IQR 65 to 75) of T3 and Tumour CD10 Median was 2 (IQR 0 to 5) of T4.
The difference in between Tumour CD10 Median and Depth of Tumour is statistically significant (P Value 0.001) (Table 3).

Table 4: Comparison of improvement in Depth of Tumor and Stromal CD10 among study group (N=30)

<table>
<thead>
<tr>
<th>Death of Tumor</th>
<th>Stromal CD10 Median (IQR)</th>
<th>P value (Kruskal Wallis Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>53 (50 to 55)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>64 (62 to 65)</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>82 (78 to 83)</td>
<td></td>
</tr>
</tbody>
</table>

Among the study participants, Stromal CD10 Median was 53 (IQR 50 to 55) of T2, Tumour CD10 Median was 64 (IQR 62 to 65) of T3 and Tumour CD10 Median was 82 (IQR 78 to 83) of T4. The difference in between stromal CD10 Median and Depth of Tumour is statistically significant (P Value 0.001) (Table 4).

Table 5: Comparison of mean STROMAL CD10 across study groups (N=30)

<table>
<thead>
<tr>
<th>GROSS</th>
<th>STROMAL CD10 Mean±STD</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer proliferative</td>
<td>70.54 ± 20.17</td>
<td>18.69</td>
<td>0.25</td>
<td>0.047</td>
</tr>
<tr>
<td>Infiltrating</td>
<td>51.85 ± 28.67</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean STROMALCD10 was 70.54 ± 20.17 in subjects with Ulcer proliferative type of growth and meanSTROMALCD10 was 51.85 ± 28.67 in subjects with Infiltrating type of growth. The mean difference across the group is (18.69). It is statistically significant (P Value 0.047) (Table 5).

Table 6: Comparison of mean TUMOUR CD10 across study groups (N=30)

<table>
<thead>
<tr>
<th>NODE</th>
<th>TUMOUR CD10 Mean±STD</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>73.49 ± 32.04</td>
<td>26.25</td>
<td>0.12</td>
<td>0.049</td>
</tr>
<tr>
<td>Absent</td>
<td>47.24 ± 37.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean TUMOURCD10 was 73.49 ± 32.04 in subjects with nodal positivity and meanTUMOURCD10 was 47.24 ± 37.12 in subjects with negative node. The mean difference across the group is (26.25). It is statistically significant (P Value 0.049).

Table 7: Comparison of mean STROMALCD10 across study groups (N=30)

<table>
<thead>
<tr>
<th>NODE</th>
<th>STROMAL CD10 Mean±STD</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>80.51 ± 11.78</td>
<td>30.38</td>
<td>15.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absent</td>
<td>50.14 ± 24.17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean STROMALCD10 was 80.51 ± 11.78 in subjects with nodal positivity and meanSTROMALCD10 was 50.14 ± 24.17 in subjects with negative node. The mean difference across the group is (30.38). It is statistically significant (P Value <0.001) (Table 7).

V. Discussion:

CD10 is an important molecule involved in integrating signals from either the cell environment or the intracellular compartment by cleaving peptides through enzymatic activity and through intracellular signalling pathways that interfere with other major signalling pathways. It is significant that CD10 expression derangement is associated with development of different tumour types. Among the study participants, 30(50.00%) were adenocarcinomas and 30(50.00%) were adenomas. Out of the 30 adenocarcinomas that were studied, 10 were well differentiated, 10 were moderately differentiated and 10 were poorly differentiated. TNM staging was used: 2(6.67%) were stage I, 12(40.00%) were stage II, 12(40.00%) belonged to stage III and 4(13.33%) were stage IV.

In the present study, CD10 expression gradually increased in adenomas from low grade dysplasia to high grade dysplasia and was maximally expressed in adenocarcinomas. The difference in mean CD10 expression between adenomas and adenocarcinomas was statistically significant (P value less than 0.001).

