Original Article

Prevalence of *Helicobacter pylori* Genes (*CagA*, *BabA*, and *HomB*) with Stem Cell Markers (LGR5 and CD133) in Severity of Gastric Disease

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Abstract

Background: Gastritis is commonly caused by *Helicobacter pylori*, which colonizes the mucosa of the gastric epithelium in more than half the population worldwide. It adheres to the gastric epithelium, with an important aspect of the pathogenicity of the microorganism facilitating the transport of certain proteins such as CagA, BabA, and Hom into that epithelium. **Objective:** This study aimed to determine the association between the co-expression of LGR5 and CD133 in patients with gastric diseases and assess the distribution of *H. pylori* outer membrane genes (*CagA*, *BabA*, and *HomB*) with gastrointestinal disease outcomes. **Materials and Methods:** For the 140 patients enrolled, *H. pylori* virulence factors including Cag, BabA, and HomB were detected using Multiplex real-time PCR, while LGR5 and CD133 expressions were determined via immunohistochemistry. **Results:** The expressions of BabA and HomB had a significant difference with gastric disease such as gastric cancer, gastric metaplasia, and gastritis with and without the colonization of *H. pylori*, while there was no significant difference of CAG gene expression with gastric disease. Co-expression of LGR5 and CD133 had significant association with gastric disease. **Conclusion:** There was a significant increase in *H. pylori* Ag (BabA and HomB) expression among patients with gastric disease. However, no significant differences were observed between *H. pylori* Cag Ag expression among patients with gastric disease, and all the patients were infected with *H. pylori* having the Cag gene (100%). It is suggested that CD133 and Lgr5 may play an important role in gastric carcinoma.

Keywords: Gastric cancer cell, *Helicobacter pylori*, virulence genes

Introduction

Helicobacter pylori is a Gram-negative, spiral-shaped, 4- to 6-flagellated, mobile bacterium colonized in the gastrointestinal tract in microaerophilic surroundings at 37°C. Flagellae, urease enzyme, and adherence ability are pivotal factors aiding in *H. pylori* colonizing the gastric mucosa, and its prevalence is found in more than half the population worldwide. The pathogenicity of *H. pylori* is significantly related to its virulence factors, including CagA, Lewis blood group antigen-binding adhesin (*BabA*), and HomB. Initially to colonize the mucosa, *H. pylori* uses the adhesion proteins *babA* and sialic acid-binding adhesin (*sabA*), which have a significant effect on the initial steps of infection. *H. pylori* is a Gram-negative bacterium that causes active gastric inflammation in the stomach. This microbe not only causes gastritis but also causes gastric cancer and gastric metaplasia[1,2]

The *babA* facilitates the *CagA* and *vacA* virulence factors to enter the gastric epithelial cell, while *sabA* binds to carbohydrates that have been reacted with sialic acid on

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neutrophil surfaces. Thus, *sabA* promotes an immune reaction. Since the current approach in screening, diagnosis, and monitoring of gastric malignancy relies on markers' predictability, it is very important to identify newer biomarkers, preferably more accurate, that can potentially measure each therapy, orienting well-guided management plans.[3] The Hom genes that encode the outer membrane proteins of *H. pylori* have allelic variations, especially at the significant intermediate regions of 300 bp. This highly polymorphic region of HomB is located at 750–1050 bp, while in *homA,* it is at 720–980 bp.[4]

One of these markers represents human intestinal stem cells that have the potential to be another cancer stem cell (CSC) marker. In biology, stem cells are defined as unspecialized cells that can self-renew, giving rise to more stem cells having the same features and identity of the mother cell.[5] LGR5 or GPR49 (leucine-rich repeat-containing and G protein-coupled receptor 5, respectively) is a stem cell. Precursors and progressed CSCs express this marker of their crypts; in addition, it is expressed at the basal prospective corpus and pyloric glands in the neonatal gastric tissue, mainly confined basally at mature pyloric glands in adults.[6] Its gene is approximately 144 kb long and is located on chromosome 12 positioned at 12q22–q23. Interestingly, Lgr5 is upregulated in different malignancies, including small bowel, colonic CA, and hair follicles.[7]

