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Original Research Article

Expression of CD10 Marker in Stromal Cells of Gastric Carcinoma: A Prospective Study

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Abstract: Stomach cancer or gastric cancer, is a cancer which develops from the lining epithelium of the stomach. Symptoms of gastric carcinoma generally include dyspepsia, pain in the epigastric region or upper part of the abdomen, nausea, anorexia and loss of appetite. Signs and symptoms in the advanced stages of cancer include severe weight loss, vomiting, difficulty in swallowing and blood in the stools. The cancer may spread from the stomach to other parts of the body by local and distant metastasis through vascular channels, distance spread particularly involves the liver, lungs, skeletal bones, lining of the abdomen and lymph nodes .According to many researchers, the most common cause of gastric carcinoma is infection by the bacterium Helicobacter pylori, which accounts approximately 60% of cases or more. A total of 40 cases were included in this study. CD10 Immmuno-histochemistry marker was used. Out of the 40 cases of gastric carcinomas, 16(40%) cases were of well differentiated, 13(32.5%) cases were of moderately differentiated and 11(27.5%) cases were of poorly differentiated grade. Most of the cases were seen in male patients and the most commonest region involved was pyloric antrum of the stomach. CD10 expression by the stromal cells plays an important role in the pathogenesis of gastric cancer and also that the proliferation of CD10-positive stromal cells is part of the mechanism of metastasis in gastric cancer.
Keywords: Gastric Carcinoma, CD 10 , Immunohistochemistry, Helicobacter pylori , Pyloric Antrum, Adenocarcinoma.

INTRODUCTION

According to the statistics worldwide, gastric carcinoma is the second-most common cancer among males and third-most among females in Asia and worldwide leading to significant morbidity and mortality [1]. The symptoms and signs of the stomach cancer are often reported late when the disease is already in advanced stages and 5-year survival is less than 30% in developed countries and around 20% in developing countries [2]. The gastric carcinoma incidence and prevalence rates show marked geographical variation, with high-risk geographical areas being Japan, China, Eastern part of Europe and few countries in Latin America. Low incidence and prevalence rates were noted among whites in North America, India, Philippines, most countries in Africa, some Western European countries and Australia [2]. In India, the number of new gastric cancer cases in 2001 was estimated to be approximately 35,675 (*n*=23,785 in men; 11,890 in women) [3]. These differences in incidence rates can be attributed to multiple factors and are particularly due to different dietary habits, and exposure to infections, particularly Helicobacter pylori.

Desmoplasia is a stromal reaction seen in many carcinomas as a component of cancer progression. The reactive stroma in cancer is characterized by stromal cell phenotypic switching, extracellular matrix remodeling, increased growth factor bioavailability, elevated protease activity, increased angiogenesis and an influx of inflammatory cells [4]. In cancers, reactive/desmoplastic stroma comprises of fibroblasts, myofibroblasts, endothelial cells of vascular channels and immune cells. Among all these cells, myofibroblasts are of particular interest as they potentially affect tumorigenesis.. Myofibroblasts in reactive stroma synthesize extracellular matrix (ECM) components such as collagen I, collagen III, fibronectin isoforms, tenascin and versican [5-9]. In addition to the mentioned mediators, myofibroblasts also express proteases, including urokinase, plasminogen activator, fibroblast activation protein (FAP) and matrix

metalloproteinases(MMPs) [10-12]. Due to the production and release of these components results in extracellular matrix (ECM) remodeling, which ultimately bring about cancer cell division, growth and migration. Therefore, myofibroblasts appear to play a key role in creating the tumor-promoting reactive stroma environment.

CD10 marker is a 90- to 110 kilo dalton cell surface zinc-dependent metalloprotease that has been called neutral endopeptidase (NEP) [13-15]. There is a wide expression of CD10 in the various tissues of the human body e.g. granulocytes, lymphoid precursor and progenitor cells, intestinal epithelial cells, placental trophoblasts such as cytotrophoblasts and syncytiotrophoblasts, epithelium of prostate gland and gall bladder, myoepithelial cells of various areas/glands, schwann cells and renal tubular epithelium [16, 17]. In addition, CD10 has been demonstrated to be expressed by the stromal cells of the normal bone marrow elements and endometrial tissue [18, 19]. Recent research reports indicate that CD10-positive stromal cells belong to the myofibroblast group, and their presence indicates poor prognosis in breast carcinoma, and they are also involved in colorectal carcinogenesis [20, 21]. In this study, we aimed to immunohistochemically investigate the correlation between CD10-positive stromal cells and invasion and metastasis of gastric carcinoma.

