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A study of RUNX3, E-cadherin and β-catenin in CagA-positive *Helicobacter pylori* associated chronic gastritis in Saudi patients

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Abstract. – OBJECTIVE: *H. pylori* is the most important risk factor for gastric carcinoma. CagA-positive *H. pylori* is associated with an increased risk for gastric cancer compared with negative strains. RUNX3 is a tumor suppressor gene, which is related to the genesis of gastric cancer. β-catenin is integrated with E-cadherin in the cell membrane, and aberrant expression of the complex was reported in gastric carcinoma. Aim of this paper is to determine of the relation between RUNX3, E-cadherin and β-catenin in chronic gastritis associated with cagA-positive *H. pylori* infection.

PATIENTS AND METHODS: Retrospective study was done on formalin fixed paraffin embedded gastric biopsies blocks of 90 patients diagnosed as *H. pylori* associated chronic gastritis. *H. pylori* was detected using modified Giemsa stain. Nested PCR was used for detection of cagA, reverse transcription-PCR for detection of RUNX3 and immunohistochemistry for detection of E-cadherin and β-catenin.

RESULTS: Fifty percent of cases were found to be cagA positive. CagA was significantly associated with the intensity of mononuclear inflammation, the intensity of neutrophilic inflammation, the degree of mucosal atrophy and loss of RUNX3 but not with the density of *H. pylori*, intestinal metaplasia, E-cadherin or β-catenin. There was significant relation between loss of RUNX3 and increasing density of *H. pylori*, intensity of neutrophilic inflammation, mucosal atrophy and intestinal metaplasia. RUNX3 was found to be significantly correlated with E-cadherin but not with β-catenin. E-cadherin showed decreased expression in 36.7% of biopsies while, β-catenin was decreased in 33% of biopsies.

CONCLUSIONS: Loss of RUNX3, E-cadherin and β-catenin was considered early events in the cascade of gastric carcinoma development. Loss of RUNX3 but neither E-cadherin nor β-catenin was related to cagA positive *H. pylori* strains.

Key Words:
H. pylori, Nested PCR, cagA gene, Reverse transcription-PCR, RUNX3gene, Immunohistochemical methods, E-cadherin, β-catenin.

Introduction

It was established that infection with *Helicobacter pylori* is one of the strongest gastric cancer risk factors[1]. Recently, it was found that patients with chronic gastritis caused by *Helicobacter pylori* infection have a threefold greater risk of gastric cancer development compared with those without *H. pylori* infection[2]. Furthermore, treatment of *H. pylori* infection by antibiotics could decrease the risk of gastric cancer. However, while the carcinogenic role of *H. pylori* is now widely accepted, the underlying pathogenetic mechanism remains to be clarified[3].

The most widely studied *H. pylori* virulence factor is the cagA antigen (cytotoxin-associated-gene), a 96-to 138-kDa protein. The cagA gene, found on a genomic region called the *cag* pathogenicity island (PAI), is considered as a marker for enhanced virulence[4]. The PAI regulates a type IV secretion system that releases the bacterial virulence factor cagA into the gastric epithelial cells[5]. There is increased risk of gastric cancer development with infection by cagA-positive *H. pylori* strains compared with infection by cagA-

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negative strains[6]. The cagA acts on different cellular proteins, resulting in different actions such as actin rearrangement, decreased cell adhesion and inflammation, which are associated with *H. pylori*-mediated gastric carcinogenesis[7].

RUNX3 (Runt-related transcription factor 3) is a transcription factor which controls lineage-specific gene expression in developmental processes and is associated with the genesis of different types of cancers. *H. pylori* cagA downregulates RUNX3 expression in the gastric epithelium[8]. RUNX3 is expressed in glandular stomach epithelium, and loss of RUNX3 expression is associated with the development of gastric carcinoma and related to tumor differentiation, metastasis and dismal prognosis of gastric cancer[9]. The tumor suppressor functions of RUNX3 are elicited by adjusting the expression of many genes associated with the mitosis, apoptosis, differentiation, angiogenesis and cell-cell adhesion[11-14].

E-cadherin (epithelial-cadherin) is transmembrane glycoprotein that has an important role in intercellular adhesion of epithelial cells. The function of E-cadherin depends on the catenin family (α-catenin, β-catenin and γ-catenin). β-catenin is the key factor in the Wnt pathway that plays important roles in cell differentiation, migration and proliferation. Normally, β-catenin is integrated with E-cadherin in the membrane, and they form a complex that mediates homocellular adherence. Aberrant expression of proteins in the complex has been reported in some epithelial cancers including gastric carcinoma[15,16]. It is postulated that changes of the proteins in the complex may inhibit glycogen synthase kinase 3 (GSK-3)-mediated phosphorylation of β-catenin, allowing it to translocate from the membrane to the nucleus where it combines with TCF/LEF (T-cell factor/Lymphoid enhancer-binding factor), interacts with transcription factors, regulates gene transcription, and consequently induces cell proliferation and even tumor formation[17]. Moreover, It was found that activation of β-catenin signaling pathways are required for the sustention of the epithelial-mesenchymal transition (EMT)[18]. EMT is a complex process which provides the immotile epithelial cells with migratory and invasiveness capacity by converting them into cells with a motile mesenchymal cell phenotype. EMT also increases cell resistance to apoptosis, thus, contributing to cancer progression[19-21]. The Wnt/β-catenin signaling pathways decreases the epithelial marker E-cadherin while increases the mesenchymal marker vimentin[22-24]. EMT provides tumor cells with a characteristic advantage for metastatic dissemination, and also it provides those cells with cancer stem cell-like characters for proliferation and drug resistance[9]. It was found that cagA can activate β-catenin through destabilizing E-cadherin/β-catenin complexes, leading to induction of intestinal metaplasia of gastric epithelial cells[25,26].

