Journal of the Egyptian National Cancer Institute (2016) **28**, 149–156

Cairo University

Journal of the Egyptian National Cancer Institute

www.elsevier.com/locate/jnci
www.sciencedirect.com

Full Length Article

Expression of cyclooxygenase 2 and vascular endothelial growth factor in gastric carcinoma: Relationship with clinicopathological parameters

Nesreen H. Hafez *, **Neveen S. Tahoun**

Department of Pathology, National Cancer Institute (NCI), Cairo University, Egypt

Received 24 February 2016; revised 18 May 2016; accepted 30 May 2016
Available online 21 June 2016

KEYWORDS

Gastric adenocarcinoma;
COX-2;
VEGF;
IHC

Abstract *Background:* Gastric cancer is one of the most common cancers and the second most common cause of cancer-related death worldwide. Identification of specific prognostic indicators might allow a better prognostic stratification and more effective therapy.
Aim: To assess the expression and relationship between COX-2 and VEGF protein in gastric adenocarcinoma and whether these markers are useful in predicting clinicopathological prognostic parameters.
Materials and methods: The study included 83 formalin-fixed paraffin embedded tissue samples of excised gastric adenocarcinoma and 20 non tumorous tissue controls. The slides were subjected to COX-2 and VEGF immunohistochemical staining using a streptavidin–biotinperoxidase according to the manufacturer's protocol. The results were assessed independently by two pathologists. The relationships among COX-2 and VEGF expression and clinicopathological parameters were statistically analyzed.
Results: COX-2 and VEGF expressions were obviously higher in carcinoma tissues compared to normal mucosae ($p < 0.001$). The expression rate of COX-2 was 54.2% and of VEGF was 68.7%. COX-2 positive tumors were significantly correlated with Lauren classification, tumor depth and *Helicobacter pylori* infection ($p < 0.001$, $p = 0.008$, $p = 0.035$). VEGF was significantly associated with lymph node metastasis and tumor depth ($p < 0.001$). There was a positive association between VEGF and COX-2 expression in gastric adenocarcinoma (Kappa value = 0.55).
Conclusion: In gastric adenocarcinoma, COX-2 expression might serve as a powerful indicator for intestinal type carcinoma, locally advanced disease and *H. pylori* infection, while VEGF was related to loco-regional progression. COX-2 might be involved in the development of angiogenesis in gastric carcinoma through VEGF upregulation.

* Corresponding author. Tel.: +22 0823150, +20 01004959148.
E-mail address: nesreennci@hotmail.com (N.H. Hafez).
Peer review under responsibility of The National Cancer Institute, Cairo University.

http://dx.doi.org/10.1016/j.jnci.2016.05.005

Introduction

Gastric cancer (GC) is one of the most common cancers worldwide with a relative frequency of 7.8% of all cancers [1,2]. More than 90% of gastric cancers are adenocarcinomas. In the latter half of the twentieth century, GC was the second most common cause of cancer-related deaths after lung carcinoma accounting to 11.3% of all cancer deaths [3]. In Egypt, GC represented 1.6% of all cancers and 2.2% of all cancer mortality [4]. According to the registry of the Egyptian National Cancer Institute, GC formed 2.12% of total malignancy and 10.3% of gastrointestinal cancers [5].

Despite advances in diagnosis and treatment, the prognosis of patients with GC has remained unsatisfactory. Nearly one-third of the patients (29.9%) experienced recurrence after gastric surgery. It is the main cause of cancer related death [6]. One major difficulty in the therapy of GC is the presence of only few prognostic indicators that can predict its clinical behavior. Therefore, identification of other specific prognostic markers might allow a better prognostic stratification and thus more effective therapy [3].

Cyclooxygenase (COX) is a key enzyme in prostaglandin synthesis from arachidonic acid. There are two enzyme forms, COX-1 isoform, a component of the normal cells that has been connected to physiological functions, and the COX-2 isoenzyme that is frequently undetectable in most normal tissues [6]. Overexpression of COX-2 protein has been detected in some tumors including GC. It was reported that its overexpression is associated with poor prognosis and reduced survival [7]. The mechanism by which COX-2 induces carcinogenesis is not known until now. COX-2 enzyme may stimulate cell proliferation, inhibit apoptosis, increase invasiveness and induce angiogenesis by elaborating some angiogenic factors such as vascular endothelial growth factor (VEGF) [8].

Angiogenesis plays a critical role in tumor progression and metastasis. In the vast majority of malignancies, including gastrointestinal neoplasms, angiogenesis has been associated with poor prognosis and relapse of the tumor. The best known and the most efficient angiogenic growth factor is VEGF [9]. VEGF is known to accelerate endothelial cellular proliferation, vascular permeability, and endothelial cell migration, and inhibit apoptosis, whereas inhibition of VEGF results in suppression of tumor growth [10].

