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Predictive Value of CDX2 and SOX2 in Chronic Gastritis and Intestinal-type Gastric Cancer

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Abstract

BACKGROUND: Worldwide gastric cancer (GC) ranks sixth in incidence and second in mortality among all malignancies. CDX2 has an essential role in the development and maintenance of intestinal differentiation in the gut and ectopic sites such as intestinal metaplasia (IM) of the stomach. SOX2 contributes to the cell lineages normally found in the stomach, suggesting contribution in gastric differentiation.

AIM: The aim of the study was to assess the expression of CDX2 and SOX2 in chronic gastritis (CG) lesions associated with *Helicobacter pylori*, IM, or dysplasia as well as in intestinal-type GC.

METHODS: Immunohistochemical staining for CDX2 and SOX2 were applied on archival paraffin blocks from 80 CG cases, 40 intestinal-type GC cases, and 10 controls. CG cases were either of non-specific inflammation or associated with *H. pylori* infection. GC cases were of intestinal-type only, excluding any other type of GC. Control cases were of minimal gastritis, negative for *H. pylori*, IM, and dysplasia.

RESULTS: CDX2 expression was correlated with CG associated with *H. pylori*, IM, and dysplasia as well as with more differentiated and less invasive pattern of intestinal-type GC, while SOX2 expression was correlated with CG negative for *H. pylori* and IM as well as with less differentiated and more invasive intestinal-type GC.

CONCLUSION: Both CDX2 and SOX2 could predict the behavior of CG disease over time and plan the suitable line of treatment and both proteins could be potential targets for novel therapeutic interventions.

Introduction

According to GLOBOCAN 2018 data, the incidence of gastric cancer (GC) ranks sixth and mortality ranks second [1]. Although GC prevalence has shown a continuous reduction since the last mid-century, it is still a common malignancy and a frequent cause of cancer-related deaths [2]. Both histological types of GC: Intestinal and diffuse, present distinct morphological, clinical, and epidemiological features and are thought to develop from the activation of independent molecular mechanisms. Intestinal GC develops through a sequence of histological changes, including diffuse chronic gastritis (CG), mucosal atrophy, intestinal metaplasia (IM), dysplasia, and finally invasive carcinoma [3].

Helicobacter pylori are Gram-negative spirochetes which infect more than half of the world's population, likely due to water contamination and less sanitary living conditions [4]. Infection with *H. pylori* and the resulting chronic inflammation is a major step in the initiation and development of almost 90% of new cases of GC [5]. The pathogenicity of *H. pylori* is attributed largely to its various virulence components [6]. Chronic infection with *H. pylori* gives rise to IM which is the most relevant pre-neoplastic lesion of the stomach affecting about 30% of the individuals infected with *H. pylori* [7].

CDX2 is an intestine-specific homeobox transcription factor which is expressed in the intestinal epithelial cells from duodenum to the rectum [8] and has an essential role in the development and maintenance of intestinal differentiation in the gut and ectopic sites such as IM of the stomach and esophagus [9]. It regulates many cellular processes such as cell differentiation, proliferation, cell adhesion, migration, and tumor genesis [10]. Its role as a prognostic marker in colorectal carcinomas is well known, whereas its role in the outcome of gastric carcinomas is not yet established [11].

SOX2 is a member of the SOX (SRY-related HMG Box) family of transcription factors that play diverse roles, starting from orchestrating the mammalian embryogenesis [12], later on contributing to the normal morphogenesis and homeostasis of the foregut-derived

epithelia of the esophagus, lung, and trachea [13]. It has been shown, in mice, that SOX2 expression contributes to all the cell lineages normally found in the stomach, suggesting an important contribution for gastric differentiation [14]. In addition, abnormal expression of SOX2 has been observed in tumors of the brain, breast, lung, and esophagus. However, in the GC context, its role remains puzzling and needs further clarification. Furthermore, its interplay with CDX2 remains unexplored [15].

This work aims to assess the expression of CDX2 and SOX2 as intestinal and gastric differentiation markers, respectively, in intestinal-type GCs and precancerous conditions, namely, chronic *H. pylori* infection, IM, and dysplasia, to evaluate the role of these markers as prognostic indicators of progression to gastric carcinoma.