It was recognized on crypt stem cells (precursor cells) in addition to precancerous lesions, usually identified in corpus and pyloric glands in stomachs of neonates and predominantly confined basally in adults' mature pyloric glands. CD133 (human prominin-1, PROM1), a 5-transmembrane glycoprotein, is primarily localized in membrane protrusions. The CD133 marker (either alone or with others) has been utilized extensively as a candidate marker to identify CSCs and predict prognosis from a variety of cancers. Evidence indicates that CD133 expression can be found in over half of human gastric cancers and is shown to be associated with poor prognosis using immunohistochemistry.[8]

Materials and Methods

Specimens' collection

The tissue samples were obtained retrospectively from 140 paraffin-embedded archived blocks from January to December 2020, at Educational Laboratories of Medical City, Al-Yarmook Teaching Hospital, Specialized Center for Gastroenterology and Hepatology, Al-Moayad Private Hospital, and Dijla Private Hospital. The samples were obtained from 68 males and 72 females (mean age 51.49 years, ranging from 20 to 86 years) divided into four groups: 30 gastric cancers (mean age 52.66 years, ranging from 20 to 85 years), 30 gastric metaplasia tissues (mean age 53.5 years, ranging from 25 to 86 years), 60 gastritis (mean age 48.5 years, ranging from 20 to 85 years), and 20 normal gastric tissue (mean age 51.8 years, ranging from 26 to 85 years). The latter group was chosen as a control (samples from patients without lesion in gastric mucosa).

After obtaining approval from each center for using the specimens, the diagnosis was reconfirmed histopathologically through reviewing freshly prepared hematoxylin and eosin-stained slides.

Material and method of immunostaining

Each paraffin block was sectioned into 5 µm. From each block, three sections were taken: one for hematoxylin and eosin (H& E stain) for histopathology revision, and two were used on positively charged slides (Fischer brand) for immunohistochemical staining with LGR5,CD133 rabbit polyclonal IgG antibodies.

The (H&E-stained sections had been reassessed for the morphological types of the tumor, grade, and other parameters by two independent pathologists. For LGR5 and CD133 immunohistochemical detection, rabbit anti-human LGR5: rabbit polyclonal IgG, H-100 and SC-9048, lot # D2613, rabbit anti-human CD133: rabbit polyclonal IgG, H-132, and SC-7927, lot # A3113 (Santa Cruz Biotechnology, Texas, USA), at 1:50 dilution with an overnight incubation at 4°C for both.

After the primary antibody reacted with the tissue antigen, a biotin-labeled secondary antibody was added to bind to the primary antibody. After that, this conjugate was added, and the biotinylated secondary antibody formed a complex with the peroxidase-conjugated streptavidin. The substrate, which contained 3, 3 diaminobenzidines (DAB) in a chromogen solution, was then added, resulting in the formation of a brown-colored precipitate at the antigen site.

The appearance of reacting brown products at the site of the targeted antigen is associated with a positive reaction in the peroxidase secondary detection system. A counterstain was then used for blue staining of the cells. Appropriate positive control slides (human kidney for CD133 and human placenta tissue for LGR5 as indicated by the manufacturer's instructions) and negative control slides (technically negative by omitting the primary antibody) were included in each run of immunohistochemical staining. Immunostaining evaluation was conducted by two independent histopathologists who were blinded to the clinical diagnosis of each tissue.

Scoring

Light microscope examination was carried out to estimate the number of cells that exhibited a positive (brown cytoplasmic staining) reaction. Estimation of the immunohistochemical staining intensity was done by counting the number of cells in each of the 10 fields (×40

magnification), and then this score was divided by the total number of cells per field, so the percentage of positively stained gastric cells (in the 10 fields) can be estimated by taking the mean of the percentages of the positively stained gastric cells in the 10 fields.

Expression of CD133 was defined as follows: (0%–5%) no expression, (5%–25%) weak expression, (26%–50%) moderate expression, and extensive/ high expression (≥ 75%) of neoplastic cells. Expression of LGR4 was defined as follows: (0%) no expression, (1%–25%) weak expression, (26%–50%) moderate expression, and extensive/ high expression (≥ 75%) of neoplastic cells. The secondary staining kit used was Goat anti-Rabbit IgG CAT #SC-2050 (Santa Cruz Biotechnology) and Goat anti-Rabbit IgG CAT #2051 (Santa Cruz Biotechnology).