The aim of the current study is to assess: COX-2 and VEGF immunohistochemical (IHC) expression in GC cases, whether these markers are useful in predicting clinicopathological prognostic parameters and whether there is an association between the expression of COX-2 and VEGF.

Materials and methods

The present retrospective study included 83 patients with histopathologically proven gastric adenocarcinoma who underwent curative surgical resections that were retrieved from the files of Pathology Department, National Cancer Institute, Cairo University between June 2008 and December 2014. The control group included 20 cases with non neoplastic gastric tissues that underwent endoscopic biopsy during the same period. All specimens were taken from the archives of the Pathology Department.

Clinicpathological data including age, sex, location, histological type, grade, depth of invasion, nodal status and *Helicobacter pylori* (*H. pylori*) infection in the non neoplastic adjacent mucosa were determined from the pathology reports. The eligibility criteria included: histopathologically proven gastric adenocarcinoma classified according to the World Health Organization classification [11], no neoadjuvant chemotherapy and/or radiotherapy and availability of complete clinicalpathological data.

Immunohistochemical method

The archival histopathological slides of all studied cases were reviewed to confirm the diagnosis, to detect *H. pylori* in the adjacent mucosa in some cases and to choose the appropriate paraffin embedded tissue blocks for sectioning and IHC staining. For each case; two serial sections, of 4 μm thickness were cut by the microtome then mounted onto positively charged slides.

The slides were subjected to IHC staining using a streptavidin–biotin-peroxidase according to the manufacturer's protocol using BenchMark XT automated slide stainer (a product of Ventana Medical Systems). All sections were deparaffinized by xylene, rehydrated by a graded series of ethanol, and treated with 0.3% H_2O_2 for 5 min at room temperature to block endogenous peroxidase activity. Heat-based antigen retrieval was performed to obtain optimal results. Sections were treated with 5% bovine serum albumin to block non-specific staining. The slides were incubated with the primary antibody, anti-COX-2 antibody (monoclonal rabbit anti-human, clone SP2, in a dilution of 1:100, Thermo Scientific, USA) and anti-human VEGF antibody (monoclonal mouse, clone VG1, M7273, DakoCytomation, Denmark, at a 1:50 dilution). Diaminobenzidine was used as a chromogen and hematoxylin as a counterstain.

Appropriate positive and negative controls were included in each IHC run. Negative controls were prepared by replacing the primary antibody with Phosphate Buffered Saline (PBS). Positive staining controls for COX-2 included sections of colonic carcinoma. Positive staining controls for VEGF included sections of hemangioma tissue

Evaluation of immunohistochemical staining

The expression of COX-2 and VEGF were assessed independently by two pathologists who were blinded to the clinicopathological parameters of the patients. COX-2 and VEGF immunoreactivity was detected in the cytoplasm of the cells. The IHC score was calculated by adding the percentage of positively stained cells to the staining intensity. The percentage of positive cells ranged between 0 and 3, i.e. 0, if less than 10% of tumor cells were stained; 1, if 10–25% of tumor cells were stained; 2, if 25–50% were positive; and 3, if $>50\%$ were positive. The staining intensity was scored as: 0, negative immunoreaction; 1, weak intensity; 2, moderate intensity; and 3, strong intensity. The sum of the two parameters varied between 0 and 6. In our study, we considered: a negative immunoreaction (−), for scores between 0 and 2; a weakly positive immunoreaction (+), for scores 3 and 4; a strongly positive immunoreaction (++), for scores 5 and 6. Cases with scores equal to or higher than 3, were considered as positive [8,12].

Statistical analysis

Statistical analyses were performed with SPSS 11.5 software (SPSS Inc, Chicago, USA). The correlation between COX-2 and VEGF protein expressions and clinicopathological characteristics were evaluated by Fisher's exact and chi-square test for categorical variables. *P* value less than 0.05 was considered statistically significant. Kappa statistics was used to assess the level of agreement between COX-2 and VEGF expression; Kappa values ranging from 0.61 to 0.8 were assumed to indicate a very good agreement.

Results

The studied patients consisted of 60 males (72.3%) and 23 females (27.7%) with male: female ratio of 2.6:1. Ages ranged between 36 and 88 years old with a mean age of 53 years with standard deviation of 6.192. The control group included 11 males (55%) and 9 females (45%) ranging between 22 and 63 years with a mean age of 48.1 years.

Analysis of COX-2 immunoreactivity

We investigated the IHC expression of the COX-2 protein in 83 GC tissues and in 20 non cancerous gastric mucosae. COX-2 expression was significantly higher in gastric cancer tissues vs. control mucosae ($p < 0.001$) (Table 1).

COX-2 expression was noted in 45 (54.2%) of the 83 studied GC cases (Table 1). The expression rate of COX-2 was 54.2%. The majority of positive cases (32/45) exhibited strong expression for COX-2 protein, score + +, while 13 cases exhibited weak expression, score + (Table 2, Figs. 1–3).