Materials and Methods

Samples

This retrospective study included formalin-fixed paraffin-embedded blocks of 130 specimens of endoscopic and surgically resected gastric lesions, divided as 10 blocks for control cases with minimal gastritis, negative for *H. pylor*i, IM, and dysplasia; 80 blocks of cases with CG and 40 intestinal-type GC blocks. Blocks were collected from the Pathology Department, Theodor Bilharz Research Institute, in a period from January 2017 to October 2019.

Specimens of CG cases were obtained as endoscopic biopsies, while specimens of GC cases were obtained as partial/total gastrectomy specimens.

Table 1: Patients' characteristics in studied groups

Groups	n	Gender		Age	p value
		Male n (%)	Female n (%)	Mean±SD	
Control	10	7 (70)	3 (30)	45.20 ± 16.37*	p < 0.01
Chronic gastritis	80	44 (55)	36 (45)	51.92 ± 16.43*	p < 0.001
Intestinal-type GC	40	26 (65)	14 (35)	60.95 ± 6.86	
Total	130	77 (59.2)	53 (40.8)		

N: Number, GC: Gastric cancer, *compared to intestinal-type GC.

Patients' characteristics were summarized in Table 1 and clinicopathological parameters of studied cases were shown in Table 2.

Table 2: Clinicopathological parameters

Patients groups			n. (%)
Control (10)			10
Chronic gastritis (80)	Intensity of inflammation	Mild	28 (35)
		Moderate	52 (65)
Associated lesions	*H. pylori* infection	Present	44 (55)
		Absent	36 (45)
	IM	Present	34 (42.5)
		Absent	46 (57.5)
	Dysplasia	Present	10 (12.5)
		Absent	70 (87.5)
Intestinal-type GC (40)	Grade of differentiation	Low grade	29 (72.5)
		High grade	11 (27.5)
	Stage of invasion	Early stage	27 (67.5)
		Advanced stage	13 (32.5)
	Vascular invasion (vascular emboli)	Present	33 (82.5)
		Absent	7 (17.5)

N: Number, *H. pylori: Helicobacter pylori*, IM: Intestinal metaplasia, GC: Gastric cancer

The protocol of this study was approved by the Institutional Review Board of Theodor Bilharz Research Institute for the protection of human subject and adopted by the 18th world medical assembly, Helsinki, Finland (2013).

Histopathological technique and evaluation

Paraffin sections from different gastric lesions were cut in 4 μm thickness stained using hematoxylin and eosin (H&E) for routine histopathological examination and diagnosis. Giemsa stain was used for the detection of *H. pylori* micro-organisms.

CG sections were evaluated for intensity of inflammation, presence/absence of *H. pylori*, IM and dysplasia.

Sections of gastric carcinoma were examined for tumor grading and staging according to International Histological Classification proposed by the World Health Organization, 2019. GC is considered as low grade (well-differentiated) or high grade (moderately or poorly differentiated) and is considered as either early (pT1) or advanced (≥pT2) [16]. GCs of antrum were found in 31/40 cases and that of corpus/funds in 9/40. All were of intestinal-type histology.

Immunohistochemical technique

One paraffin-embedded block was selected from each case and was cut into 4 μm sections. The sections were put in the oven at 60°C for 4 h, deparaffinized in xylene, rehydrated in a graded ethanol series, and treated with 3% hydrogen peroxide solution for 10 min. Antigen retrieval was done by microwaving the tissue in 10 mmol/L citric acid buffer for 12 min, then cooling at room temperature for 2 h. The sections were incubated with an anti-CDX2 monoclonal antibody (code no. CMC23531040, Abcam, Cambridge, MA, USA) and anti-SOX2 monoclonal antibody (code no. ab97959, Abcam, Cambridge, MA, USA), at dilution of 1:100 for overnight at 4°C. Sections were then washed 3 times for 5 min in phosphate-buffered saline. Non-specific staining was blocked with 0.5% casein and 5% normal serum for 30 min at room temperature. Finally, staining was developed with diaminobenzidine substrate and sections were counterstained with hematoxylin, dehydrated with graded ethanol, and mounted.

For each setting, positive and negative controls were routinely used. Negative controls were carried out in which phosphate-buffered saline was used instead of the primary antibody. Positive control slides were pancreatic tissue for CDX2 and squamous cell carcinoma of the lung for SOX2.