Recently, it was confirmed that the decreased expression of RUNX3 is also associated with nuclear translocation of β-catenin which induces cell proliferation[27]. However, the effects of cagA-positive *H. pylori* infection on RUNX3/ E-cadherin/ β-catenin pathway in gastric epithelium have not been fully elucidated[28]. Therefore, this study was carried out to determine the relation between RUNX3, E-cadherin and β-catenin in chronic gastritis associated with cagA-positive *H. pylori* infection.

Patients and Methods

This investigation was done from Jan 2011 to Dec 2014. The study included all patients diagnosed as *H. pylori* associated chronic gastritis at Al Burg Pathology lab, Al-Madinah Al-Monawarah, KSA during this period. Sections from formalin fixed paraffin embedded gastric biopsies blocks were stained with Harris hematoxylin and eosin for histological evaluations[29]. Classification and grading of gastritis were based on Updated Sydney System[30,31]. *H. pylori* was detected using modified Giemsa stain[32] (Kwik-Diff stain kit, Thermo Scientific, Cat No. 9990702). Nested PCR was chosen to detect the presence of the cagA gene. Reverse transcription-PCR was selected to detect RUNX3 gene. Immunohistochemical methods were used for the detection of E-cadherin and β-catenin.

DNA Extraction From Paraffin Embedded Gastric Tissue

One to three μm thick paraffin sections were placed on positive slides. Sections were dewaxed by incubation with xylene at 65°C for 20 minute, followed by washing in 500 μl 100% ethanol. A 200 μl proteinase K buffer (500 μg/ml proteinase K (Gibco, Gaithersburg, MD, USA), 50 mM Tris/HCl, pH 7.4, and 5 mM EDTA, pH 8) was used for lysis. Then phenol/chloroform was used for nucleic acid extraction, which was precipitated with 300 mM sodium acetate and isopropanol[33].

Nested Polymerase Chain Reaction Amplification of cagA gene

Nested PCR was chosen as a sensitive and specific method to detect the presence of the cagA gene. Primers were selected based on the sequences of the cagA genes (MWG-Biotech, Ebersberg, Germany). The first amplification was performed with cagA1, the sense primer (5'-TGGCAGTGGGTTAGTCATAGCAG-3'), and cagA1, the antisense primer (5'-AGGACTCTTGCAGGCGTTGGTG-3'), in a 15 µL reaction mixture containing 1.5 µL of 10 × PCR buffer (Roche; 25 mM MgCl2, 100 mM Tris/HCl, and 500 mM KCl; pH 8.3 at 20°C), 5 pmol of each primer, 10 ng of DNA, 200 µmol/L aliquots of each dNTP; 1 mM (Roche, Penzberg, Germany), and 1 unit of *Taq* polymerase; 5 units/ml (Roche, Penzberg, Germany), resulting in a 481-base pair DNA fragment. For the second amplification, 2 µL of the primary amplification product was used in a 15 µL reaction mixture with cagA2, the sense primer (5'-ATAATGCTAAATTAGACAACTTGAGCGA-3'), and CagA2, the antisense primer (5'-TTAGAATAATCAACAAACATCACGCCAT-3'). Both PCR amplifications consisted of 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. The final amplicon (expected size, 298 base pairs) was analyzed by electrophoresis on a 2% agarose gel[33] (Figure 1).

RNA Isolation and Reverse Transcription-PCR of RUNX3 Gene

Total RNA was extracted using a commercially available kit (Easy-Blue, Intron, Seongnam, South Korea). The RT reaction was performed using 1 µg of total RNA with SuperScript III First-strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). The following primer sets were used: 5' TCTGCTCCGTGCTGCCCTCGCACTG-3' and 5' AGGCATTGCGCAGCTCAGCGGAGTA-3' for RUNX3 (151 bp). Amplification was carried out in a 15 µL reaction volume containing 0.1 µg of cDNA and 1.5 µL of 10 × PCR buffer with 20 mmol/L MgCl2, 3 µL of a GC-rich solution, 5 pmol of each primer, 200 µmol/L aliquots of each dNTP, and 1 unit of Fast start *Taq* DNA polymerase. PCR was performed in the PCR Thermal Cycler with an initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 62.3°C for 1 min, 72°C for 1 min and a final extension at 72°C for 7 min. The PCR product was separated on a 3% agarose gel[4] (Figure 2).