Thirty four cases (41%) of gastric carcinoma were associated with intestinal metaplasia in neighboring mucosae. In 16 cases (47.1%), epithelial cells in the adjacent intestinal metaplastic region showed COX-2 staining (score +). Ten out 83 gastric carcinoma cases (12%) were associated with dysplasia, 4 cases (40%) were associated with COX-2 expression in these dysplastic epithelial cells (score +).

Clinical and pathological characteristics of the 83 studied GC patients are listed in Table 3. We assessed the relationship between COX-2 expression and various clinicopathological parameters (Table 3). COX-2 positive tumors were noted in 33 (73.3%) GC cases of the intestinal-type and in 12 (31.6%) of the diffuse type, the expression was significantly higher in the intestinal than in the diffuse carcinomas ($p < 0.001$). The COX-2 expression was significantly correlated with the depth of invasion ($p = 0.008$). COX-2 positive tumors were significantly detected among *H. pylori* infected patients ($p = 0.035$). Among the histological subtypes, COX-2 expression was lower in signet-ring cell carcinoma and undifferentiated carcinoma than other subtypes; however the statistics were not valid as the numbers of some subtypes were very low.

Table 2 Scores of COX-2 and VEGF expression in the 83 studied GC cases.

Score	COX-2	VEGF
+ +	32	22
+	13	35
−	38	26
Total	83	83

There were no significant relationships between the levels of COX-2 expression and each of age, sex, location, grade of the tumor and lymph node status ($p > 0.05$)

Analysis of VEGF immunoreactivity

VEGF expression was observed in the cytoplasm of cancer cells. Fifty seven out of eighty three cases (68.7%) were VEGF positive. VEGF expression was significantly higher in gastric cancer tissues vs. the control mucosae ($p < 0.001$) (Table 1). Weak expression, score +, was found in 35 cases while strong expression, score + +, was observed in 22 cases (Table 2). Ten out of thirty four cases with intestinal metaplasia in the adjacent mucosa (29.4%) were positive for VEGF (score +) in the metaplastic area (score +). Five of ten (50%) dysplasias of the stomach were positive for VEGF (score +) (Figs. 4 and 5).

The associations between VEGF expression and the clinicopathological parameters are shown in Table 4. VEGF positivity was significantly higher in patients with lymph node metastasis than in those without ($p < 0.001$). VEGF expression was significantly correlated with the depth of invasion. The frequency of VEGF positive tumors was significantly higher in stages T3 and T4 than in the T1 and T2 ($p < 0.001$).

There were no significant relationships between VEGF expression and each of age, sex, location, grade of the tumor, Lauren classification, histological subtypes and *H. pylori* infection ($p > 0.05$)

Relationship between COX-2 and VEGF

In order to evaluate the relation between the IHC expression of COX-2 and tumor angiogenesis, we have evaluated the association between VEGF and COX-2 expression (Table 5). VEGF was higher in patients with COX-2 expression than in those without. Kappa value was 0.55 indicating a moderate agreement between COX-2 and VEGF expression.

Table 1 COX-2 and VEGF protein expression in cancer and control cases.

Cases	No.	COX-2 expression		*P* value	VEGF expression		*P* value
		+ ve	− ve		+ ve	-ve	
Gastric cancer	83	45	38	< 0.001	57	26	< 0.001
Control	20	1	19		0	20	

Table 3 Relationship between COX-2 expression and the clinicopathological factors of the 83 studied GC cases.

Clinicopathological factors	Total	COX-2 expression		*P* value
		+ve (n = 45)	−ve (n = 38)	
Age				
< 60 years	44	23 (52.3%)	21 (47.7%)	0.706
⩾ 60 years	39	22 (56.4%)	17 (43.6%)	
Sex				
Male	60	35 (58.3%)	25 (41.7%)	0.224
Female	23	10 (43.5%)	13 (56.5%)	
Location				
Antrum	41	22 (53.7%)	19 (46.3%)	0.943
Body	20	11 (55%)	9 (45%)	
Fundus	17	9 (52.9%)	8 (47.1%)	
Cardia	5	3 (60%)	2 (40%)	
Lauren classification				
Intestinal type	45	33 (73.3%)	12 (26.7%)	< 0.001
Diffuse type	38	12 (31.6%)	26 (68.4%)	
Histologic subtypes				
Tubular adenocarcinoma	33	25 (75.8%)	8 (24.2%)	NA*
Papillary adenocarcinoma	6	4 (66.7%)	2 (33.3%)	
Mucinous adenocarcinoma	7	5 (71.4%)	2 (28.6%)	
Signet ring adenocarcinoma	31	10 (32.3%)	21 (67.7%)	
Undifferentiated carcinoma	6	1 (16.7%)	5 (83.3%)	
Grade				
1	6	3 (50%)	3 (50%)	0.732
2	50	29 (58%)	21 (42%)	
3	27	13 (48.1%)	14 (51.9%)	
Lymph nodes				
−ve	25	12 (38%)	13 (52%)	0.455
+ve	58	33 (56.9%)	25 (43.1%)	
Depth of invasion				
T1	7	0	7 (100%)	0.008
T2	13	5 (38.5%)	8 (61.5%)	
T3	23	14 (60.9%)	9 (39.1%)	
T4	40	26 (65%)	14 (35%)	
H. pylori infection				
+ve	69	41 (59.4%)	28 (40.6%)	0.035
−ve	14	4 (28.6%)	10 (71.4%)	

* NA, not applicable.