AIMS AND OBJECTIVES

1. To demonstrate the expression of CD10 in the stromal cells of gastric carcinomas.
2. To analyse the distribution of CD10 positivity according to histopathological grades.
3. To compare the CD10 positivity with depth of invasion and metastasis.
4. To compare the present study with other studies by other authors.

MATERIALS AND METHODS

Materials

The study was done at Upgraded department of pathology, Osmania general hospital, Hyderabad. A total of 40 cases of gastric carcinomas were picked out from 2008 to 2012. The tissues were fixed in 10% formalin, processed and embedded in paraffin.

Inclusion criteria for selection of cases :

- Gastrectomy specimens with diagnosis of primary gastric adenocarcinoma.
- No prior treatment.
- Complete clinicopathologic data (age, sex, histopathological diagnosis, tumor stage, nodal status).

Exclusion criteria:

- Small biopsies.
- Non carcinomatous gastric tumors.

Methods

Two sections of 4-5 micron thickness were prepared from the corresponding paraffin blocks, one on albumin coated slide for H&E staining and the other on poly- L-lysine coated slide for immunohistochemical staining.

Standard procedure for H&E staining was employed using Harris haematoxylin and aqueous Eosin. The kits for CD10 immunohistochemical staining were obtained from DAKO Company. Staining was done according to the manufacturer's protocol using lymph node sections as positive control.

Evaluation of immunostaining

When more than 10% of the stromal cells around the neoplastic glands or tubules were positive for CD10, the expression was judged to be positive.

To determine whether the stromal cells positive for CD10 are the myofibroblastic cells, we performed immunohistochemistry of CD10 and a-smooth muscle actin on the serial sections of gastric carcinoma, as α-smooth muscle actin is the marker used to identify myofibroblasts. We found that α-smooth muscle actin was expressed in the stromal cells and in the smooth muscle cells of the vessel walls, and CD10 was positive in the stromal cells and granulocytes. The distribution of CD10-positive stromal cells corresponded to that of α-smooth muscle actin-positive stromal cells.

Correlation between CD10 expression of stromal cells and clinico pathological factors was evaluated using the chi squared test. P-values <0.05 were considered to be significant.

OBSERVATIONS AND RESULTS

The age of the patients ranging from 29yrs to 70yrs, majority of the patients were in sixth decade of life followed by the patients of seventh decade.

Table 1: Age wise distribution of cases

Age range	No. of cases	Percentage(%)
≤30yrs	02	05
31-40yrs	04	10
41-50yrs	07	17.5
51-60yrs	18	45
61-70yrs	09	22.5

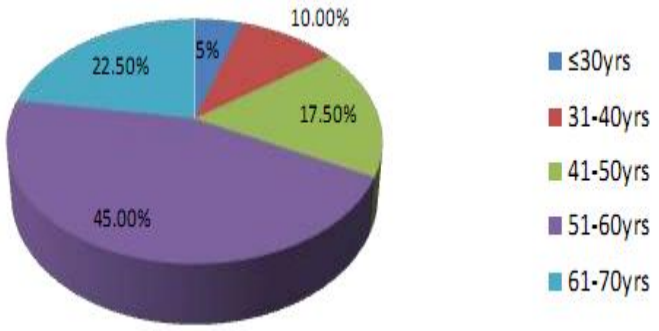

Fig-1: Age wise distribution of cases

Out of 40 cases, 29 cases were males constituting 72.5% and 11 were females constituting 27.5%. The male to female ratio is 2.6: 1.

Table 2: Sex wise distribution of cases

Sex	No. of cases	Percentage (%)
Males	29	72.5
Females	11	22.7
Total	40	100

Distribution of cases according to site

Of the 40 cases, majority of the cases were located in the region of pyloric antrum, followed by body, cardia and fundus.

