Significance of TGF-β1 and Catheps in E expression in gastric adenocarcinoma and precancerouslesions

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Abstract: Background and aim: 40 cases with gastric lesions comprise the material of this study. These include 40 paraffin blocks selected from gastric specimens (30 cases were obtained by endoscopy and 10 cases were surgical specimens) received at the pathology department, Faculty of Medicine, Zagazig University in the period from September 2013 to September 2014. The aim of this study to examine the expression of transformed growth factor beta 1 (TGF-β) and Cathepsin E (CTSE) in gastric cancer and gastric precancerous lesions and to correlate between these markers and clinicopathological parameters. **Methods:** Immunohistochemical analysis of TGF beta and Cathepsin E were performed using 40 cases for chronic superficial gastritis, chronic atrophic gastritis, chronic atrophic gastritis with intestinal metaplasia, gastric dysplasia and gastric cancer. **Results:** In cases of chronic gastritis, we observed only TGF-β1 reactivity in the cytoplasm of inflammatory cells mainly plasma cells. In intestinal metaplasia and dysplastic lesions, the reactivity to TGF-β1 was more intense than in normal mucosa, especially in the cytoplasm of the Goblet cells. TGF-β1 was expressed in 88.8% of incomplete intestinal metaplasia. TGF-β1 expression was detected in intestinal type of gastric adenocarcinomas in 100% of well differentiated adenocarcinoma, 50 % of moderately differentiated adenocarcinoma and 66.6% of poorly differentiated adenocarcinoma. TGF-β1 was expressed in 50% of papillary carcinoma and mucinous carcinoma. TGF-β1was expressed in 100% of signet ring carcinoma. In chronic gastritis without atrophy or intestinal metaplasia, expression of cathepsin E is clearly observed in the fundic and pyloric glands of stomach. CTSE immunestaining was detected in 100% of type II (incomplete intestinal metaplasia) it is negative in type I IM (complete intestinal metaplasia). CTSE was expressed in 57.14% of gastric cancer. More undifferentiated gastric tumors tend to increase expression of CTSE in tumor lesions (poorly differentiated adenocarcinoma> moderately differentiated adenocarcinoma> well differentiated adenocarcinoma). Cathepsin E was negative in mucinous and papillary carcinoma (100%) negative expression. Cathepsin E was positively expressed in 100% of signet ring carcinoma. **Conclusion:** The alterations of expression of TGF and cathepsin E can determine the histological variants of gastric cancer (GC). Cathepsin E could be a marker for signet ring carcinoma Understanding the expression pattern of TGF beta and Cathepsin E in gastric cancer allows them to be used as future therapeutic targets.

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Key words: cancer stomach, TGF-β1,cathepsin E, Immunohistochemistry.

1. Introduction

Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide. Gastric cancer is known to increase with age with the peak incidence occurring at 60-80 years ***(Jemal et al., 2011)***.

In Egypt, gastric cancer is in the eleventh rank constituting 2.1% of all cancers. At the National Cancer Institute (NCI), Cairo University, gastric cancer constituted 1.8% of all cancers and 10.3% of gastrointestinal cancers with a median age 53 compared to a median age 70 years in the USA***(El Bolkainy et al., 2013).***

Gastric cancer presents various histological features. The most widely used classification of gastric cancer is Lauren classification which distinguishes intestinal (differentiated) type GC and diffuse (undifferentiated) type GC ***(Lauren, 1965***).

The recent years have been witnessed for progresses in cancer therapeutics and chemotherapy development, with improved survival periods for these patients. Gastric cancer treatment follows the introduction of new anti-target drugs based on a more thorough understanding of molecular mechanisms of cell-cycle deregulations in cancer ***(Cunningham et al., 2006).***

Transformed growth factor beta (TGF-β) signaling has been shown to play important roles in the metastatic spread of cancer cells such as migration, invasion and epithelial to-mesenchymal transition (EMT) ***(Massagué, 2009).*** TGF-β signaling had become an important therapeutic target, several reports of such molecular targeted therapies relying on the interference with the adhesion and proliferation ***(Komuro et al., 2009).***