Discussion

Previous studies have concluded that COX-2 and VEGF expressions played important roles in the growth and metastasis of many human tumors including gastrointestinal cancers. Because of their high expression in tumors, they constitute potential targets in cancer prevention and treatment. Their expressions were also associated with a variety of clinicopathological parameters [3,6,12,13]. However, these associations remained controversial as some studies showed no such association [14,15]. These studies prompted us to evaluate COX-2 and VEGF expression at protein levels in tissues with GC and assess the relationship with clinicopathological data.

Several studies have reported that COX-2 expression is elevated in GC when compared with control normal mucosae [3,6,8,13,16,17]. Our results were concordant with this previous observation, confirming that COX-2 protein plays an important role in gastric carcinogenesis.

In the present study, COX-2 protein expression was detected in 54.2% of the studied GC cases. This finding was comparable to previous studies [3,8]. Higher and lower expression figures were reported by others [13,15,6]. These discrepancies may be related to the use of different scoring systems, change in specificity and sensitivity of antibodies employed in IHC or patient heterogeneity. Another possibility was suggested by Wang et al., 2014 [18] and Sierra et al., 2013 [19] who reported that *H. pylori infection* causes up-regulation of COX-2 mRNA expression in GC cases. Since it is proven that *H. pylori* infection varies from area to area in the world, the expression of COX-2 protein also varies. In an agreement with these conclusions, 59.4% of our *H. pylori* infected cases were positive to COX-2 protein. Hussein, 2010 [20] observed reduction, but not elimination, in COX-2 expression after treatment of *H. pylori infection*.

Among the 34 cases of intestinal metaplasia and the 10 cases with dysplasia in neighboring mucosae, 16 cases

Table 4 Relationship between VEGF expression and the clinicopathological factors of the 38 studied GC cases.

Clinicopathological factors	Total	VEGF expression		*P* value
		+ve ($n = 57$)	−ve ($n = 26$)	
Age				
< 60 years	44	28 (63.6%)	16 (36.4%)	0.293
⩾60 years	39	29 (74.4%)	10 (25.6%)	
Sex				
Male	60	42 (70%)	18 (30%)	0.674
Female	23	15 (65.2%)	8 (34.8%)	
Location				
Antrum	41	30 (73.2%)	11 (26.8%)	0.847
Body	20	13 (65%)	7 (35%)	
Fundus	17	11 (64.7%)	6 (35.3%)	
Cardia	5	3 (60%)	2 (40%)	
Lauren classification				
Intestinal type	45	30 (66.7%)	15 (33.3%)	0.668
Diffuse type	38	27 (71.1%)	11 (28.9%)	
Histologic subtypes				
Tubular adenocarcinoma	33	22 (66.7%)	11 (33.3%)	NA*
Papillary adenocarcinoma	6	4 (66.7%)	2 (33.3%)	
Mucinous adenocarcinoma	7	5 (71.4%)	2 (28.6%)	
Signet ring adenocarcinoma	31	22 (71%)	9 (29%)	
Undifferentiated carcinoma	6	4 (66.7%)	2 (33.3%)	
Grade				
1	6	4 (66.7%)	2 (33.3%)	1.000
2	50	34 (68%)	16 (32%)	
3	27	19 (70.4%)	8 (29.6%)	
Lymph nodes				
−ve	25	10 (40%)	15 (60%)	< 0.001
+ve	58	47 (81%)	11 (19%)	
Depth of invasion				
T1	7	2 (28.6%)	5 (71.4%)	< 0.001
T2	13	3 (23.1%)	10 (78.9%)	
T3	23	17(73.9%)	6 (26.1%)	
T4	40	35 (87.5%)	5 (12.5%)	
H. pylori infection				
+ve	69	48 (69.6%)	21 (30.4%)	0.698
−ve	14	9 (64.3%)	5 (35.7%)	

* NA, not applicable.

Table 5 Relationship between COX-2 and VEGF immunostaining in the studied cases of GC.