Genomic DNA extraction from bacterial isolates

gDNA was extracted from the gastric tissue biopsy by a specific kit based on the MagPurix technology, a fully automated and highly innovative system for rapid molecular biology sample extraction. This cutting-edge platform uses magnetic beads to extract nucleic acids from samples. From samples to outcomes, the platform provides true walk-away automation in nucleic acid purification. The matrix technology provides high-quality results in three simple steps: load samples, execute the pre-programmed protocol, and collect findings. The purification procedure includes stages such as lysis, binding, washing, and elution. The MagPurix FFPE DNA Extraction Kit is intended to be used with MagPurix® Instruments for the extraction of genomic DNA from FFPE (formalin-fixed, paraffin-embedded) tissue samples.

Instrumentation and equipment

A number of instruments and different types of equipment were used for processing the Genomic DNA extraction, such as reagent cartridge, reaction chamber, tip holder, filter tip, piercing pin, sample tube (2 mL), elution tube (1.5 mL), Proteinase K (10 mg/ mL) (1 mL), BL4 buffer (25 mL), DP buffer (15 mL), filter column, collection tube, barcode paper, and selection guide.

PCR detection of *H. pylori* gene (CagA gene, Bab A gene, and HomB gene)

Samples were prepared for amplifying specific regions of Cag, BabA, and HomB genes. Beacon Designer V: 8.21software was used to design the primers. Alpha DNA Company supplied primers that were used according to the manufacturer's instruction to yield a primer with a concentration of 10 pmol/μL, as shown in Table 1. They were used for detection of CagA for *H. pylori* and BabA gene and HomB genes, as shown in Table 2. The PCR amplification program is shown in Table 3.

Table 1: The sequence, product size, and reference of the used primer

Name of primer	Sequence	Product length (bp)	Reference
HP: CagA Detection	F:GAGGCTAGTAAGGAAGCA R: GTGGTGTTGGAAGGTTAA	122	Design
G1: BabA	F:GAGGCTAGTAAGGAAGCA R: GCCAAAGTCTTTCAAATTCTTG	111	Design
G2: HomB	F:TCCGCATCCTTAAACAGA R: TGCACAAACGCAAGAAAA	126	Design

Table 2: PCR components for amplifying the targeted fragments for detection of virulence factors for *H. pylori* (multiplex real-time PCR by HRM)

Components	Volume (μL)
Forward primer (CagA)	0.5
Reverse primer (CagA)	0.5
Forward primer (BabA)	0.5
Reverse primer (BabA)	0.5
Forward primer (HomB)	0.5
Reverse primer (HomB)	0.5
DNA template	3.5
D.W.	3.5
PCR remix (ready to use) EVA green	10
Final volume	20

Table 3: Shows the PCR amplification program

Step	Temp (°C)	Time	Number cycle
Initial denaturation	95	5 min	1
Denaturation	95	10–30 s	30–40
Annealing	55–68	10–60 s	30–40
Melting curve	65–95	2–5 s/step	1

Statistical analysis

Analysis of data was carried out using the Statistical Package for Social Sciences SPSS-28, IBM Corp., Armonk, NY, USA). Descriptive data were represented as frequencies, percentages, means, and standard deviations. To examine the statistical difference between two numerical values, independent-samples *t* test was used, whereas paired-samples *t* test was used to examine the significance of change in paired observations. The analysis of variance test was done for comparison of variances among more than two numerical variables. The chi-square test (χ^2-test) with application of Yates' correction or Fisher's exact test whenever applicable was used to test the significance of association between categorical variables. *P* value less than 0.05 was considered significant throughout the study.

Ethical approval:

The experimental design was applied out based on the guidelines of the laboratory and according to the protocol

approved by the Department of Microbiology/College of Medicine/Mustansiriyah University, Baghdad, Iraq, with the ethical clearance number 693 dated January 20, 2021.

Results

The immunohistochemical expression for both markers (CD133 and LGR4) was cytoplasmic with different intensities, as demonstrated, and multiplex real-time PCR was conducted for diagnosis of CagA, BabA, and HomB genes. The study sample was divided into four groups, which included gastric carcinoma 30 (21.4%), gastric metaplasia 30 (21.4%), gastritis with and without *H. pylori* 60 (42.8%), and apparently normal tissue control samples 20 (14.2). The Cag gene was expressed in all gastric tissue samples (100%), indicating no significant difference between gastric carcinoma, gastric metaplasia, *H. pylori*-positive gastritis, *H. pylori*-negative gastritis, and healthy controls, as shown in Table 4.