Assessment of immunostaining

The sections were examined using a light microscope (Scope A1, Axio, Zeiss,

Germany). Photomicrographs were taken using a microscope-camera (AxioCam, MRc5, Zeiss, Germany). Two experienced pathologists independently examined nuclear CDX2 and SOX2 staining while blind to the clinicopathologic data of patients. At least 10 high-power fields at ×400 were chosen randomly for each section. Cases with >5% positive gastric/tumor cells in a section were regarded as positive expression. Both immunopositivity (number of positive cases) and extent of expression (mean percentage of positively stained neoplastic cells) were evaluated.

Statistical analysis

Analyses were performed using SPSS version 23 (IBM Corp., Armonk, New York, USA). The significance of differences in means was calculated using One-way ANOVA and the T-test. Chi-square and Fischer's exact tests were used to assess the significance of differences in clinicopathological characteristics across categories of CDX2 and SOX2 expression. Differences were considered statistically significant whenever the $p < 0.05$.

Results

In this study, 130 paraffin blocks with gastric lesions were enrolled. Seventy-seven patients were male (59.2%) and the rest were female (40.8%). The mean age for CG patients was 51.92 years and for GC patients were 60.95 years; these values were significantly higher than that of controls (45.20 years) (Table 1).

CDX2 immunoreactivity

Our data revealed that CDX2 immunopositivity and CDX2 expression were significantly associated with male sex in both CG and GC cases (Table 3).

Table 3: Relationship between CDX2 immunostaining and gender

Gender in studied groups (N.)	CDX2 immunopositivity			CDX2 expression (% of positive cells)	
	Positive n (%)	Negative n (%)	p value	Mean ± SD	p value
Chronic gastritis (80)	40 (50)	40 (50)		17.88 ± 21.36	
Male (44)	35 (79.5)	9 (20.5)	p > 0.001	27.72 ± 16.48	p < 0.001
Female (36)	5 (14)	31 (86)		6.94 ± 11.91	
Gastric adenocarcinoma (40)	37 (92.5)	3 (7.5)		50.38 ± 25.53	
Male (26)	26 (100)	0	p > 0.05	56.07 ± 25.52	p > 0.05
Female (14)	11 (78.6)	3 (21.4)		47.31 ± 18.23	

N: Number, GC: Gastric cancer.

CDX2 immunopositivity showed a significant difference between studied groups, while it was undetectable in controls; 50% of CG cases and 92.5% of GC cases were CDX2 positive. Moreover, CDX2 expression was significantly increased from 0 in controls to 18.37% in CG to 50.38% in GC cases (Table 4) (Figure 1).

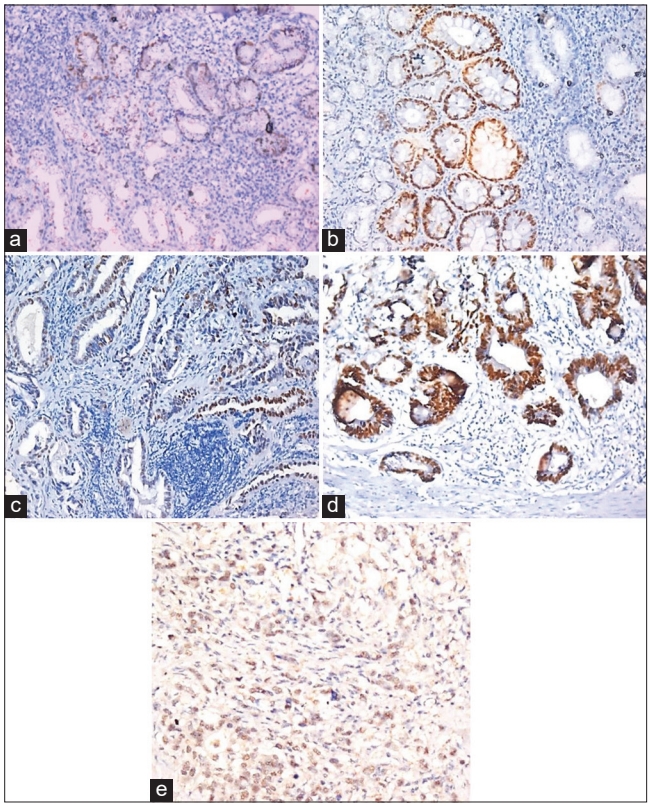

Figure 1: Immunohistochemical expression of CDX2. (a) Moderate chronic gastritis with Helicobacter pylori, positivity in ~40% of gastric cells (×200), (b) Moderate chronic gastritis with intestinal metaplasia, positivity in ~ 40% of gastric cells (×200), (c) Moderate chronic gastritis with intestinal metaplasia and dysplasia, positivity in ~ 80% of gastric cells (×200), (d) Low-grade intestinal-type gastric cancer, positivity in ~ 80% of malignant gastric cells (×200), (e) High-grade intestinal-type gastric cancer, positivity in ~ 60% of malignant gastric cells (200)