VEGF expression	COX-2 expression		Kappa value
	+ve	−ve	
+ve	42 (50.6%)	15 (18.1%)	0.55
−ve	3 (3.6%)	23 (27.7%)	

(47.1%) and 4 cases (40%) showed weak expression for COX-2 (scores+), respectively. These findings confirmed previous observations that COX-2 might be involved in the early stages of gastric cancer development [21]. Our result was almost concordant with others who reported immunoreactivity for COX-2 protein in 37.8% of cases with intestinal metaplasia and 41.7%, of intestinal dysplasia cases. They found that COX-2 expression in the metaplasia or dysplasia tissues was related to *H. pylori* infection [18]. In our series, 12 out of 16 (75%) COX-2 positive intestinal metaplasia cases and 100% of dysplasia positive cases were infected with *H. pylori*.

Our data revealed that COX-2 was expressed predominantly by the intestinal type GC in contrast to carcinoma of diffuse type ($p < 0.001$). Similarly, this finding was concordant with most of the previous studies [8,13,17,22]. The explanation of these results was related to the fact that *H. pylori* infection has been identified in almost 90% of intestinal type carcinoma which induced COX-2 expression in GC cells [18,22]. In the present study, *H. pylori* infection was identified in 100% of COX-2 positive intestinal type GC. However, this outcome was inconcordant with the results reported in a previous study [3].

This study indicated that the expression of COX-2 was significantly associated with deeper depth of invasion ($p = 0.008$) suggesting that this protein might be involved in the local progression of GC. This result was consistent with other reports [7,8,13,16] yet contradicted with others' [3,15].

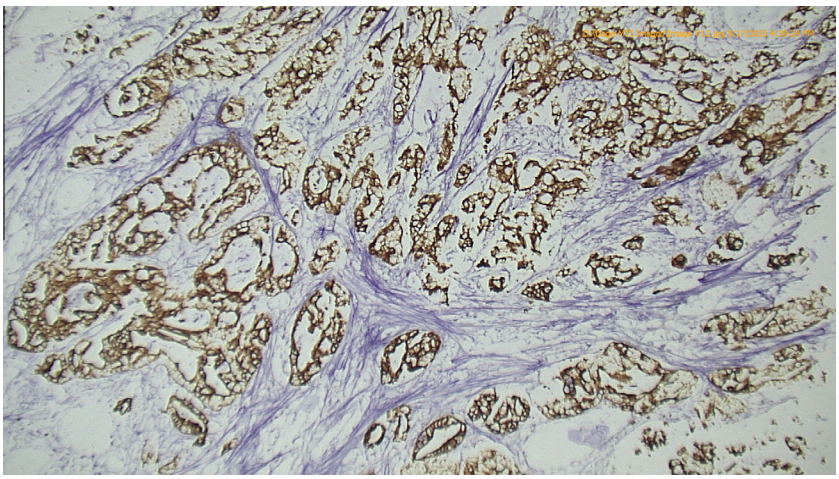

Figure 1 Immunohistochemical staining of COX-2 in tubular adenocarcinoma showing score + + positivity (×200).

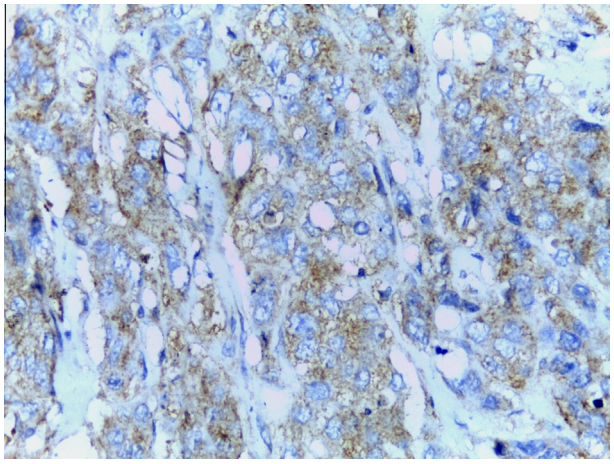

Figure 2 Immunohistochemical staining of COX-2 in undifferentiated carcinoma revealed score + positivity (×400).

Among the histological subtypes, COX-2 expression was significantly low in the signet-ring adenocarcinoma (32.3%) and in undifferentiated carcinoma (16.7%).

In agreement with others [14,15,17], no significant association was found between COX-2 expression and age, sex, tumor location, grade and lymph node status. These results were not in concert with the results of Lazar et al. (2008) [8] who revealed that COX-2 expression was significantly associated with tumor differentiation and lymph node status. Others reported an association between COX-2 expression and tumor grade [13]. Mao et al. (2007) [16] demonstrated that the expression was related to lymph nodes metastasis. Thiel et al. (2011) [23] concluded that the COX-2 expression is more frequent in proximal than in distal gastric location.

Tumor angiogenesis and its clinical significance have been evaluated in many human cancers. VEGF was confirmed to be a useful marker for the assessment of angiogenesis [12,13]. Based on our IHC evaluation, VEGF were highly expressed in GC tissues, but not in control normal mucosae. The difference in the expression was extremely significant ($p < 0.001$). Our result was in line with previous publications [12,13,17,24,25].