This observation of gradually increased expression of CD10 in adenomas and significantly increased expression in adenocarcinomas makes CD10 a potentially exploitable target of anti-cancer therapy with maximal targeting of the tumour and minimal damage to the normal epithelium. Also the significant increase is observed in CD10 expression from adenoma to adenocarcinoma suggests that CD 10 has an important role in colorectal tumorigenesis and malignant transformation of adenomas (the adenoma-carcinoma sequence).
The mean difference of Tumour CD10 and stromal CD10 expression in subjects with carcinoma and adenoma was 41.41. It is statistically significant (p value 0.001). This result of the present study correlates with that of Jang et al in 2013 stated that tumour CD 10 Expression significantly increased from 14% in low grade adenoma(3 cases out of 22 cases), to 22% in high grade adenomas(6 cases out of 27 cases) and 44% in invasive colorectal carcinoma(14 cases out of 32 cases) and this support the involvement of CD10 in progression and carcinogenesis of colorectal carcinoma.

Wang et al in 2013 reported there was progression in tumour CD10 Expression from 0.8% in low grade adenoma to 9.1% in high grade adenomas and 40% in invasive colorectal carcinoma.

Hirano et al in 2012, Koga et al in 2008, Iwase et al in 2005 also supported that CD 10 Expression were reported more frequently in invasive phenotype rather than adenomas.

The present study includes 30 cases of colorectal adenocarcinomas of which 10 were seen in T2 depth of tumour (Tumour invades muscularis propria), 11 were seen in T3 depth of tumour (Tumour invades through the muscularis propria in to the pericolorectal tissue) and 9 were seen in T4 depth of tumour (Tumour penetrates the visceral peritoneum). The difference in between Tumour CD 10 median and depth of invasion is statistically significant (P value less than 0.001). A significant correlation is observed between Tumour and Stromal CD10 expression and depth of invasion.

Hirano et al reached similar conclusions in their study on colorectal carcinomas which showed that CD 10 expression correlated significantly with depth of tumour invasion.

The results of the present study are in agreement with koga et al who in their study conducted in 2008 comprising 48 cases of colorectal adenocarcinomas concluded that CD10 expression correlated with the depth of tumour invasion.

Ogawa et al analysed the expression of CD10 in the cell stroma and showed significantly more positivity in protruding lesion. Waisberg et al also reported positivity for CD10 more in exophytic appearance of tumour. Yao et al in 2002 also supported the findings of significant CD 10 positivity high in proliferative lesion than in infiltrating lesion.

On comparing the CD10 expression between stage I and II adenocarcinomas and stage III adenocarcinomas it was found that the difference in mean cd10 expression between the two groups was not statistically significant showing that CD10 expression does not correlates with the stage of colorectal adenocarcinomas.

Fujita et al in 2007 also showed expression of CD 10 did not show any statistical significance with the clinical staging of colorectal adenocarcinoma. Waisberg et al in 2012 analysed the expression of CD 10 in various stage of colorectal carcinoma and found no positive correlation.

VI. Conclusion:

The aim of the present study was to examine the expression of CD10 cell membrane metallopeptidase in colorectal neoplasia, its role in the transition sequence from adenoma to adenocarcinoma and its association with various clinicopathological characters of adenocarcinomas. The following are the conclusions of the present study:

- CD 10 expression showed a significant increase from adenoma to adenocarcinoma. This signifies that CD10 plays an important role in all stages of the adenoma-carcinoma sequence, which includes the early event of adenoma formation from normal epithelium and its malignant transformation.
- The expression of CD10 showed significant correlation with depth of tumour invasion and nodal metastasis.
- These results highlight the association of CD 10 expression with malignant behaviour of colorectal adenocarcinomas and CD10 could prove to be a new biomarker for aggressiveness and prognostic information in these tumours.
- There was however no correlation between CD 10 expression and the age, gender of the patient and stage of the tumour.
- The finding of gradually increasing CD 10 expression in adenoma to significantly higher expression in adenocarcinomas makes CD 10 an attractive and potential therapeutic target, which when implemented will result in maximal targeting of cancerous and also pre-cancerous tissues with minimal damage to the surrounding normal mucosa.

References:
[4]. Tae Jung Jang, Jeong Bae Park and Jong Im Lee, The Expression of CD10 and CD15 is progressively increased during Colorectal carcinoma development, Korea Journal of Pathology,47(4):340-347.