Regarding CG cases, CDX2 immunopositivity and expression were significantly associated with the intensity of inflammation, *H. pylori* infection, IM, and dysplasia (Table 4).

Notably, there was a significant increase in CDX2 expression in CG cases with IM (30%) to dysplasia (47.5%) then to carcinoma (50.38%) (Table 4).

Regarding intestinal-type GC cases, CDX2 immunopositivity was higher in low grade and early-stage cancers as well as cancers negative for vascular emboli compared with their counterparts, but these relationships did not achieve significant values. On the other hand, CDX2 expression was significantly associated with low grade and early-stage cancers (Table 4).

SOX2 immunoreactivity

Our data revealed that the immunopositivity and expression of SOX2 were significantly higher in males than in females regarding GC cases (Table 5).

We found SOX2 immunopositivity in 70% of control cases in scattered foci of foveolar cells. Only 22.5% of CG cases were positive for SOX2 compared

Table 4: CDX2 immunostaining in studied groups

Diagnosis (n.)			CDX2 immunopositivity			CDX2 expression (% of positive cells)	
			Positive n (%)	Negative n (%)	p value	Mean ± SD	p value
Control (10)			0	10	p < 0.01	00 ± 00	p > 0.001
Chronic gastritis (80)			40 (50)	40 (50)		18.37 ± 17.86	
Intensity of inflammation		Mild (28)	8 (28.6)	20 (71.4)	p < 0.01	10.17 ± 8.66	p > 0.01
		Moderate (52)	32 (61.5)	20 (38.5)		22.79 ± 19.93	
Associated lesions	*H. pylori* infection	Present (44)	27 (61.4)	17 (38.6)	p < 0.05	23.52 ± 15.27	p > 0.01
		Absent (36)	13 (36)	23 (64)		12.08 ± 18.95	
	IM	Present (34)	27 (79.4)	7 (20.6)	p < 0.001	30.00 ± 17.71	p > 0.001
		Absent (46)	13 (28.3)	33 (71.7)		9.78 ± 12.34	
	Dysplasia	Present (10)	10 (100)	0	p < 0.001	47.50 ± 6.34†	p > 0.001
		Absent (70)	30 (43)	40 (57)		14.21 ± 14.81	
Intestinal-type GC (40)			37(92.5)*	3 (7.5)		50.38 ± 25.53*,‡,§	
Grade of differentiation		Low grade (29)	28 (96.6)	1 (3.4)	p > 0.05	61.38 ± 19.73	p > 0.001
		High grade (11)	9 (81.8)	2 (18.2)		21.36 ± 13.25	
Stage of invasion		Early stage (27)	26 (96.3)	1 (3.7)	p > 0.05	60.74 ± 20.12	p > 0.001
		Advanced stage (13)	11 (84.6)	2 (15.4)		28.85 ± 22.28	
Vascular invasion		Present (33)	30 (91)	3 (9)	p > 0.05	48.03 ± 27.24	p > 0.05
		Absent (7)	7 (100)	0		61.43 ± 10.29	

N: Number, *H. Pylori: Helicobacter pylori,* IM: Intestinal metaplasia, GC: Gastric cancer, *p < 0.001 compared to control and CG, †p < 0.01 compared to IM, ‡p < 0.05 compared to dysplasia, §p < 0.001 compared to IM.