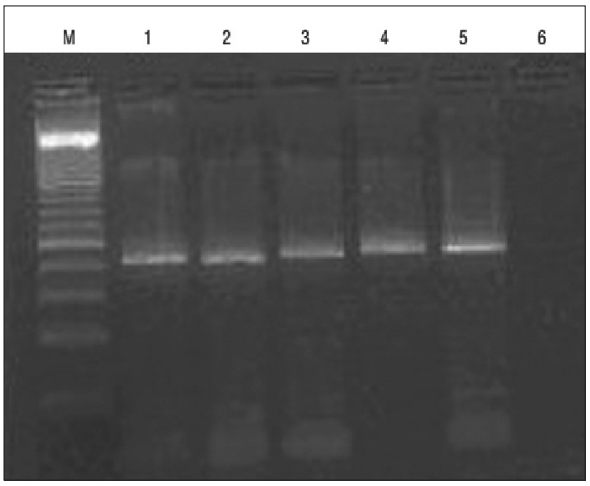

Figure 1. Polymerase chain reaction final amplification product of *Helicobacter pylori* cagA. M indicates molecular size standard (DNA ladder). Lanes 1 through 5: *H. pylori* cagA positive (298 bp); lane 6: negative control.

Immunohistochemistry of E-cadherin, and β-catenin

Immunohistochemistry was used for the detection of E-cadherin and β-catenin. The used primary antibodies were anti-E-cadherin (Ventana Medical Systems, Tucson, AZ, USA, Catalog Number: 790-4497) and anti-β-catenin (Ventana Medical Systems, USA, Catalog Number: 760-4242), monoclonal IgG antibodies. An indirect, biotin-free system was used for detecting the pri-

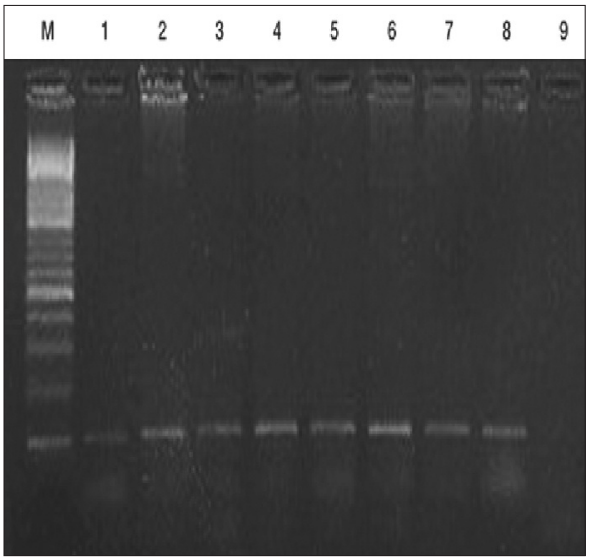

Figure 2. Polymerase chain reaction amplification product of RUNX3. M indicates molecular size standard (DNA ladder). Lanes 1 through 8: RUNX3 positive (151 bp); lane 9: negative control.

mary antibodies (ultra View Universal DAB Detection Kit Ventana Medical Systems, Germany, and Catalog Number: 760-500). Briefly, 4 μ-thick paraffin sections were placed on positive slides. Sections were deparaffinized using xylene and rehydrated in descending concentrations of ethanol. Endogenous peroxidase activity was blocked by incubating sections with 3% H_2O_2 for 20 min. Antigen retrieval was done by microwaving sections for 15 min in 10 mM citrate buffer pH 6.0, then cooling sections for 30 min at room temperature. Nonspecific binding were blocked with 1% bovine serum albumin for 30 min, followed by incubation of sections with the primary antibodies overnight at 4°C. Immunohistochemical staining was performed using the Ventana 320 ES automated immunostainer (Ventana Medical Systems, Tucson, AZ). Mayer's hematoxylin was used as counterstain. Normal gastric tissues were considered positive controls. Negative control was accomplished by using phosphate buffered saline (PBS), instead of the primary antibody[34].

Interpretation of Immunostaining

A semi quantitative estimation of E-cadherin and β-catenin expression was made, using a combined score done by adding the values of the intensity of immunoreaction and the quantity of immunoreactive cells[34]. Briefly, the intensity of immunoreaction was graded from 0 to 3: 0 for absence of staining, 1 for cytoplasmic staining, 2 for mixture of both normal and abnormal staining areas, and 3 for membranous staining. The quantity of positive cells was graded from 0 to 4: 0: for positive cells < 5%, 1: 5-25%, 2: 26-50%, 3: 51-75%, 4: 76-100%. The combined score 6 or 7 was considered preserved expression. The scores between 0 and 5 were considered decreased expression.

Statistical Analysis

Statistical analysis was done using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). The correlation between two variables was evaluated using chi-square, Fisher's exact and Student *t*-tests, where $p \leq 0.05$ was considered significant.

Results

This study included 90 patients with *H. pylori* associated chronic active gastritis (Figure 3). They belonged to 66 males and 24 females with mean age 36.5 ± 9.686 (range 16-54 years).