There was a significant difference between the level of the BabA1 gene in gastric carcinoma, gastric metaplasia, *H. pylori* +ve gastritis, *H. pylori* −ve gastritis, and healthy controls ($P = 0.0001$). In comparing each of the healthy controls with gastric carcinoma and gastric metaplasia, significant differences were observed ($P = 0.0001$). In comparing healthy controls with *H. pylori* +ve gastritis, significant differences were observed ($P = 0.013$), but no significant differences between healthy controls and *H. pylori* −ve. In comparing *H. pylori* −ve gastritis with gastric CA and gastric metaplasia, there were significant differences ($P = 0.0001$). Similar to that, there was a significant difference between *H. pylori* −ve gastritis and *H. pylori* +ve ($P = 0.006$), but no significant difference was observed between *H. pylori* −ve with healthy controls. In comparing *H. pylori* +ve and gastric metaplasia, there was no significant difference with all study groups in Table 5.

There was a significant difference in the level of the HomB gene in gastric CA, gastric metaplasia, *H. pylori* +ve gastritis, *H. pylori* −ve gastritis, and healthy controls ($P = 0.008$). In comparing healthy controls with gastric CA, there was a significant difference ($P = 0.007$). In comparing healthy controls with gastric metaplasia, there was no significant difference. In comparing healthy controls with *H. pylori* +ve, there was a significant difference with $P = 0.050$. However, no significant differences were observed between healthy controls and *H. pylori* −ve gastritis. In comparing *H. pylori* −ve gastritis with gastric CA, there was a significant difference ($P = 0.003$). In comparing *H. pylori* −ve gastritis with gastric metaplasia, there was a significant difference ($P = 0.032$). In comparing *H. pylori* −ve gastritis and *H. pylori* +ve gastritis, there was a significant difference ($P = 0.029$). However, no significant difference was observed between *H. pylori* −ve gastritis and healthy controls. Additionally, there were no significant differences in both *H. pylori* +ve gastritis and gastric metaplasia with all study groups [Table 6].

Table 7 and Figure 1 illustrate a significant difference in the mean level of CD133 stem cell expression between healthy controls and the gastric CA and gastric metaplasia groups ($P = 0.0001$) and a significant difference in comparing *H. pylori* +ve gastritis ($P = 0.017$), but no significant difference between *H. pylori* −ve and healthy controls. In comparing *H. pylori* −ve with gastric CA and gastric metaplasia, there was a significant difference ($P = 0.0001$) and a significant difference in comparing *H. pylori* +ve with of ($P = 0.036$), *b*ut no significant difference with healthy controls. In comparing *H. pylori* +ve with gastric CA and gastric metaplasia, there was a significant difference with ($P = 0.0001$). However, there was no significant difference in the mean level of CD133 stem cell marker expression between *H. pylori* +ve group and the *H. pylori* −ve and healthy controls. In comparing the gastric metaplasia group with the gastric CA, there was a significant difference with $P = 0.0001$. While there was no significant difference in the mean level of CD133 stem cell marker expression between the gastric metaplasia group, the *H. pylori* +ve, *H. pylori* −ve gastritis, and the healthy controls.

Table 4: Distribution of the *H. pylori* Cag gene in study groups

Variables		Gastric carcinoma		Gastric metaplasia		HP-positive gastritis		HP-negative gastritis		Healthy controls		*P* value
		No	%	No	%	No	%	No	%	No	%	
HP cag	Positive	30	100	30	100	35	100	–	–	–	–	–
	Negative	–	–	–	–	–	–	25	100	20	100	
P value compared to	HC	–		–		–		–		–		
	HP neg	–		–		–		–		–		
	HP post	–		–		–		–		–		
	G MP	–		–		–		–		–		

HC: healthy controls, HP neg: HP-negative gastritis, HP post: HP-positive gastritis, GMP: gastric carcinoma

*P value < 0.05

Table 5: Distribution of the *H. pylori* BabA1 gene in study groups

Variables		Gastric carcinoma		Gastric metaplasia		HP-positive gastritis		HP-negative gastritis		Healthy controls		*P* value
		No	%	No	%	No	%	No	%	No	%	
BabA1	Positive	14	46.7	12	40.0	9	25.7	–	–	–	–	0.0001*
	Negative	16	53.3	18	60.0	26	74.3	25	100	20	100	
P value compared to	HC	0.0001*		0.0001*		0.013*		–		–		
	HP neg	0.0001*		0.0001*		0.006*		–		–		
	HP post	0.078		0.220		–		–		–		
	GMP	0.602		–		–		–		–		