Cathepsin E (CTSE), a non-lysosomal intracellular aspartic protease, is one of the cathepsin

family proteases. CTSE is mainly expressed in cells of the immune systems such as macropahges, lymphocytes and dendritic cells. The enzyme participates in processing of the neurotensin precursors and other bioactive peptidesas well as functions in antigen processing in dendritic cells ***(Chain et al., 2005)***. The enzyme has a substantial role in host defense against tumor cells through TRAIL-dependent apoptosis without affecting normal cells ***(Kawakubo et al., 2007)***. In particular, it was shown that tumor growth arrest through inhibition of angiogenesis is induced by stable expression of Cathepsin E ***(Shin et al., 2007***). Expression of CTSE in the stomach had been reported ***(Matsuo et al., 1996).*** The physiological and pathological function of gastric CTSE remains unknown ***(Zaidiand Kalbacher, 2008).***

2. Material and methods

40 cases with gastric lesions comprise the material of this study. These include 40 paraffin blocks selected from gastric specimens (30 cases were obtained by endoscopy and 10 cases were surgical specimens) received at the pathology department, Faculty of Medicine, Zagazig University in the period from September 2013 to September 2014.

Aim of this study to

Examine the expression and the role of TGF-β1 and cathepsin E in gastric cancer and gastric precancerous lesions and to correlate between these markers and clinicopathological parameters.

Methods:

I- For histopathological evaluation: Paraffin blocks of all cases were sectioned at 3-4 micron thickness and stained with hematoxylin and eosin stain ***(Kiernan, 2001)*** to reevaluate and confirm the diagnosis.

II- For immunohistochemical evaluation:

Paraffin sections 3-5 um were deparaffinized in the oven at 56 ˚C for 30 minutes, and inserted in xylene for 30 minutes. Tissues were rehydrated in descending grades of alcohol 95%, 85% and then 75% for 5 minutes each. Slides were rinsed with distilled water for 5 minutes. Antigen retrieval was performed by boiling in sodium citrate buffer (0.001M, pH 6) for 15 minutes in microwave. Endogenous peroxidase activity was blocked by incubation with hydrogen peroxide for 10 minutes. Then rinse with distilled water. Apply primary anti-human CTSE (goat polyclonal antibody Cataloged (Cat.) from Thermo Scientific/Lab Vision Corporation, Fermont, USA, and clone: AF1294. 0.09% sodium azide. Dilution 1:100) and anti TGF-β1 (Mouse monoclonal Cataloged (Cat.) from Thermo Scientific/Lab Vision Corporation, Fermont, USA, and clone: MCA797T, 0.09% sodium azide. Dilution 1:100) overnight at 4˚C. After 3 wash with PBS and sections were incubated with biotinylated secondary antibodies at for 30 min. This is followed by incubation with streptavidin-biotin-peroxidase complex. After 3 rinses with PBS, The slides were incubated with diaminobenzidine for 15 min. The slides were rinsed with H2O and counterstained with hematoxylin for 3 minutes. This was followed by washing in cold running water, then wash in distilled water. Sections were dehydrated in ascending grades of alcohol and cleared with xylene, then cover slipped and examined.

Negative-control were done by omitting the primary antibodies.

Positive control for Cathepsin E were Hodgkin lymphoma or spleen Scoring criteria.

TGF β1 reactivity was present in more than 10% of the tumor cells, and negative if staining was present in less than 10% of the tumor cells ***(Docea et al., 2012).***

CTSE positive staining (from 1 to 4) were decided as follows: 1, percentage of cells with immunoreactivity of CTSE ranges from 0% to 10%; 2, percentage of cancer cells with immunoreactivity of CTSE ranges from 10% to 50%; 3, percentage of cancer cells with immunoreactivity of CTSE ranges from 50% to 90%; 4, percentage of cancer cells with immunoreactivity of CTSE is greater than 90% ***(Shimizu et al., 2013).***

Statistical Analysis

Categorical variables were expressed as a number (percentage). Percent of categorical variables were compared using the Pearson's Chi-square ($\chi 2$) test. All tests were two sided, p-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA).