Cytoplasmic staining for VEGF was observed in 68.7% of the studied GC cases. Our findings were similar to other studies [9,12,26] but higher than results of others [10,27,28]. Zhao et al. (2006) [13] reported a higher positivity rate of 76.1%.

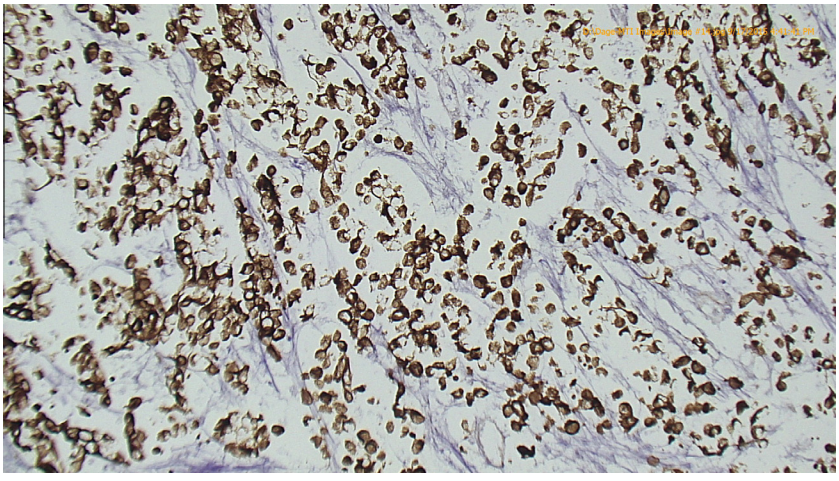

Figure 3 Strong cytoplasmic expression of COX-2 in signet ring adenocarcinoma, score + + (×200).

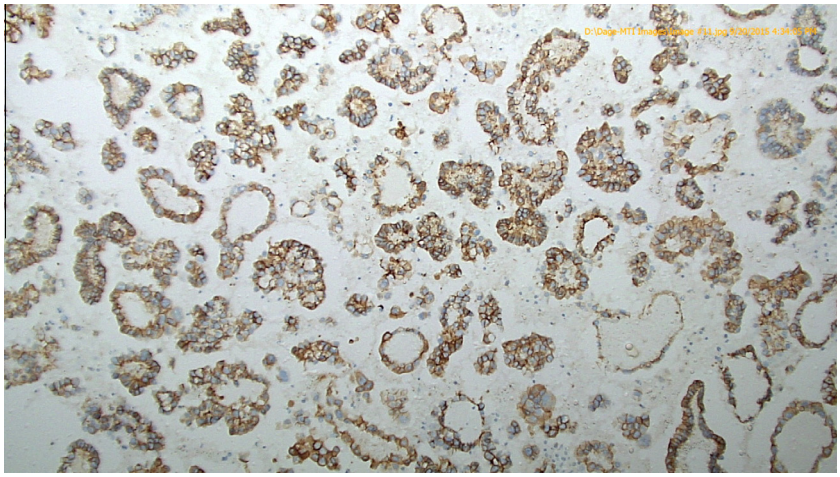

Figure 4 Immunohistochemical staining of VEGF in tubular adenocarcinoma showing score + + positivity (×200).

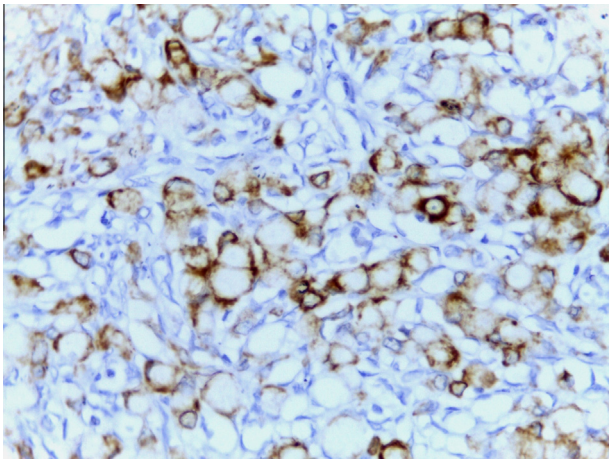

Figure 5 Immunohistochemical staining of VEGF in signet ring adenocarcinoma showing score + positivity (×400).

The variation between various studies could be related to sample size, different scoring system, different antibodies used or patient heterogeneity.

In the current study, 10 out of 34 cases with intestinal metaplasia in the adjacent mucosa (29.4%) were weakly positive for VEGF (score +). Positive reaction was also found in 6 out of 10 (60%) cases with gastric dysplasia. In the same context, others found positivity in 28.6% and 66.7% of their studied intestinal metaplastic and dysplasia cases and they reported that it is the expression of an early tumor angiogenesis during the natural evolution from the normal mucosa to carcinoma [29].