HC: healthy controls, HP neg: HP-negative gastritis, HP post: HP-positive gastritis, GMP: gastric carcinoma
*P value < 0.05

Table 6: Distribution of the *H. pylori HomB* gene in study groups

		Gastric carcinoma		Gastric metaplasia		HP-positive gastritis		HP-negative gastritis		Healthy controls		*P* value
		No	%	No	%	No	%	No	%	No	%	
HomB	Positive	9	30.0	5	16.7	6	17.1	-	-	-	-	0.008*
	Negative	21	70.0	25	83.3	29	82.9	25	100	20	100	
P value compared to	HC	0.007*		0.054		0.050*		–		–		
	HP neg	0.003*		0.032*		0.029*		–		–		
	HP post	0.220		0.959		–		–		–		
	GMP	0.222		–		–		–		–		

HC: healthy controls, HP neg: HP-negative gastritis, HP post: HP-positive gastritis, GMP: gastric carcinoma
*P value < 0.05

Table 7: The statistical analysis and difference of CD133 immunoexpression among different studied subjects

CD133% stem cell marker		Gastric carcinoma	Gastric metaplasia	HP-positive gastritis	HP-negative gastritis	Healthy controls
Mean ± SD		67.4 ± 16.7	50.18 ± 24.0	29.85 ± 21.7	18.64 ± 14.7	15.63 ± 13.7
Standard error of mean		3.05	4.53	3.77	3.14	3.41
(Range)		25–85	5–85	5–80	5–50	5–50
Percentile 05th		30	10	5	5	5
25th		65	35	15	5	5
50th (median)		75	50	25	13	10
75th		77	75	35	30	25
95th		85	80	80	40	50
99-		85	85	80	50	50
Compared to	HC	0.0001#	0.0001#	0.017#	0.635	–
	HP neg	0.0001#	0.0001#	0.036#	–	–
	HP post	0.0001#	0.0001#	–	–	–
	MP	0.0001#	–	–	–	–
	All	0.0001^				

HC: healthy controls, HP neg: HP-negative gastritis, HP Post: HP-positive gastritis, GMP: gastric carcinoma
#*P* value < 0.05 independent samples-*t* test. ^*P* value < 0.05 analysis of variance-test

According to the data presented in Table 8 and Figure 2, the mean level of Lgr5 stem cell expression in healthy controls is significantly different compared to the gastric carcinoma and gastric metaplasia groups ($P = 0.0001$). However, there was no significant difference in the mean level of Lgr5 stem cell marker expression between the healthy control group and both *H. pylori* +ve gastritis and *H. pylori* −ve gastritis. In comparing *H. pylori* −ve gastritis with gastric carcinoma and gastric metaplasia, there was a significant difference with ($P = 0.0001$). However, there was no significant difference in the mean level of Lgr5 stem cell marker expression between *H. pylori* −ve gastritis group and both *H. pylori* +ve gastritis and healthy control groups. In comparing *H. pylori* +ve gastritis with gastric carcinoma and gastric metaplasia, there was a significant difference with ($P = 0.0001$).