3. Results

The results are shown in Tables 1-10 and Figures 1-9.

Table1:Age and gender of studied cases

Gastric lesions	Male	female	Mean age
Chronic gastritis	3	4	48.8±12
chronic atrophic gastritis with intestinal metaplasia	9	4	58.5±15
chronic atrophic gastritis with dysplasia	2	4	54.3±22.2
gastric cancer	8	6	55.9±13.4

Table 2: Histopathologic results of studied cases

Histopathologic diagnosis	No	%
Chronic gastritis (chronic superficial and atrophic gastritis)		
• Chronic superficial gastritis	2	5%
• Chronic atrophic gastritis	5	12.5%
Gastric metaplasia		
• Complete intestinal metaplasia (IM)	4	10%
• Incomplete intestinal metaplasia (IM)	9	22.5%
Gastric dysplasia		
• Low grade dysplasia	4	10%
• High grade dysplasia	2	5%
Gastric carcinoma		
• Tubular adenocarcinomas (intestinal type)	7	17.5%
• papillary carcinoma	2	5%
• mucinous carcinomas	2	5%
• signet ring carcinoma	3	7.5%
Total	40	100%

Immunohistochemical results

Table 3: Expression of TGF β1 in gastric lesions

	Total number of cases	TGF beta1 expression				χ^2	p-value
		Negative expression (≤10%) (n=9)		Positive expression (>10%)(n=31)			
		No	%	No	%		
Chronic superficial gastritis & chronic atrophic gastritis	7	0	0 %	7	100 %	2.463	0.117
Completeintestinal metaplasia (IM)	4	4	100 %	0	0 %	15.309	<0.001*
Incomplete IM	9	1	11.1 %	8	88.8 %	0.864	0.353
Gastric dysplasia	6	1	16.6 %	5	83.3 %	0.138	0.711
Gastric cancer	14	4	28.6 %	10	71.5 %	0.455	0.500

Table 4: Expression of cathepsin E in gastric lesions

	Total number of cases	Cathepsin E expression				χ^2	p-value
		Negative expression (n=13)		Positive expression (n=27)			
		No	%	No	%		
Chronic superficial gastritis & chronic atrophic gastritis	7	0	0 %	7	100 %	4.085	0.043
Completeintestinal metaplasia (IM)	4	4	100 %	0	0 %	9.231	0.002*
Incomplete IM	9	0	0 %	9	100 %	5.591	0.018
Gastric dysplasia	6	2	33.3 %	4	66.7 %	0.002	0.962
Gastric cancer	14	7	50 %	7	50 %	3.007	0.083

Table 5: Expression of TGF β1 in gastric cancer

	N	Positive expression				Total positive		X2	P
		Low Less than 10% (negative)		High more than 10% Positive					
		N	%	N	%	N	%		
Adenocarcinoma									
GI	2	0	0%	2	100%	2	100%	0.01	0.9
G2	2	1	50%	1	50%	1	50%	0.01	0.9
G3	3	1	33.3%	2	66.6%	2	66.6%	0.27	0.6
Papillary	2	1	50%	1	50%	1	50%	0.01	0.9
Mucinous	2	1	50%	1	50%	1	50%	0.01	0.9
Signet ring	3	0	0%	3	100%	3	0%	0.27	0.6
Total	14	4	28.57%	10	71.42%	10	71.42%		

Table 6: Cathepsin E (CTSE) expression in gastric cancer

	Negative		Positive expression							
Histological Typing of Gastric Cancer	NO	%	4	3	2	1	Total positive		X2	p
							NO	%		
Well differentiated tubular adenocarcinoma 2 cases	1	50%	0	0	1	0	1	50%	2.92	0.4
Moderately differentiated tubular adenocarcinoma 2 cases	1	50%	0	0	1	0	1	50%	2.92	0.4
Poorly differentiated adenocarcinoma 3 cases	1	33.3%	0	2*	0	0	2	66.6%	8.91	0.03*
Papillary adenocarcinoma 2 cases	2	100%	0	0	0	0	0	0%	2.33	0.5
Mucinous adenocarcinoma 2 cases	2	100%	0	0	0	0	0	0%	2.33	0.5
Signet-ring cell carcinoma 3 cases	0	0%	3*	0	0	0	3	100%	14.0	0.002*
Total 14	7	50%	3	2	2	0	7	50%		