We related VEGF expression to clinicopathological data of the studied GC cases. Expression of VEGF was found to be significantly linked to the depth of invasion ($p < 0.001$) and lymph node metastasis ($p < 0.001$). These results indicated that VEGF promoted GC local invasion and metastasis. Our results were in agreement with the results obtained by others [12,17,26]. However, other researchers failed to prove these significant relations [9,24]. In the current study, we did not observe a statistically significant relation between VEGF overexpression and some clinicopathological features, such as age, sex, location, grade, Lauren classification, histological subtypes and *H. pylori* infection ($p > 0.05$). Our results were confirmed by others [15,17]. Previous studies have demonstrated a positive association with tumor location [28] as well as histological type and grade [13,25]. Others reported a positive relation between VEGF expression in and *H. pylori* gastric cancer cells [29].

In order to evaluate the contribution of COX-2 expression to tumor angiogenesis, we have evaluated the association between the VEGF and COX-2 expression. Our result revealed a positive association (Kappa value = 0.55). These data suggest that COX-2 is involved in the development of angiogenesis in GC cases through VEGF upregulation. Our results were in agreement with previous studies where COX-2 expression was significantly associated with VEGF [13,30]. In contrary to the above results, a study has shown no relationship [15]. Similar association was reported in lung adenocarcinoma cases and in breast carcinoma [31].

In conclusion, our study demonstrates that expression of COX-2 and VEGF is significantly higher in GC compared to control samples. They might be used as biomarkers predicting tumor behavior and prognosis in GC. COX-2 expression is significantly related to intestinal type carcinoma, locally advanced disease and *H. pylori* infected patients. VEGF expression is significantly associated with loco-regional progression. VEGF showed significant association with the expression of COX-2 suggesting the involvement of COX-2 in tumor angiogenesis.

In the current study, there was an important limitation that needs to be addressed. The sample size was relatively small; therefore, it is not possible to reliably conclude that there were no associations between biomarkers and different clinicopathological parameters. Further works with larger sample size are required to evaluate the negative relationships.

Conflict of interest

None declared.

References

[1] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64(1):9–29.

[2] Chen H, Guan R, Lei Y, Chen J, Ge Q, Zhang X, et al. Lymphangiogenesis in gastric cancer regulated through Akt/mTOR-VEGF-C/VEGF-D axis. BMC Cancer 2015;15:103.

[3] Ugraş N, Ozgun G, Ocakoğlu G, Yerci Ö, Öztürk E. Relationship between HER-2, COX-2, p53 and clinicopathologic features in gastric adenocarcinoma. Do these biomarkers have any prognostic significance? Turk J Gastroenterol 2014;25(Suppl. 1):176–81.

[4] Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014;2014:437971.

[5] Malignant Digestive System Tumors. In: Mokhtar N, Gouda I, Adel I, editors. Cancer pathology registry 2003–2004 and time trend analysis. Department of Pathology, NCI, Cairo University; 2007. p. 59–60 (El Sheraa for advertising).

[6] Spolverato G, Ejaz A, Kim Y, Squires MH, Poultsides GA, Fields RC, et al. Rates and patterns of recurrence after curative intent resection for gastric cancer: a United States multi-institutional analysis. J Am Coll Surg 2014;219(4):664–75.

[7] Samaka RM, Abdou AG, Abd El-Wahed MM, Kandil MA, El-Kady NM. Cyclooxygenese-2 expressions in chronic gastritis and gastric carcinoma, correlation with prognostic parameters. J Egypt Natl Canc Inst 2006;18(4):363–74.

[8] Lazar D, Taban S, Ardeleanu C, Simionescu C, Sporea I, Cornianu M, et al. Immunohistochemical expression of the cyclooxygenase-2 (COX-2) in gastric cancer. The correlations with the tumor angiogenesis and patients' survival. Rom J Morphol Embryol 2008;49(3):371–9.

[9] Ding S, Li C, Lin S, Han Y, Yang Y, Zhang Y, et al. Distinct roles of VEGF-A and VEGF-C in tumor metastasis of gastric carcinoma. Oncol Rep 2007;17(2):369–75.

[10] Nikiteas NI, Tzanakis N, Theodoropoulos G, Atsaves V, Christoni Z, Karakitsos P, et al. Vascular endothelial growth factor and endoglin (CD-105) in gastric cancer. Gastric Cancer 2007;10:12–7.

[11] Pathology and genetics: tumors of the stomach. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO Classification of Tumors of the Digestive System, 3. Lyon: IARC Press; 2010. p. 37–67.

[12] Zhao ZQ, Yang S, Lu HS. Expression of midkine and vascular endothelial growth factor in gastric cancer and the association of high levels with poor prognosis and survival. Mol Med Rep 2012;5:415–9.