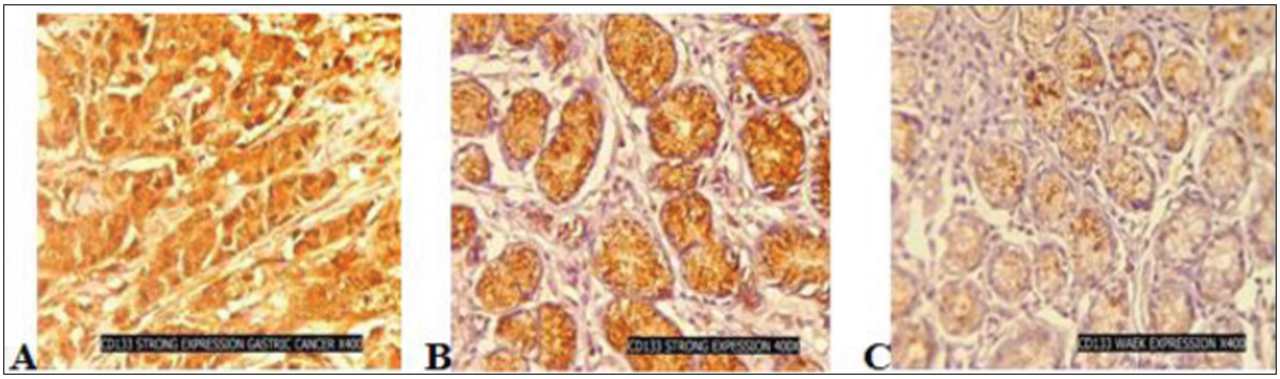

Figure 1: (a) Immunohistochemical staining for CD133 in the gastric carcinoma tissue, strong expression ×100, (b) immunohistochemical staining for CD133 in the gastric metaplasia tissue, strong expression, and (c) immunohistochemical staining for CD133 in the gastritis tissue, weak expression

Table 8: The statistical analysis and difference of Lgr5 immunoexpression among different studied subjects

Lgr5% stem cell marker		Gastric carcinoma	Gastric metaplasia	HP-positive gastritis	HP-negative gastritis	Healthy controls
Mean ± SD		58.8 ± 16.6	39.7 ± 22.162	21.6 ± 12.8	22.2 ± 12.6	13.0 ± 10.3
Standard error of mean		3.09	4.11	2.38	3.16	3.27
(Range)		25–80	10–80	5–50	5–50	5–25
Percentile 05th		35	10	5	5	5
25th		50	25	10	10	5
50th (Median)		50	35	25	25	5
75th		75	50	25	25	25
95th		80	80	45	50	25
99th		80	80	50	50	25
P value compared to	HC	0.0001#	0.0001#	0.161	0.170	–
	HP neg	0.0001#	0.0001#	0.902	–	–
	HP post	0.0001#	0.0001#	–	–	–
	MP	0.0001#	–	–	–	–
	All	0.0001^				

HC: healthy controls, HP neg: HP-negative gastritis, HP Post: HP-positive gastritis, GMP: gastric carcinoma

#P value < 0.05 independent samples-t test. ^P value < 0.05 analysis of variance-test

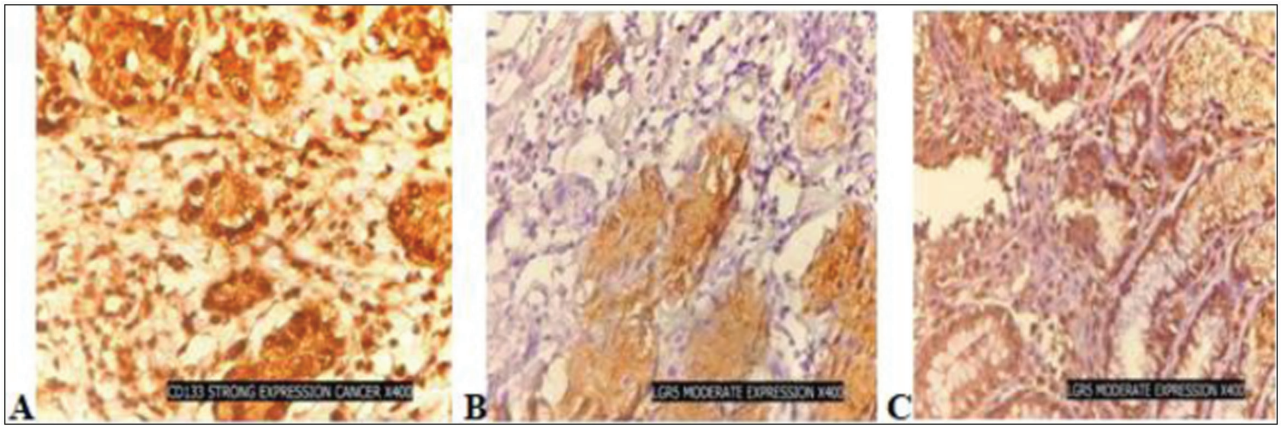

Figure 2: (a) Immunohistochemical staining for Lgr5 in the gastric carcinoma tissue, strong expression. (b) Immunohistochemical staining for Lgr5 in the gastric metaplasia tissue, moderate expression. (c) Immunohistochemical staining for Lgr5 in the gastritis tissue, moderate expression

However, there was no significant difference in the mean level of Lgr5 stem cell marker expression between the *H. pylori* +ve gastritis group and both *H. pylori* −ve gastritis and healthy controls groups. In comparing gastric metaplasia with gastric carcinoma, there was a significant difference ($P = 0.0001$), but no significant difference with the other study groups.