Nested PCR was used for detection of the cagA gene. Forty five cases were found to be cagA positive (50%). Table I showed the relation between cagA and the different studied parameters. The mean age of cagA positive patients was 38.87 years compared with 34.13 years for cagA negative patients. The difference was statistically significant ($p = 0.02$). On the other hand there was no relation between cagA and sex of patients ($p = 1.00$). On studying the relation between cagA and the different histopathological findings, cagA was found to be associated with the intensity of mononuclear inflammation ($p = 0.007$), the intensity of neutrophilic inflammation ($p = 0.001$) and the degree of mucosal atrophy ($p = 0.000$). However, there was no relation between cagA and the density of *H. pylori* ($p = 0.176$) or intestinal metaplasia ($p = 0.118$). RUNX3 ($p = 0.046$) but neither E-cadherin ($p = 0.662$) nor β-catenin ($p = 0.263$) were found to be associated with cagA.

Reverse transcription PCR were used for detection of RUNX3 RNA. As Table II showed, there was no significant relation between RUNX3 and the age ($p = 0.143$) or the sex ($p = 1.00$) of patients. On the other hand, there was significant relation between loss of RUNX3 and increasing density of *H. pylori* ($p = .003$), intensity of neutrophilic inflammation ($p = 0.002$), mucosal atrophy ($p = 0.036$) and intestinal metaplasia ($p = 0.000$). However, although there was increasing of intensity of mononuclear inflammation with loss of RUNX3 the difference did not reach statistical significance ($p = 0.059$). RUNX3 was found to be significantly correlated with E-cadherin ($p = 0.040$) but not with β-catenin ($p = 0.263$).

Both β-catenin and E-cadherin were detected using IHC (Figure 4). Regarding E-cadherin thirty three biopsies (36.7%) showed decreased expression. As table III showed decreased E-cadherin was significantly associated with younger age ($p = 0.027$) and female gender ($p = 0.000$). It was also significantly associated with intensity of mononuclear inflammation ($p = 0.003$). However, there was no significant relation between decreased E-cadherin and the other studied histopathological factors. On the other hand β-catenin showed decreased expression in thirty biopsies (33%). There was an association between decreased β-catenin and intensity of neutrophilic inflammation ($p = 0.001$). However, no

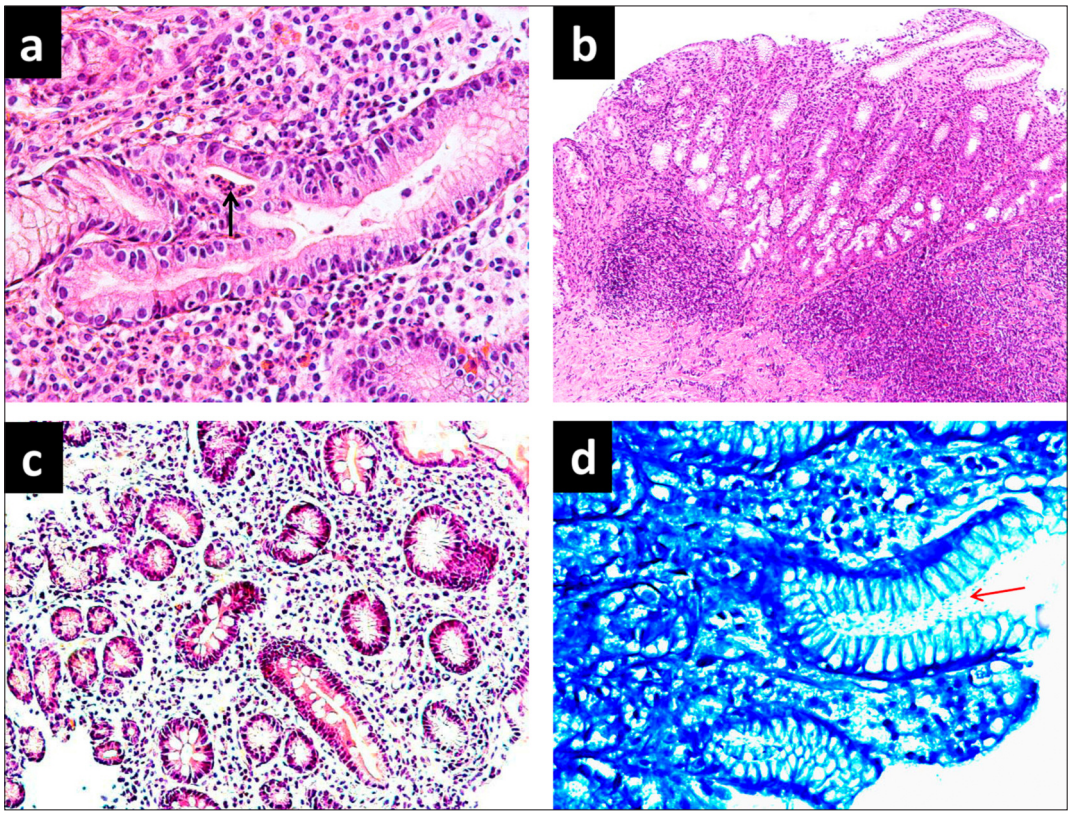

Figure 3. Chronic active *H. pylori* associated gastritis. ***a,*** Intense infiltration of the gastric glands and the lamina propria by neutrophils. A pit microabscess appeared in the center of the section (*black arrow*) (H&E × 400). ***b,*** Severe mononeuclear inflammation of the mucosa and the lamina propria (H&E × 40). ***c,*** Gastric glands with intestinal metaplasia and moderate glandular atrophy (H&E × 200). ***d,*** Numerous *H. pylori* bacilli were observed in the lumen of a gastric pit (*red arrow*) (Kwik-Diff × 400).