[13] Zhao HC, Qin R, Chen XX, Sheng X, Wu JF, Wang DB, et al. Microvessel density is a prognostic marker of human gastric cancer. World J Gastroenterol 2006;12:7598–603.

[14] Sun WH, Sun YL, Fang RN, Shao Y, Xu HC, Xue QP, et al. Expression of cyclooxygenase-2 and matrix metalloproteinase-9 in gastric carcinoma and its correlation with angiogenesis. Jpn J Clin Oncol 2005;35(12):707–13.

[15] Gou HF, Chen XC, Zhu J, Jiang M, Yang Y, Cao D, et al. Expressions of COX-2 and VEGF-C in gastric cancer: correlations with lymphangiogenesis and prognostic implications. J Exp Clin Cancer Res 2011;30:14.

[16] Mao X, Wang X, Lv X, Xu L, Han CB. COX-2 expression in gastric cancer and its relationship with angiogenesis using tissue microarray. World J Gastroenterol 2007;13(25):3466–71.

[17] Shi H, Xu JM, Hu NZ, Xie HJ. Prognostic significance of expression of cyclooxygenase-2 and vascular endothelial growth factor in human gastric carcinoma. World J Gastroenterol 2003;9(7):1421–6.

[18] Wang Z, Chen J, Liu J. COX-2 inhibitors and gastric cancer. Gastroenterol Res Pract 2014;2014:132320.

[19] Sierra JC, Hobbs S, Chaturvedi R, Yan F, Wilson KT, Peek Jr RM, et al. Induction of COX2 expression by *Helicobacter pylori* is mediated by activation of epidermal growth factor receptor in gastric epithelial cells. Am J Physiol Gastrointest Liver Physiol 2013;305(2):G196–203.

[20] Hussein NR. *Helicobacter pylori* and gastric cancer in the Middle East: a new enigma? World J Gastroenterol 2010;16(26):3226–34.

[21] Zhang Y, Pan KF, Zhang L, Ma JL, Zhou T, Li JY, et al. *Helicobacter pylori*, cyclooxygenase-2 and evolution of gastric lesions: results from an intervention trial in China. Carcinogenesis 2015;36(12):1572–9.

[22] Yamac D, Ayyildiz T, Coskun U, Akyürek N, Dursun A, Seckin S, et al. Cyclooxygenase-2 expression and its association with angiogenesis, *Helicobacter pylori*, and clinicopathologic characteristics of gastric carcinoma. Pathol Res Pract 2008;204:527–36.

[23] Thiel A, Mrena J, Ristimäki A. Cyclooxygenase-2 and gastric cancer. Cancer Met Rev 2011;30(3):387–95.

[24] Guo R, Li Q, Meng L, Zhang Y, Gu C. P53 and vascular endothelial growth factor expressions are two important indices for prognosis in gastric carcinoma. West Indian Med J 2008;57:2–6.

[25] Yu YF, Zhang Y, Shen YN, Zhang RY, Lu XQ. Effect of VEGF, P53 and telomerase on angiogenesis of gastric carcinoma tissue. Asian Pac J Trop Med 2014;7(4):293–6.

[26] Raica M, Mogoanta L, Cimpean AM, Alexa A, Ioanovici S, Mărgăritescu C, et al. Immunohistochemical expression of vascular endothelial growth factor (VEGF) in intestinal type gastric carcinoma. Rom J Morphol Embryol 2008;49:37–42.

[27] Kolev Y, Uetake H, Iida S, Ishikawa T, Kawano T, Sugihara K. Prognostic significance of VEGF expression in correlation with COX-2, microvessel density, and clinicopathological characteristics in human gastric carcinoma. Ann Surg Oncol 2007;14:2738–47.

[28] Iordache S, Saftoiu A, Georgescu CV, Ramboiu S, Gheonea DI, Filip M, et al. Vascular endothelial growth factor and microvessel density – two useful tools for the assessment of prognosis and survival in gastric cancer patients. J Gastrointest Liver Dis 2010;19:135–9.

[29] Liu N, Wu Q, Wang Y, Sui H, Liu X, Zhou N, et al. *Helicobacter pylori* promote VEGF expression via the p38 MAPK-mediated COX-2-PGE2 pathway in MKN45 cells. Mol Med Rep 2014;10(4):2123–9.

[30] Zhang J, Ji J, Yuan F, Zhu L, Yan C, Yu Y-Y, et al. Cyclooxygenase-2 expression is associated with VEGF-C and lymph node metastases in gastric cancer patients. Biomed Pharmacother 2005;59(Suppl. 2):285–8.

[31] Liu H, Yang Y, Xiao J, Lv Y, Liu Y, Yang H, et al. COX-2-mediated regulation of VEGF-C in association with lymphangiogenesis and lymph node metastasis in lung cancer. Anat Rec (Hoboken) 2010;293:1838–46.