Table 3: Distribution of cases according to site

Site	No. of cases	Percentage (%)
Cardia	02	5
Fundus	01	2.5
Body	12	30
Antrum pylorus	25	62.5
Total	40	100

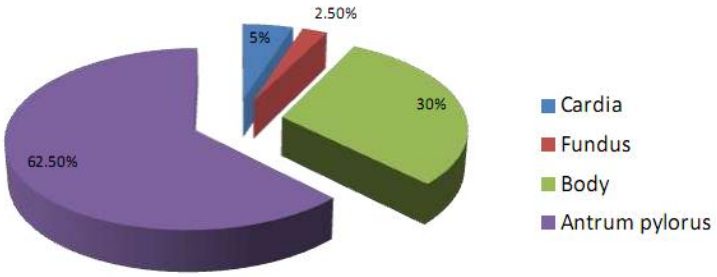

Fig-2: Distribution of cases according to site

Out of the 40 cases of gastric carcinomas, 16(40%) cases were of well differentiated, 13(32.5%) cases were of moderately differentiated and 11(27.5%) cases were of poorly differentiated grade.

The depth of invasion was classified as invasion into submucosa, muscularis propria, serosa and sub serosa, there were 0(0%), 12(30%), 22(55%) and 6(15%) respectively.

There were 23(57.5%) cases showing lymph nodal metastases and 17(42.5%) cases without lymph nodal metastases.

According to TNM staging, there were 16(40%) cases of stage I, 19(47.5%) cases of stage II, 4(10%) cases of stage III and 1(2.5%) case of stage IV.

Expression of CD10 by stromal cells in relation to differentiation:

There were 16 well differentiated adenocarcinomas, of which 11(68.75%) cases showed CD10 positivity and 5(31.25%) cases were CD10 negative.

There were 13 cases of moderately differentiated adenocarcinomas, of them 8(61.538%) cases were CD10 positive and 5(38.462%) cases were CD10 negative.

There were 11cases of poorly differentiated adenocarcinomas, of them 4 (36.364%) cases were CD10 positive and 7(63.636%) cases were CD10 negative.

Expression of CD10 by stromal cells in relation to depth of invasion:

In 12 cases there was invasion till muscularis propria, of them 6(50%) cases showed CD10 positivity and 6(50%) cases showed CD10 negativity.

In 22 cases there was invasion till serosa, of them 13(59.091%) cases showed CD10 positivity and 9(40.909%) cases showed CD10 negativity.

In 6 cases there was invasion beyond the level of serosa, of them 3(50%) cases showed CD10 positivity and 3(50%) cases showed CD10 negativity.

Expression of CD10 by stromal cells in relation to lymph nodal metastases:

There were 23 cases of gastric carcinomas which showed metastases to lymph nodes. Of them 17(73.913%) cases showed CD10 positivity and 6(26.087%) cases showed CD10 negativity.

There were 17 cases of gastric carcinomas which did not showed metastases to lymph nodes. Of them 6(35.294%) cases showed CD10 positivity and 11(64.706%) cases showed CD10 negativity.

Expression of CD10 by stromal cells in relation to TNM stage:

There were 17 cases of TNM stage I of them 7(41.176%) cases showed CD10 positivity and 10(58.824%) cases showed CD10 negativity.

There were 18 cases of TNM stage II, of them 13(72.222%) cases showed CD10 positivity and 5(27.222%) cases showed CD10 negativity.

There were 4 cases of TNM stage III, of them 3(75%) cases showed CD10 positivity and 1(25%) case showed CD10 negativity.

There was only one case of TNM stage IV which showed CD10 positivity.

Table 4: Expression of CD 10 by the stromal cells in relation to differentiation, depth of invasion, nodal metastases and TNM staging in gastric cancer patients

S.No.	Parameter	Total number	CD10 +ve	CD10 -ve	p- value
1.	**Histological type**				
	Well differentiated	16	11(68.75%)	05(31.25%)	
	Moderately differentiated	13	08(61.538%)	05(38.462%)	0.232
	Poorly differentiated	11	04(36.364%)	07(63.636%)	
2.	**Depth of invasion**				
	Muscularis propria	12	6(50%)	6(50%)	
	Serosa	22	13(59.091%)	09(40.909%)	
	Subserosa	06	03(50%)	03(50%)	0.848
3.	**Lymph node metastases**				
	Present	23	17(73.913%)	06(26.087%)	
	Absent	17	06(35.294%)	11(64.706%)	**0.015**
4.	**TNMstage**				
	Stage I	17	07(41.176%)	10(58.824%)	
	StageII	18	13(72.222%)	05(27.777%)	
	Stage III	04	03(75%)	01(25%)	0.197
	Stage IV	01	01(100%)	00(0%)	

Above table presents the significant statistical correlation between CD10 expression and lymph nodal metastases (p value – 0.015). There was no statistically significant correlation between CD10 expression and histologic type, depth of invasion and TNM staging.