significant relation was detected between decreased β-catenin and the other variables (Table IV). E-cadherin and β-catenin were found to be significantly related to each other ($p = 0.002$).

Discussion

H. pylori infection is prevalent in Saudi Arabia. In one study conducted at Makah the prevalence of *H. pylori* infection in the healthy individuals was 51%, and reaches up to 61% as the age advances[35]. The prevalence rates rises up to 60-88% in patients with gastrointestinal diseases[36-39]. Many studies assumed that the different infection rates of *H. pylori* as well as the incidence and/or severity of *H. pylori*-related gastric diseases may be partly related to the different distribution of *H. pylori* virulence factors among different countries[40,41]. The most important determinant factor is the cag island. It is 40 kb, containing 31 genes. CagA, the terminal gene of the island was considered a marker for the entire locus[42].

In the current study the prevalence of the virulence factor cagA was 50%. This result was different from other previous studies in different areas. For example in one study conducted in Egypt the prevalence of cagA was 62.2%. It was 40% in non-ulcer dyspepsia, 66.7% in peptic ulcer and 89% in gastric cancer. They used ELISA for detection of anti-cagA antibodies[43]. In Tunisian patients suffering from gastritis, peptic ulcer, gastric carcinoma and MALT lymphoma, the prevalence of cagA was 61.6%. *H. pylori* was first cultured and cagA was detected using PCR[44]. In one study carried out in Cyprus the prevalence was 41.5%[45] and in another study in Brazil it was only 29.6%[42]. The prevalence of cagA in Colombian patients was 71%[46]. Differences in results not only related to the geographic distribution of different studies but also to the

Table I. The relation between cagA and the different studied variables.

Variable	cagA		*p* value	Significance
	-ve	+ve		
Age				
Mean (± std)	34.13 (9. 5)	38.87 (9. 38)	0.02	S
Sex				
Male	33	33	1.00	NS
Female	12	12		
Density of *H. pylori*				
Mild	18	15	0.176	NS
Moderate	24	21		
Severe	3	9		
IMI				
Mild	15	6	0.007	S
Moderate	24	21		
Severe	6	18		
INI				
Mild	21	6	0.001	HS
Moderate	9	24		
Severe	15	15		
Atrophy				
Mild	24	3	0.000	HS
Moderate	21	33		
Severe	–	9		
Metaplasia				
-ve	42	36	0.118	NS
+ve	3	9		
RUNX3				
-ve	18	27	0.046	S
+ve	27	18		
E-cadherin				
Decreased	15	18	0.662	NS
Preserved	30	27		
β-catenin				
Decreased	12	18	0.263	NS
Preserved	33	27		

IMI: intensity of mononuclear inflammation, INI: intensity of neutrophilic inflammation, S: significant, HS: highly significant, NS: not significant.

method used for detection of the virulence factor. The highest prevalence of cagA positive strains was found in studies used ELISA for detection of the cagA compared with those using PCR. This is attributed to the presence of circulating antibodies for a long period even after eradication of infection[47]. Moreover, DNA extraction from isolates obtained from colonies of *H. pylori* grown in culture can lead to different results compared to a technique that performs extraction directly from gastric biopsy specimens[48]. Direct PCR from biopsy specimens tends to show less prevalence of cagA, especially when bacterial density is low. The difference of results may be also related to the different types of studied diseases ranged from gastritis to peptic ulcer and gastric carcinoma[49]. Bearing in mind that our study included only patients with chronic gastritis and cagA was detected using PCR with DNA extracted directly from paraffin sections of gastric tissues, the 50% prevalence of cagA is considered relatively high.

The current study showed that cagA positive strains tend to infect older patients ($p = 0.02$). However, there was no relation detected between cagA and sex of patient ($p = 1.00$). Queiroz et al[47] revealed that colonization by cagA positive strains seems to increase with age. They suggested that this susceptibility may be related to the different expression of adhesion molecules in the gastric mucosa, which changes over time. They also noted that cagA positive *H. pylori* was more prevalent in adults compared with children.

Table II. The relation between RUNX3 and the different studied variables.