Table 7: Expression of Cathepsin E and TGF-β1 in gastric adenocarcinoma

	NO of cases	CathepsinE		TGF β1	
		Total Positive cases			
		N	%	N	%
Tubular adenocarcinoma	7	4	57.14%	5	71.42%
Papillary carcinoma	2	0	0%	1	50
Mucinous carcinoma	2	0	0%	1	50%
Signet ring carcinoma	3	3	100%	3	100%
Total	14	7	50%	10	71.42%
X2		7.14		2.1	
p		0.06		0.55	

Table 8: Immunohistochemical reactivity for Cathepsin E and TGF beta1 expression in well, moderately and poorly differentiated gastric adenocarcinoma

	N	Cathepsin E				TGF β1			
		-		+		-		+	
		N	%	N	%	N	%	N	%
Well Differentiated	2	1	50	1	50%	0	0%	2	100%
Moderately differentiated	2	1	50%	1	50%	1	50%	1	50%
Poorly differentiated	3	1	33.3%	2	66.6%	1	33.3%	2	66.6%
Total	7	3	42.85%	4	57.14%	2	28.57	5	71.42%
X^2		0.19				1.28			
P		0.9				0.52			

Table 9: ***Variability of TGF beta1 immunoexpression with the clinicopathological parameters***

	negative <10%		positive >10%		**X2**	P
Age						
<50	0	0%	5	50%		
>50	4	100%	5	50%	0.31	0.25
Gender						
Male	1	25%	9	90%	5.49	0.019*
Female	3	75%	1	10%		
Gastric adenocarcinoma						
Intestinal	3	75%	6	66.6%		
Papillary	1	25%	1	10%		
Diffuse	0	0%	3	33.3%	1.75	0.41
Grade						
I	2	50%	4	40%		
II	1	25%	1	10%	0.93	0.62
III	1	25%	5	50%		
Stage (T)						
T1	2	50%	4	40%		
T2	1	25%	0	0%	3.55	0.31
T3	1	25%	4	40%		
T4	0	0%	2	20%		
Lymph node						
Negative	2	50%	5	50%	0.0	1.0
Positive	2	50%	5	50%		

Table 10: ***Variability of Cathepsin E immunoexpression with the clinicopathological parameters***

	negative		Positive		**X2**	P
Age						
<50	2	40%		33.3%		
>50	3	60%		66.6%	0.11	0.73
Gender						
Male	3	60%	7	77.8%	0.01	0.92
Female	2	40%	2	22.2%		
Gastric adenocarcinoma						
Intestinal	3	60%	6	66.6%		
Papillary	2	40%	0	0%	5.29	0.07
Diffuse	0	0%	3	33.3%		
Grade						
I	4	80%	2	22.2%		
II	0	0%	2	22.2%	4.56	0.01
III	1	20%	5	55.6%		
Stage (T)						
T1	2	40%	4	44.4%		
T2	1	20%	0	0%	2.97	0.39
T3	2	40%	3	33.3%		
T4	0	0%	2	22.2%		
Lymph node						
Negative	2	40%	5	55.6%	0.0	1.0
positive	3	60%	4	44.4%		