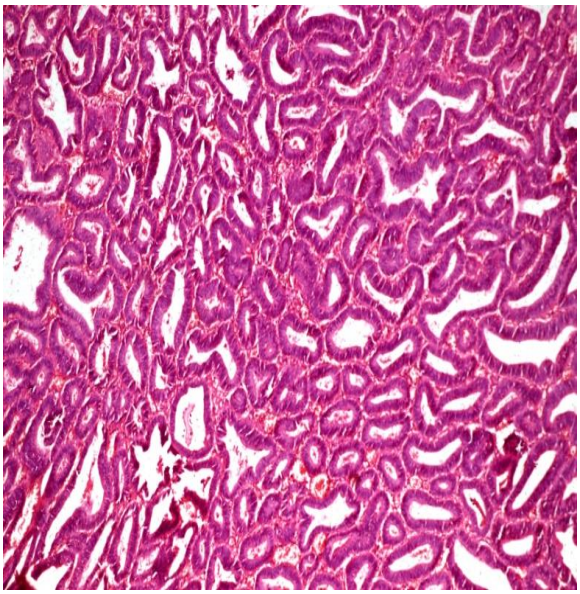

Fig-3: Well differentiated gastric carcinoma- Hematolxylin and Eosin stain

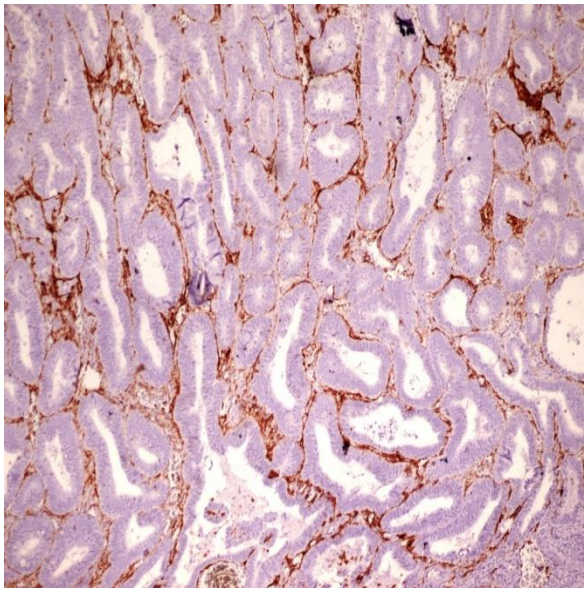

Fig-4: CD 10 Positivity of the Stromal cells in Gastric Carcinoma

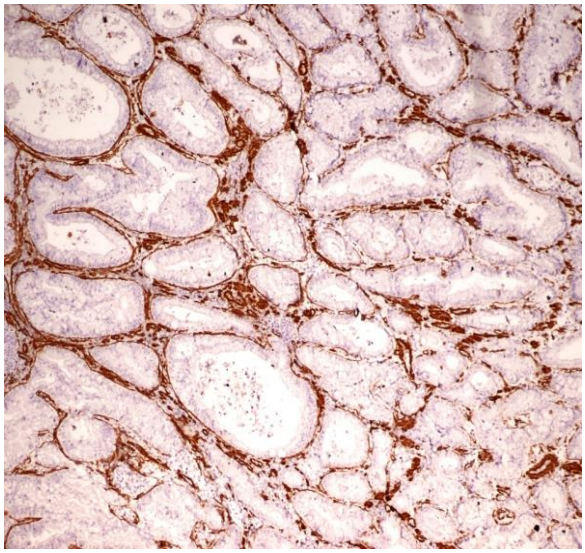

Fig-5: Smooth Muscle Actin (SMA) positivity in the stromal cells of gastric carcinoma