Table 5: Relationship between SOX2 immunostaining and gender

Gender in studied groups (n.)	SOX2 immunopositivity			SOX2 expression (% of positive cells)	
	Positive n (%)	Negative n (%)	p value	Mean ± SD	p value
Control (10)				9.00 ± 6.58	
Male (7)	4 (57)	3 (43)	p < 0.05	10.71 ± 5.35	p < 0.05
Female (3)	3 (100)	0		5.0 ± 8.66	
Chronic gastritis (80)				52.38 ± 28.82	
Male (44)	10 (22.7)	34 (77.3)	p >0.05	13.41 ± 25.78	p < 0.05
Female (36)	8 (22.2)	28 (77.8)		9.17 ± 19.44	
Intestinal-type GC (40)					
Male (26)	25 (96.2)	1 (3.8)	p < 0.001	60.96 ± 16.91	p < 0.01
Female (14)	7 (50)	7 (50)		36.43 ± 39	

N: Number; GC: Gastric cancer.

to positivity in 80% intestinal-type GC with a significant difference between both groups (Table 6). Furthermore, SOX2 expression significantly increased from 11.5% in CG to 52.38% in intestinal-type GC (Figure 2).

In CG cases, intensity of inflammation – either mild or moderate – did not affect immunoreactivity for SOX2. CG with dysplasia significantly showed higher SOX2 immunopositivity and expression than in CG without dysplasia (Table 6).

Regarding intestinal-type GC cases, SOX2 immunopositivity was higher in high grade and in advanced stage GCs than with low grade and early-stage cancers, but with no statistically significant difference. However, we found that SOX2 expression was significantly increased in high grade and advanced-stage cancers compared with low grade and early-stage cancers. Vascular invasion did not affect immunoreactivity for SOX2 (Table 6).

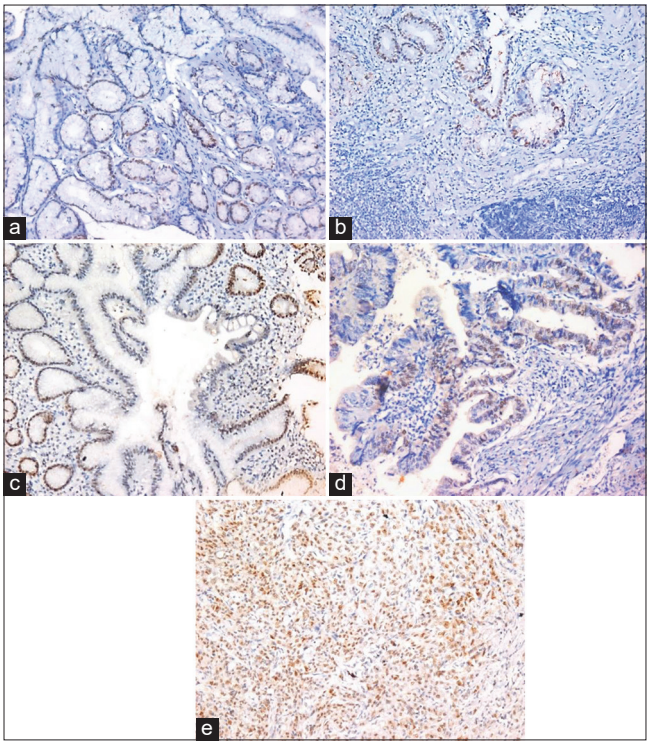

Figure 2: Immunohistochemical expression of SOX2. (a) Mild chronic gastritis, positivity in ~ 40% of gastric cells (×200), (b) Moderate chronic gastritis with Helicobacter pylori, positivity in ~ 30% of gastric cells (×200), (c) Chronic gastritis with H. pylori and intestinal metaplasia, positivity in ~ 30% of gastric cells (×200), (d) Low-grade intestinal-type gastric cancer, positivity in ~ 40% of malignant gastric cells (×200), (e) High-grade intestinal-type gastric cancer, positivity in ~ 80% of malignant gastric cells (×200)

Discussion

Considering GC as one of the main cancer-causing deaths and being a disease with multiple outcomes that cannot be predicted by clinicopathological features alone [17], finding precise prognostic factors in patients with GC is an urgent need. Moreover, GC represents an example of inflammation-linked cancer. The progression of *H. pylori*-infected CG facilitates the development of IM, which has been extensively studied as a premalignant condition of gastric carcinoma [18].