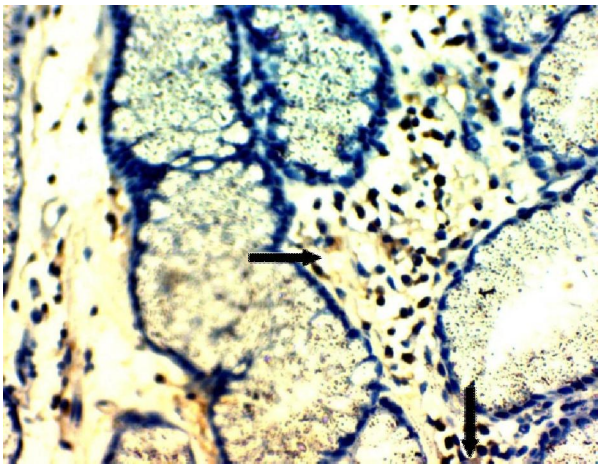

Figure 1. Chronic atrophic gastritisshowing cytoplasmic immune-reactivity for TGF beta1in chronic inflammatory cells mainly in plasma cells (ABC, DAB x400).

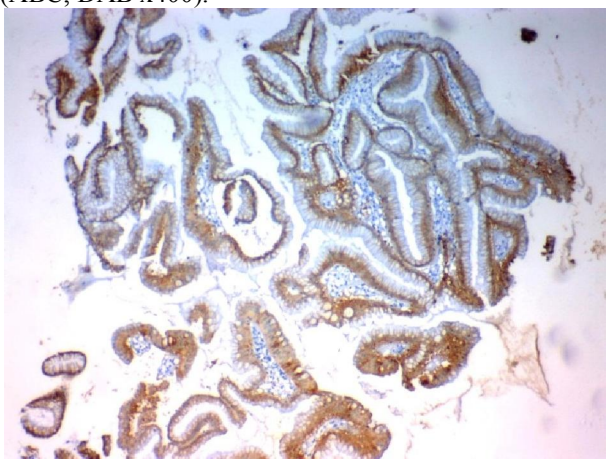

Figure 2. Incomplete gastric metaplasia; showing cytoplasmic immune-reactivity for cathepsinE (ABC, DAB x100).

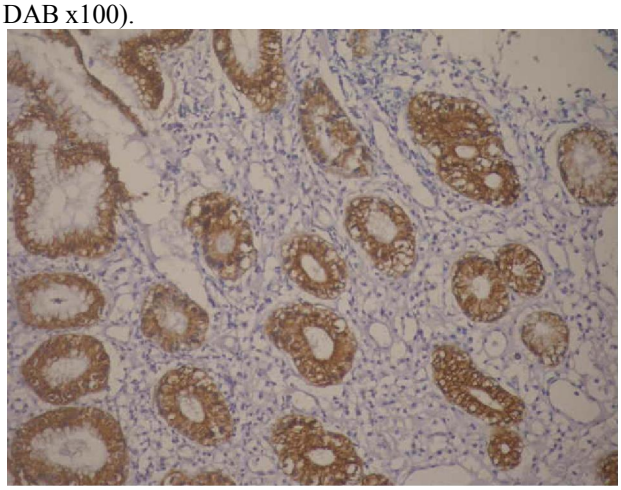

Figure 3. **Low grade gastric dysplasia; showing cytoplasmic immune-reactivity for TGF beta1 (ABC, DAB x100).**

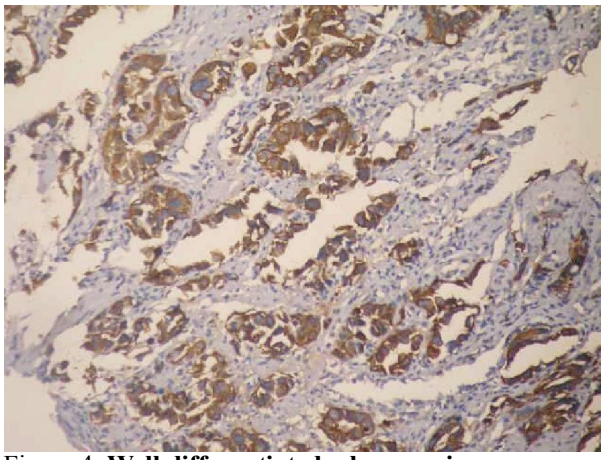

Figure 4. **Well differentiated adenocarcinoma; showing cytoplasmic immune-reactivity for TGF beta1. (ABC, DAB x100).**