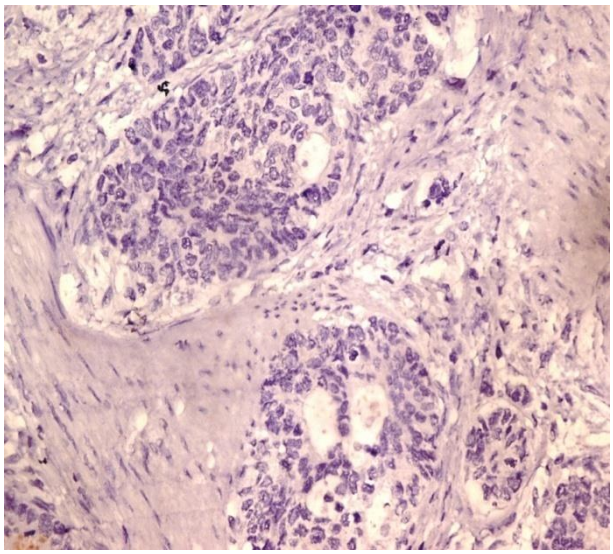

Fig-6: CD 10 Negative in the stromal cells of gastric carcinoma

DISCUSSION

Our study shows that the distribution of CD10-positive cells corresponds to that of the stromal cells expressing α-SMA. This suggests the possibility that CD10-positive stromal cells and myofibroblasts in the invasive front are the same cells, because α-SMA has become the marker most often used to identify myofibroblasts by immunohistochemistry [22]. Most studies measured epithelial CD10 expression by immunohistochemistry and cDNA array, but few studies measured stromal CD10 expression by immunohistochemistry.

Iwaya *et al* [19] investigating CD10 expression by the stromal cells in 123 cases of breast cancer by immunohistochemistry, showed that 18% of tumors exhibited stromal CD10 expression, which was undetectable in all non-invasive ductal carcinomas or normal breast tissue. They also proved that the frequency and increased number of positive CD10 stromal staining was positively correlated with the axillary lymph-node metastasis and also had a effect on prognosis. These results directly show that stromal expression of CD10 is an important novel prognostic factor in breast carcinoma.

Ogawa *et al* [20] also showed that the stromal expression of CD10 is an integral part of colorectal carcinogenesis.

Carl MC Grath *et al* demonstrated that CD10 is upregulated in gastric carcinoma and lymph node metastases and that, in cell proliferation assays, the inhibition of CD10 significantly reduced growth of cell lines indicating that the ability of CD10 to degrade

gastrointestinal peptides may play an important role in pathobiology of gastric carcinoma.

Wen-Bin Huang et al demonstrated that stromal cells expressing CD10 may play an important role in gastric carcinogenesis. CD10 expression by stromal cells seems to promote invasion and metastasis of differentiated gastric carcinoma [24].

The present study showed that CD10 was over expressed in patients with primary gastric cancer compared with normal gastric mucosa. We demonstrated a significant correlation between stromal CD10 expression and lymph nodal metastasis. Stromal CD10 expression, however, did not show any significant correlation with differentiation, invasion and TNM stage. This suggests that CD10 expression by the stromal cells may play an important role in the pathogenesis of gastric cancer and also that the proliferation of CD10-positive stromal cells is part of the mechanism of metastasis in gastric cancer.

Recently, Pan *et al* [23] demonstrated that CD10 is capable of cleaving CPI-0004Na and related peptide prodrugs such as N-succinyl-b-alanyl-L-isoleucyl-L-alanyl-L-leucyl-Dox (sAIAL-Dox), which have an improved antitumor efficacy profile with reduced toxicity compared with Dox. Therefore, this data can be applied to new modalities of cancer therapy which blocks the induction of CD10-positive stromal cells in gastric cancerous tissues. Further studies on the molecular basis of CD10 expression in stromal–cancer interaction will be required to pursue such new therapeutic strategies.

We compared our study by the author Wen-Bin Huang *et al* [24] who studied CD10 expression in stromal cells of 116 cases of gastric carcinomas. They observed a statistically significant correlation between CD10 expression by stromal cells and differentiation, depth of invasion, lymph nodal metastases vascular invasion. They also observed there is no statistically significant correlation between CD10 expression by stromal cells and TNM staging [24].

We studied CD 10 expression in stromal cells of 40 gastric carcinoma cases. We found there was statistically significant correlation between CD 10 expression in stromal cells and lymph nodal metastases. There was no statistically significant correlation between CD 10 expression in stromal cells and differentiation, depth of invasion and TNM staging.

CONCLUSION

In our study, we demonstrated a significant correlation between CD10 expression and lymph nodal metastases. Significant correlation was not found between the level of CD10 expression and differentiation, depth of invasion and TNM staging. CD10 expression by the stromal cells plays an important role in the pathogenesis of gastric cancer and also that the proliferation of CD10-positive stromal cells is part of the mechanism of metastasis in gastric cancer.

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