In this study, we assess immunoreactivity of CDX2 and SOX2 in different gastric lesions according to the number of positive cases (immunopositivity) and mean percentage of positive gastric and tumor cells (expression). CDX2 and SOX2 were considered immunopositive when immunoreactivity was observed in >5% of gastric/tumor cells, leading to a higher prevalence of CDX2 and SOX2 immunopositivity in our study (92.5% and 80%, respectively) than that

Table 6: SOX2 Expression in studied groups

Diagnosis (N.)			SOX2 immunopositivity			SOX2 expression (% of positive cells)	
			Positive n (%)	Negative n (%)	p value	Mean ± SD	p value
Control (10)			7 (70)	3 (30)	p < 0.001	9.00 ± 6.58	p > 0.05
Chronic gastritis (80)			18 (22.5)	62 (77.5)		11.50 ± 23.10	
Intensity of inflammation		Mild (28)	6 (21.4)	22 (78.6)	p > 0.05	13.21 ± 26.25	p > 0.05
		Moderate (52)	12 (23)	40 (77)		10.58 ± 21.43	
Associated lesions	*H. pylori* infection	Present (44)	5 (11.4)	39 (88.6)	p < 0.01	p > 0.05	p > 0.05
		Absent (36)	13 (36)	23 (64)		16.67 ± 24.93	
	IM	Present (34)	6 (17.6)	28 (82.4)	p > 0.05	8.53 ± 17.21	p > 0.05
		Absent (46)	12 (26)	34 (74)		13.7 ± 26.61	
	Dysplasia	Present (10)	5 (50)[†]	5 (50)	p < 0.05	28 ± 31.2 [‡]	p > 0.01
		Absent (70)	13 (18.6)	57 (81.4)		9.14 ± 20.95	
Intestinal-type GC (40)			32 (80)*[§,‖]	8 (20)		52.38 ± 28.82*,**	
Grade of differentiation		Low Grade (29)	22 (76)	7 (24)	p > 0.05	44.14 ± 25.85	p > 0.05
		High Grade (11)	10 (91)	1 (9)		77.73 ± 28.93	
Stage of invasion		Early Stage (27)	20 (74.1)	7 (25.9)	p > 0.05	42.59 ± 26.11	p > 0.001
		Advanced stage (13)	12 (92.3)	1 (7.7)		72.69 ± 23.68	
Vascular invasion		Present (33)	25 (75.8)	8 (24.2)	p > 0.05	51.97 ± 31.74	p > 0.05
		Absent (7)	7 (100)	0		54.29 ± 4.50	

N: Number, *H. pylori: Helicobacter pylori*, IM: Intestinal metaplasia, GC: Gastric cancer, [†]p < 0.05 compared to IM, *p < 0.001 compared to control and CG, [§,‖]p < 0.05 and p < 0.001 compared to dysplasia and IM, respectively, [‡]p < 0.01 compared to IM, **p < 0.05 and p < 0.001 compared to dysplasia and IM, respectively.

reported in other studies as Bao *et a*l. [19] (35.1%), Fan *et al*. [20] (~76%), and Harras *and Mowafy* [21] (81.25%) regarding CDX2 immunopositivity, and studies by Camilo *et al*. [22] (52%) and Yang *et al*. [23] (41.7%) regarding SOX2 immunopositivity.

Clinicians encounter sex disparities in diagnostic and therapeutic responses. Data in our study revealed that male:female ratio was 1.2:1 in CG cases and 1.9:1 in GC cases. Furthermore, we found an association between male sex and both CDX2 and SOX2 expression. This is in line with the findings of Bao *et al*. [19] and Camilo *et al*. [22].

In our study, the mean age for intestinal-type GC cases was of 60.95 years, with male:female ratio 1.9:1. However, Saha *et al*. [24], in their study, found a median age of 55 years with male:female ratio of 2.7:1. In Halder *et al*. [11] study, the mean age was 51.16 years with a male:female ratio of 1.63:1.

In the current study, CDX2 was undetectable in control cases, this goes with studies of Fan *et al*. [20] and Bao *et al*. [19], who reported negative CDX2 staining in normal gastric mucosa and stromal cells. CDX2 immunopositivity and expression were sequentially increased from CG to intestinal-type GC. This is parallel to the fact that GC is one of the inflammation-linked cancers [25].

In concordance with Saito *et al*. [26], who reported a relationship between CDX2 expression and intensity of inflammation, we found a significant association between CDX2 (immunopositivity and expression) with a moderate intensity of inflammation in CG cases.