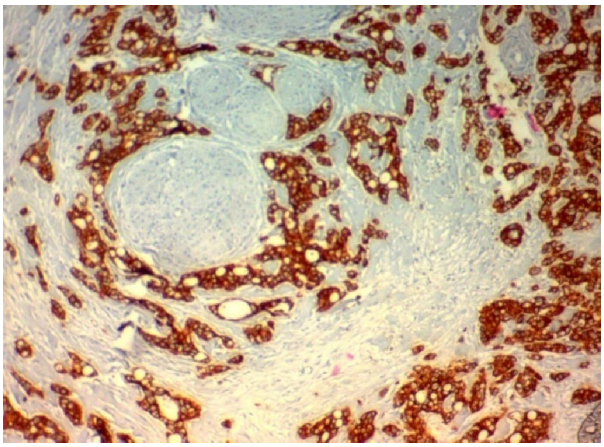

Figure 5. **Poorly differentiated adenocarcinoma with muscle invasion; showing cytoplasmic and membranous immune-reactivity for TGF beta1 (ABC, DAB x100).**

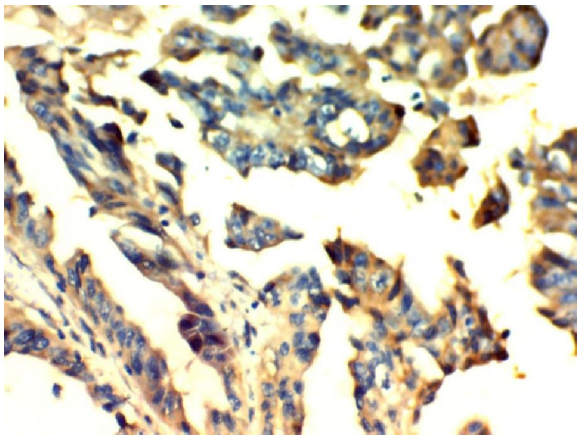

Figure 6. **Papillary adenocarcinoma; showing cytoplasmic immune-reactivity for TGF beta1 (ABC, DAB x400).**

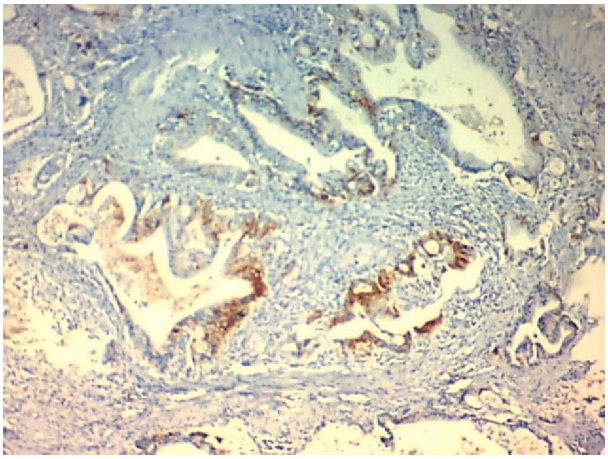

Figure 7. **Well differentiated adenocarcinoma; showing cytoplasmic immune-reactivity for cathepsin E (ABC, DAB x100).**

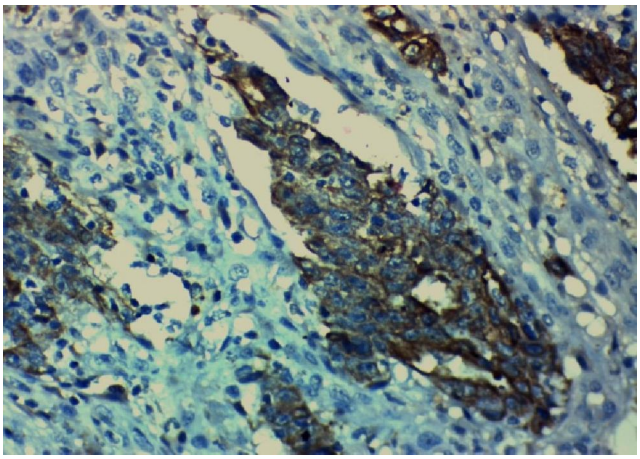

Figure 8. **Poorly differentiated adenocarcinoma; showing cytoplasmic and membranous immune-reactivity for cathepsinE (ABC, DAB x100).**