Variable	RUNX3 -ve	RUNX3 +ve	*p* value	Significance
Age				
Mean (± std)	35 (9.195)	38 (10.032)	0.143	NS
Sex				
Male	33	33	1.00	NS
Female	12	12		
Density of *H. pylori*				
Mild	24	9	0.003	S
Moderate	15	30		
Severe	6	6		
IMI				
Mild	15	6	0.059	NS
Moderate	18	27		
Severe	12	12		
INI				
Mild	21	6	0.002	
Moderate	12	21		
Severe	12	18		
Atrophy				
Mild	18	9	0.036	S
Moderate	21	33		
Severe	6	3		
Metaplasia				
-ve	33	45	0.000	HS
+ve	12	0		
E-cadherin				
Decreased	21	12	0. 040	S
Preserved	24	33		
β-catenin				
Decreased	18	12	0.263	NS
Preserved	27	33		

IMI: intensity of mononuclear inflammation, INI: intensity of neutrophilic inflammation, S: significant, HS: highly significant, NS: not significant.

Several previous studies revealed that severe gastritis, glandular atrophy and intestinal metaplasia are considered risk factors for the development of gastric carcinoma. It was presumed that the steps for developing gastric adenocarcinoma could be chronic gastritis, mucosal atrophy, intestinal metaplasia, followed by dysplasia and finally adenocarcinoma[48]. Our study showed that cagA is associated with the severity of gastritis. There was a relation between cagA and the intensity of mononuclear inflammation ($p = 0.007$), the intensity of neutrophilic inflammation ($p = 0.001$) as well as the degree of mucosal atrophy ($p = 0.000$). However, no relation was detected between cagA and the density of *H. pylori* ($p = 0.176$) or intestinal metaplasia ($p = 0.118$). Our results were in concordance with other studies. For example, Umit et al. revealed a significant relationship between chronic inflammation and cagA-positive strains in gastric cardiac biopsies. They also found a significant relationship between cagA and neutrophilic inflammation and mucosal atrophy, but not with density of *H. pylori*, the degree of chronic inflammation, or metaplasia in the antrum specimens[49]. Nogueira et al[50] also detected association between cagA positive genotype and higher degrees of lymphocytic and neutrophilic infiltrates and atrophy. However, in contrast with our results they found association between cagA and both *H. pylori* density and intestinal metaplasia. Prinz et al[51] also correlated cagA with activity and chronicity of gastritis.

These results may be attributed to the relation between cag island and the release of pro-inflammatory cytokines from gastric epithelial cells. Several previous studies revealed that there are different genes within the cag island are respon-