In our study, CDX2 immunopositivity was significantly higher in *H. pylori* positive cases than negative ones, a finding consistent with previous reports which have shown that *H. pylori* induces CDX2 expression in the human stomach before the development of IM [9], [27]. Several studies have demonstrated that *H. pylori* infection leads to the expression of CDX2 in areas of IM and also in foci of non-metaplastic cells [28], [29]. However, other studies have demonstrated that CDX2 expression is higher in *H. pylori*-negative patients than positive patients [30], [31].

As an intestine-specific transcription factor, CDX2 has a key role in regulating the proliferation and differentiation of intestinal cells and maintaining intestinal phenotypes in the gastric epithelium [19], [32]. Consistent with this literature, we found a significantly higher CDX2 immunopositivity and expression in CG with IM than cases without IM.

Reinforcing the role of CDX2 as a biomarker of progression in the preneoplastic stages of gastric carcinogenesis, we observed a significant increase in CDX2 expression from IM to dysplasia, this matches the finding of Camillo *et al*. [33] that CDX2 is acquired *de novo* in IM and maintained in dysplasia, on the contrary, Kim *et al*. [34] observed a significant reduction in CDX2 expression in the foci of gastric epithelial dysplasia when compared with the adjacent metaplastic gastric mucosa.

We found that all cases of CG associated with dysplasia were immunopositive for CDX2. Similarly, Rugge *et al*. [35] reported positive CDX2 expression in all their studied dysplastic lesions. Although we observed a gradual decrease of CDX2 immunopositivity from dysplasia to early GCs to advanced cancers, CDX2 expression increased from dysplasia to early GC then reduced in advanced cancers. In contrast to our results, Mizoshita *et al*. [36] reported a gradual decrease of CDX2 expression from dysplasia to early to advance GCs. This controversy can be attributed to the small number of our dysplastic lesions. Immunopositivity and expression of CDX2 between dysplasia and cancer were not different. The same finding was reported by Kang *et al*. [32].

Regarding CDX2 expression in GC, we found CDX2 immunopositivity in 92.5% of studied GC cases. It has been suggested that the intestinal-type gastric carcinoma may be transformed from IM [37]. This may account for the high positivity of CDX2 in intestinal-type gastric carcinomas. However, we found lower expression in high grade and advanced-stage cancers than in low grade and early-stage ones. This

is consistent with previous reports by Satio *et al*. [25] and Wang *et al*. [38], which stated that CDX2 was expressed more at a low grade and early stage of gastric carcinogenesis intestinal phenotypic elements and could be associated with the shift from gastric to intestinal phenotype expression. In a study done by Qin *et al*. [39], a significant negative association between expression of CDX2 and stage of GC was detected and also they found CDX2 positive patients had longer survival than those who were CDX2 negative. Mizoshita *et al*. [40] also reported that CDX2 expression was associated with a favorable outcome. Furthermore, Roessler *et al*. [41] indicated that reduction of CDX2 may represent a marker of tumor progression.

Consistent with Bao *et al*. [19], we did not find a correlation between CDX2 immunopositivity and CDX2 expression with vascular invasion.

SOX2 can act both as an oncogene and a tumor suppressor in different types of cancer, suggesting that the role of transcription factors in cancer initiation may depend on several factors, including the other oncogenic mutations involved in cell transformation and the cell type of origin [42]. Although transcription factors are not classical drug targets, approaches to SOX2-targeted therapy are already being addressed in breast cancer [43].

In the current study, we found SOX2 expression in scattered deep gastric glands of control cases. This was in agreement with Camilo *et al*. [33], who reported a consistent SOX2 expression in normal gastric mucosa, mostly in the neck region. Then, SOX2 expression was observed in CG and increased significantly in intestinal-type GC cases. These findings identified that SOX2 expression was evident in normal mucosa and maintained in CG and GC, which reinforce its association with gastric differentiation. Our results were consistent with previous literature by Basat *et al*. [44] and Hutz *et al*. [45], who reported significant SOX2 overexpression in GC relative to the adjacent normal tissues, concluding that SOX2 has a potential role on oncogenesis, epithelial to mesenchymal transition, tumor progression, and metastasis. However, other studies reported downregulated SOX2 expression in gastric tumor tissue and suggested that SOX2 can function as a tumor suppressor by regulating the cell cycle and apoptosis [46], [47]. This paradoxical role of SOX2 in GC was reported by Carrasco-Garcia *et al*. [48].