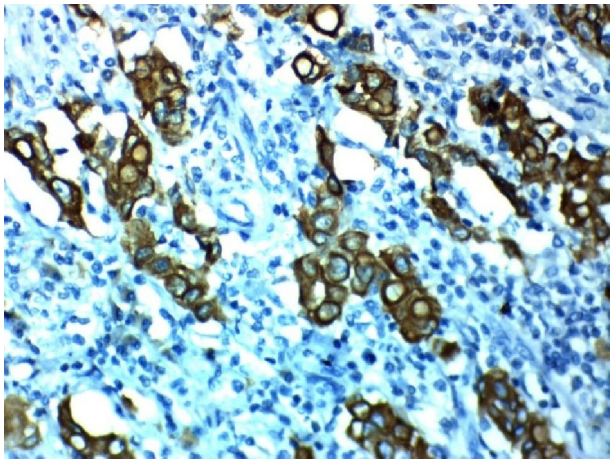

Figure 9. **Signet ring carcinoma; showing cytoplasmic and nonspecific membranous immune-reactivity for cathepsinE (ABC, DAB x400).**

4. Discussion

The potential roles of TGF-βs in the normal gastric mucosa was suggested that it may participate in the autocrine and paracrine regulation of gastric mucosal functions and that some aspects of his regulation may be TGF-β isoform-specific ***(Naef et al., 1997)***. ***Mishra et al. 1999*** raised the hypothesis that TGF-β and Wnt proteins are key morphogens that ultimately influence cell division, so that gut endodermal stem cells enter the cell cycle and undergo cell division that leads to differentiated cells such as gastric parietal cells ***(Mishra et al., 2005)***. Thus, disruptions and errors in this process can lead to gastric adenocarcinomas. The implication of TGF-βs and its receptors in gastric carcinogenesis was demonstrated by Kim et al., which noticed a continually TGF-β1, -β2 and TGFBR1 increasing expression along the normal epithelium– atrophic gastritis–dysplasia–carcinoma sequence ***(Kim et al., 2008).***

Examination of TGF-β1 stained sections revealed positive expression in the cases of chronic gastritis especially in inflammatory cells. The result is in agreement with ***Docea et al., 2012***.

In the present study, TGF-β1 is expressed in cases with intestinal metaplasia (IM) and gastric dysplasia, the intensity was more than normal stomach. The present findings are in agreement with several former studies by ***Docea et al., 2012***and ***Kim et al., 2008***.

As regards TGF-β1 expression in gastric cancer, the present study showed positive expression in 71.42% (5/7) of tubular adenocarcinoma (intestinal type), 50% (1/2) for papillary carcinoma, 50% (1/2) for mucinous carcinoma and 100% for the 3 cases of diffuse type (signet ring carcinoma).

The current study is consistent with previous studies ***Ananiev et al 2011.*** (73.8%) who investigated 42 specimens of gastric carcinomas and by ***Vagenas et al. 2007*** (71%) who studied 110 gastrectomycases.

In contrast, however, ***Saito et al. 1999*** found TGF-β1 expression in 22.8% who investigated 101 gastric carcinoma cases.

This difference was attributed to the small number of intestinal type gastric cancer cases (only 7) in the present study.

As regards TGF-β1 immunoreactivity in different histological subtypes of gastric carcinoma, the current study showed TGF-β1 expression in diffuse type adenocarcinoma more than intestinal type the results are in agree with ***Kai et al. 1996*** who noticed a strong staining for TGF-β1 only in diffuse type carcinoma, especially in that carcinoma cells that are scattered as single cells or as small nests, on the contrary ***Ananiev et al. 2011*** and ***Zolota et al. 2002*** observed that carcinomas of the intestinal type were

more frequently positive for TGF-β1 when compared to the diffuse type ones.