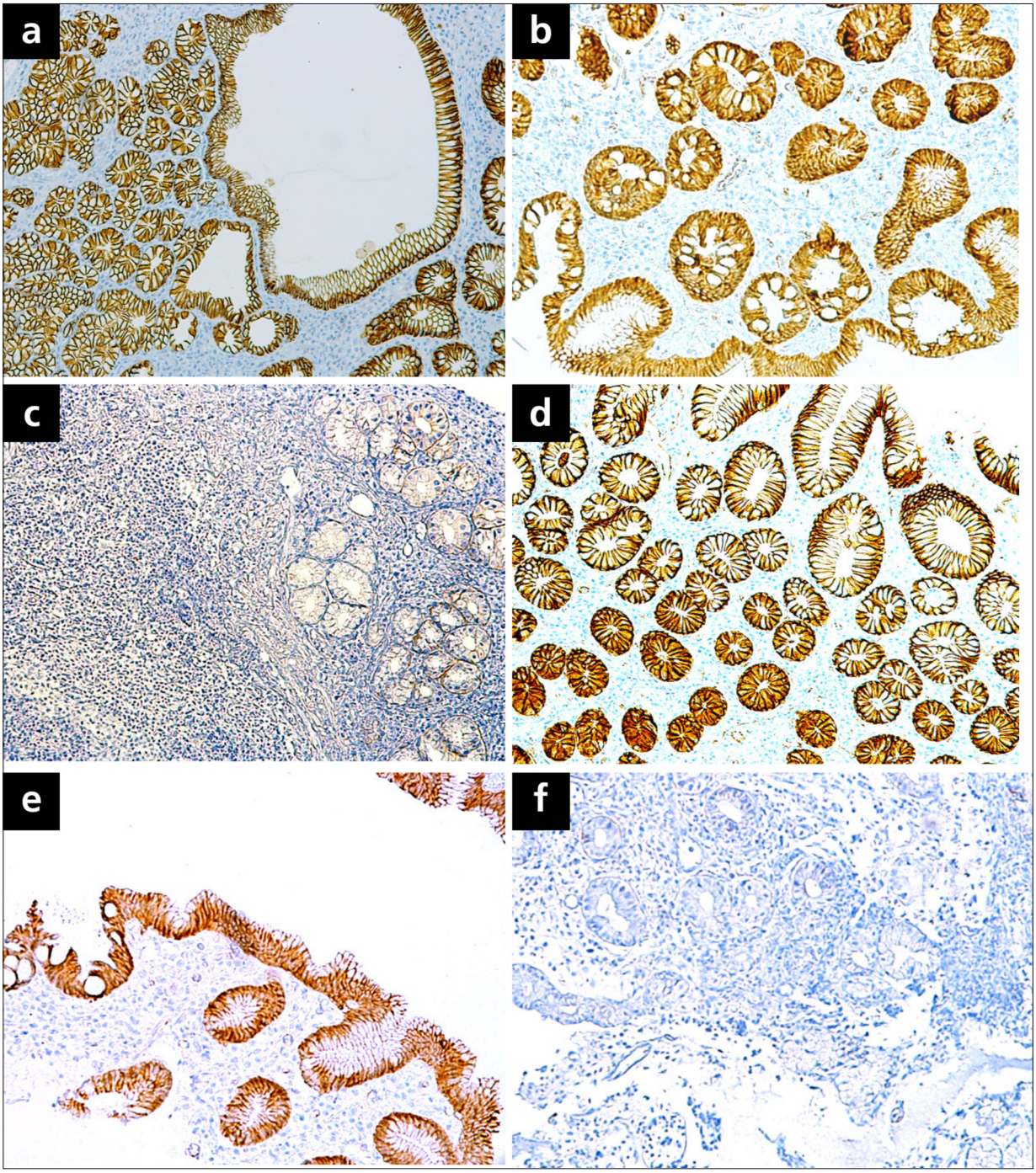

Figure 4. Immunohistochemical staining of E-cadherin and β-catenin (DAB). ***a,*** Membranous and cytoplasmic expression of E-cadherin (score 6) in a case with mild atrophy. Some gastric glands were cystically dilated (×100). ***b,*** Membranous and cytoplasmic immunostaining of E-cadherin (score 6) in chronic gastritis with intestinal metaplasia and moderate atrophy (×200). ***c,*** Decreased E-cadherin expression; heterogeneous staining of less than 5% of the gastric epithelium (score 2) with severe mononeuclear inflammation (×100). ***d,*** Both membranous and cytoplasmic expression of β-catenin (score 6) in chronic gastritis with mild gastric atrophy (×200). ***e,*** β-catenin immunostaining (score 6) in a case with intestinal metaplasia and severe atrophy (×200). ***F,*** Complete loss of β-catenin immunostaining (score 0) in a case with severe neutrophilic inflammation and severe gastric atrophy (×200).

Table III. The relation between E-cadherin and the different studied variables.

Variable	E-cadherin		*p* value	Significance
	Decreased	Preserved		
Age				
Mean (± std)	33.55 (9.93)	38.21 (9.2)	0.027	S
Sex				
Male	15	51	0.000	HS
Female	18	6		
Density of *H. pylori*				
Mild	9	24	0.303	NS
Moderate	18	27		
Severe	6	6		
IMI				
Mild	3	18	0.003	S
Moderate	15	30		
Severe	15	9		
INI				
Mild	6	21	0.160	NS
Moderate	15	18		
Severe	12	18		
Atrophy				
Mild	9	3	0.144	NS
Moderate	18			
Severe	06			
Metaplasia				
-ve	30	48	0.524	NS
+ve	3	9		
β-catenin				
Decreased	18	12	0.002	S
Preserved	15	45		

IMI: intensity of mononuclear inflammation, INI: intensity of neutrophilic inflammation, S: significant, HS: highly significant, NS: not significant.

sible for the induction of interleukin (IL)-8 among other many cytokines[52-54]. These genes also inactivate NF-B and MAPK signal transduction pathway, resulting in the production of inflammatory cytokines[55]. Moreover, studies on animal models infected with *H. pylori* showed that loss of the cag locus decreased the severity of gastritis and mucosal atrophy[56,57].

RUNX3 is a tumor suppressor gene which plays an essential role in the evolution of gastric cancer[58]. The current study examined the relation between loss of RUNX3 and the early events in the cascade of gastric adenocarcinoma development. We showed that loss of RUNX3 is associated with increasing *H. pylori* density ($p = 1.00$). Previous studies revealed that *H-pylori* can activate DNA methyltransferase through the induction of nitric oxide, resulting in methylation of many tumor suppressor genes, including RUNX3[59-63]. RUNX3 methylation could be a cause of its decreased expression[64]. Moreover, recent studies found that RUNX3 is related to the innate immunity of the gastric epithelium. RUNX3 is mandatory for IL23A induction by the TNF-a/ NF-κB pathway. IL23A is a part of NF-κB -driven antibacterial response of gastric epithelial cells. Defect in this pathway resulted in defective clearance of *H. pylori*[65]. This could be a reason for the increasing density of *H. pylori* with RUNX3 loss.

The current study revealed a relation between RUNX3 and intensity of neutrophilic inflammation ($p = 0.002$), mucosal atrophy ($p = 0.036$) and intestinal metaplasia ($p = 0.000$). There was also increasing intensity of mononuclear inflammation with loss of RUNX3. However, the difference was statistically insignificance ($p = 0.059$). In concordance with our results Li et al[64] in a population based study found a relation between decreased RUNX3 expression and gastric inflammation, atrophy and intestinal metaplasia. Several other studies revealed a relation between RUNX3 and intestinal metaplasia[66,67]. Loss of RUNX3 is associated with the transformation of

Table IV. The relation between β-catenin and the different studied variables.