We found lower SOX2 immunopositivity and expression in CG with *H. pylori* than in negative ones. This goes with the results of Camilo *et al*. [33] and Yoon *et al*. [49], who stated that SOX2 expression is strongly downregulated by *H. pylori* and attributed this finding to *H. pylori* CagA protein, which induces decreasing in SOX2 expression and increasing CDX2 expression.

In agreement with Tsukamoto *et al*. [50], we detected lower SOX2 expression in CG with IM than those without. SOX2 and CDX2 are inversely expressed in IM, this is explained as SOX2 was suggested as a CDX2 repressor since down-regulating its levels led to upregulation of CDX2 expression [51].

We observed a significant increase in SOX2 expression from IM to dysplasia to intestinal-type GC. These results indicated a link between these lesions based on the profile of SOX2 expression. This observation supported by findings that IM is a lesion that is difficult to reverse or is even a “point of no return” [52].

Our study revealed a significant increase in SOX2 immunopositivity and expression from IM to dysplasia; however, Camilo *et al*. [33] showed reverse results. Furthermore, we found a gradual increase in SOX2 immunopositivity and expression with progression from dysplasia to low-grade GC to high-grade cancers, suggesting its oncogenic role.

It is well-established that SOX2 is associated with gastric differentiation [14]. According to our results, this is maintained in GC as we found higher SOX2 immunopositivity and expression in high grade and advanced intestinal-type GCs compared with lower grade and early-stage cancers. This is parallel to the results of Basat *et al*. [44] and Du *et al*. [53], who reported a positive association between SOX2 expression with poor differentiation and advanced tumor stage. On the contrary, Lin *et al*. [54] stated that SOX2 overexpression was associated neither with the overall survival nor with the other clinicopathological factors including grade and stage, while Yang *et al*. [23] found that SOX2 positive expression was associated with shorter survival in patients at early-stage cancers, but not at an advanced stage. These conflicting results can be attributed to a lack of understanding the role of SOX2 as an oncogene or tumor suppressor in GC.

In accordance with Camilo *et al*. [22] and Basat *et al*. [44], we found no significant association between vascular invasion and SOX2 immunoreactivity; however, Du *et al*. [53] detected a positive correlation between high SOX2 expression with vascular invasion.

SOX2 expression is inversely correlated with CDX2 expression ($p = 0.004$, $r = –0.242$). CDX2 was associated with CG with *H. pylori* and with IM as well as intestinal-type GCs with low grade and early stage, while SOX2 was associated with CG without *H. pylori* and without IM as well as intestinal-type GCs of high grade and advanced stage. These results suggest that the progression from normal gastric to IM to intestinal-type GC occurs with a gain of intestinal differentiation and loss of gastric differentiation. These results are in line with Camillo *et al*. [22]; Cobler *et al*. [55]; and Yoon *et al*. [49], who reported that there was a significant inverse correlation between the expression of SOX2 and CDX2 in gastric adenocarcinomas, and SOX2+/CDX2− profile was associated with a poorer prognosis. Different studies with mice models suggest that CDX2 negatively regulates SOX2 and also the reverse [56], [57].

The present study had several advantages compared to previous studies. First, it investigated CDX2 and SOX2 in CG and GC lesions, while most of the previous studies covered only one of both lesions. Second, it evaluated the effect of *H. pylori* infection and IM associated with CG on the expression of CDX2 and SOX2. However, the study had limitations by the relatively small number of studied cases.

Conclusion

The inverse relationship between CDX2 and SOX2 suggests that both could be markers for evaluating GC progression and outcome. This study revealed that CDX2 positive expression was related to CG associated with *H. pylori* infection, IM, dysplasia, as well as to more differentiated and less invasive pattern of intestinal-type GC, while SOX2 positive expression was related to CG without *H. pylori* infection or IM as well as to less differentiated and more invasive intestinal-type GC. Hence, both could predict how CG disease will behave over time and plan a suitable line of treatment and could be potential targets for novel therapeutic interventions.

Authors' Contributions

NH interpreted all data and wrote the manuscript; ZO, TA, and AB designed the content and structure of the manuscript; MY, AA, and MA provided specimens and clinical data needed for this study. MM designed and revised the manuscript.

All authors read and approved the final manuscript.

Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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