As regards expression of TGF-β1 expression and the degree of tumor differentiation the expression was high in well differentiated tumors 100% positive expression; this is parallel to results obtained by ***Vagenas et al., 2007 and Saito et al., 1999***. But the difference was statistically in significant. The results are different from ***Ito et al. 1992*** found that TGF-β1 expression was higher in gastric high-grade malignancy lesions**.** In contrast, however, other investigators noticed that TGF-β1 reactivity in gastric cancer did not depend on differentiation ***Maehara et al., 1999 and Park et al., 1997.***

In the current work, no significant difference found between TGF β1 expression and the depth of invasion or lymph node status in contrast, however, several former studies by ***Vagenas et al., 2007 and Saito et al., 2000 and Maehara et al., 1999*** showed a significant correlation of TGF-β1 reactivity with progression and prognosis in primary gastric cancers ***Saitoet al. 1999*** showed that nodal involvement correlated with TGF-β1 mRNA expression in early and advanced carcinomas. This is supported by the observation of ***Maehara et al., 1999*** according to which TGF-β1 expression in gastric cancer cells was closely related to the higher rate of lymph node metastasis. The authors concluded that the preferential expression of TGF-β1 in lymph node metastases suggests a clonal selection of tumor cells with TGF-β1 expression, specific for the higher potential of lymph node metastasis in tumor advance, and that TGF-β1 has a role in the malignant progression of gastric cancer. ***Yu et al. 2003*** reported that TGF-β1 was highly expressed in gastric carcinoma tissues with lymph node metastasis and distant metastasis. The discrepancy attributed to small number of cases in the current study.

CathepsinE(CTSE) was reported to have some anti-oncogenic potential ***Kawakubo et al. 2007*** demonstrated that CTSE specifically induces growth arrest and apoptosis in human prostate cancer cell lines by catalyzing the proteolytic release of soluble tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) from the cell surface.

The expression of Cathepsin E was also studied in the current work. It was expressed in all cases of chronic superficial gastritis and chronic atrophic gastritis. It was strongly expressed, in the cytoplasm of mucous neck cells, secretory cells of the body and deeper glands of the antrum.

Studying Cathepsin E expression in complete intestinal metaplasia versus incomplete intestinal metaplasia has shown a complete negative expression in the former in contrast to its expression in the latter. This is in agree with ***Shimizu et al. 2013.***

The decreased expression of Cathepsin E in gastric carcinomas was confirmed in the current study where Cathepsin E expression 50% in well and moderately differentiated adenocarcinoma and 66.6% in poorly differentiated gastric adenocarcinoma. This is parallel to that of ***Shimizu et al. 2013.***

In the present work negative Cathepsin E expression in papillary carcinoma, the result was different from ***Shimizu et al. 2013.*** The discrepancy may be due to different number of studied groups.

The current study showed a statistically significant positive cathepsin E expression in 100% of diffuse- type gastric cancer (signet ring carcinoma) in contrast to the 100% negative expression in mucinous carcinoma. The present result is in agreement with that of ***Shimizu et al. 2013.***

Positive Cathepsin E was significant in signet ring carcinoma and poorly differentiated gastric adenocarcinomathis indicates that Cathepsin E could be a marker of diffuse type of gastric carcinoma. The present result is in agreement with that of ***Shimizu et al. 2013.***

In the present study, no significant difference found between Cathepsin E expression and the depth of invasion or lymph node status.

A sum-up of data concerning change in expression of these two markers was carried out in the present study to shed light on their importance both together or separately to evaluate their role during tumorigenesis of gastric neoplasia and in differentiation between gastric cancer intestinal type and diffuse (signet ring type).

Conclusion

The alterations of expression of TGF and Cathepsin E can determine the histological variants of gastric cancer (GC). Cathepsin E could be a marker for signet ring carcinoma. Understanding the expression pattern of TGF beta and Cathepsin E in gastric cancer allows them to be used as future therapeutic targets.

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