Variable	β-catenin		p value	Significance
	Decreased	Preserved		
Age				
Mean (± std)	37.9 (7.2)	35.8 (10.7)	0.335	NS
Sex				
Male	24	42	0.449	NS
Female	6	18		
Density of *H. pylori*				
Mild	12	21	0.774	NS
Moderate	15	30		
Severe	3	9		
IMI				
Mild	6	15	0.128	NS
Moderate	12	33		
Severe	12	12		
INI				
Mild	3	24	0.001	HS
Moderate	12	21		
Severe	15	15		
Atrophy				
Mild	6	21	0.325	NS
Moderate	21	33		
Severe	3	6		
Metaplasia				
-ve	24	54	0.204	NS
+ve	6	6		

IMI: intensity of mononuclear inflammation, INI: intensity of neutrophilic inflammation, HS: highly significant, NS: not significant.

gastric epithelial cells into intestinal type cells in animal models. RUNX3 acts with Smads resulting in induction of TGF-β/activin signals. Some attributed intestinal metaplasia to the activation of this pathway[68-73]. TGF-β is also considered an anti-inflammatory cytokine[77]. Fainaru et al[75] revealed that RUNX3 regulates dendritic cell function through the induction of TGF-β. They found that RUNX3$^{-/-}$ mice develop spontaneous inflammation of lung.

Our study showed a relation between cagA and loss of RUNX3. Tsang et al. showed that cagA-positive *H. pylori* inactivates RUNX3[28]. CagA recognizes RUNX3 through its WW domain which combines with the PY motif of RUNX3. This combination results in degradation of RUNX3. CagA also inhibits RUNX3 expression through Src/MEK/ERK and p38 MAPK pathways[76].

β-catenin is the key factor in the Wnt pathway that plays important roles in cell differentiation, migration and proliferation. Normally, β-catenin is integrated with E-cadherin in the membrane, and they form a complex that mediates cellular adherence. Aberrant expression of these genes has been reported in some malignant tumors including gastric adenocarcinoma[77].

This study showed decreased expression of E-cadherin and β-catenin in 36.7% and 33% of cases respectively. Both markers are not related to cagA. Previous studies showed that E-cadherin methylation occurs early in the cascade of gastric carcinoma development. This methylation is related to *H-pylori* infection. *H. pylori*, both cagA positive and cagA negative, is associated with up-regulation of IL-1b which plays an important role in E-cadherin methylation[78]. In one study methylation of E-cadherin was detected in 31% of patients suffering from *H. pylori* associated dyspepsia[79]. Another study[16] showed a rate of 46%. Chan et al[78] found an association between E-cadherin methylation and its decreased expression by immunostaining. Tamura et al[79] reported that decreased E-cadherin expression due to methylation, is associated with reduced the content of combined β-catenin in the complex. These lead to decreased expression of both E-cadherin and β-catenin as we detected in this study. Moreover, recently it was found that *H.*

pylori can activate protease calpain in gastric epithelial cells. This activation results in cleavage of E-cadherin and induces relocalization of E-cadherin and β-catenin. The action is not related to cagA[80]. *H. pylori* can also stimulate release of β-catenin which in turn is translocated into nucleus. This occurs through the activation of LRP6, Dvl2 and Dvl3 independent of the cagA or vacA[81].

Concomitant with our results many studies showed E-cadherin and β-catenin decreased expression in the early events of gastric cancer development such as chronic gastritis, intestinal metaplasia and dysplasia[82-84]. However, others did not find these changes. Different results could be contributed to the different antibodies and different scoring systems used[34].

This study showed that decreased E-cadherin expression is associated with increasing intensity of mononuclear inflammation. Recent studies found that interferon gamma (IFNγ), the important pro-inflammatory cytokine, regulates E-cadherin. It leads to E-cadherin ubiquitination and subsequent proteasomal degradation. This function is dependent upon Fyn kinase[85]. Our study also showed that decreased expression of β-catenin is associated with increasing the intensity of neutrophilic inflammation. Recently, it was found that Wnt/β-catenin activation is associated with the expression of several inflammatory cytokines during bacterial infections. β-catenin signaling was found to induce an inflammatory program through transcription and activation of the NF-κB pathway, LECT2 and iNKT[86].

The current study revealed a relation between E-cadherin and β-catenin. Zali et al[34] and Huiping et al[87] also detected the same relation. Also, concomitant with our results Tanaka et al[88] found a significant relation between E-cadherin and RUNX3. They revealed that RUNX3 reverses EMT in hepatocellular carcinoma through expression of E-cadherin. On the other hand we did not find a relation between β-catenin and RUNX3. These results is in contradiction with results detected by Lin et al[27]. However, they studied the relation between RUNX3 and β-catenin in gastric cancer cell lines, not in gastritis as in this study.

Conclusions

Loss of RUNX3, E-cadherin and β-catenin were considered early events in the cascade of gastric carcinoma development. RUNX3 but neither E-cadherin nor β-catenin, was related to cagA positive *H. pylori* strains. Loss of RUNX3 was associated with decreased E-cadherin expression. E-cadherin and β-catenin were related to each other.

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Ethical Approval

Ethical Committee of scientific research of Taibah University in Al-Madinah Al-Monawarah approved the study.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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