In evaluation of the relationship between Lgr5 and the immunohistochemical expression of CD133, the mean level of CD133 and lgr5 stem cell marker expression were

Table 9: The relationship between CD133 and Lgr5 stem cell markers in study groups

CD133% stem cell marker		Lgr5% stem cell marker				
		Negative (0%)	Mild (1%–24%)	Moderate (25%–49%)	Strong (≥ 51%)	*P* value
Gastric carcinoma	No	1	–	7	22	
	Mean ± SD	35.0 ± 0.0	–	66.0 ± 16.9	76.4 ± 8.5	0.044^
Gastric metaplasia	No	–	5	12	11	
	Mean ± SD	–	39.2 ± 24.2	44.0 ± 25.4	65.0 ± 15.7	0.023^
HP Posit. Gastritis	No	4	11	17	1	
	Mean ± SD	10.0 ± 10.0	28.6 ± 18.7	32.7 ± 21.6	75.0 ± 0.0	0.037^
HP Neg. gastritis	No	7	5	9	1	
	Mean ± SD	6.4 ± 3.8	13.0 ± 9.8	27.8 ± 12.0	50.0 ± 0.0	0.0001^
Healthy controls	No	7	5	4	–	
	Mean ± SD	20.0 ± 16.6	7.0 ± 2.7	18.8 ± 13.8	–	0.244

#*P* value < 0.05 independent samples-t test. ^*P* value < 0.05 analysis of variance-test

significantly different in all patient study groups. CD133 and Lgr5 stem cell markers increased non-significantly with gastric carcinoma ($P = 0.44$), gastric metaplasia ($P = 0.023$), *H. pylori*-positive gastritis (P = 0.037), and *H. pylori*-negative gastritis ($P = 0.0001$), but there was no significant difference between CD133 and Lgr5 with healthy controls [Table 9].

Discussion

In this study, we used a combination of histopathology and PCR for diagnosing *H. pylori* by examining the numerous virulence factors released from *H. pylori* as CagA, bab, and hom are related to gastric diseases. Among 140 subjects, 120 patients were infected by *H. pylori,* and 20 subjects as the control (samples from patients without lesions in gastric mucosa) were approved by both histopathology and PCR.

In this study, it was revealed that all the patients infected with *H. pylori* have the Cag gene (100%) with no significant association between gastric disease and Cag gene. In an Egyptian study, El-Sabbagh *et al.*[9] identified the CagA gene in 30 (50%) of *H. pylor*i-positive patients, and it was associated with gastritis (51.6%), ulcer (44%), and gastric CA (75%). In another Egyptian study by Amer[10], the reported rate of the CagA gene was 65%. However, El-Shenawy *et al.*[11] also reported a positive CagA gene only among 26.6% of *H. pylori* +ve patients, whereas Kadi *et al.*[12] reported that the CagA gene was predominantly found in patients with gastritis (85%) rather than metaplasia (77%). In a Cuban study, Feliciano *et al.*[13] found CagA in 56% of their study sample and reported no differences in the distribution of this gene between patients with peptic ulcer disease and gastric metaplasia. These differences might be due to individual variability among various races, countries, small sample sizes, and diverse social factors.

In this study, the babA gene had a significantly higher concentration in cells with gastric cancer (47.7%) and gastric metaplasia (40%) compared to gastritis (25.7%). Many authors worldwide have studied the association of the *H. pylori* babA gene with the risk of gastric diseases; however, the reported findings may be attributed to the relatively small sample size. In a meta-analysis carried out by Kpoghomou *et al.*[14] the association between the *H. pylori* babA gene and the risk of gastric cancer was studied. They enrolled 20 studies including an overall number of 1289 patients and 1081 controls and reported that the targeted gene significantly increased the risk for developing gastric CA. However, a difference among races was observed by the same authors, as they reported that in patients from South America, the *H. pylori* babA gene did not increase the risk for gastric cancer, whereas among other races, it is quite the contrary as Abdi *et al.*[15] reported that this gene was significantly involved in the risk of gastric CA in Asians. Román-Román *et al.*[16] in Mexico reported that among the population in the south of their country, the vacA s1m1 genotype rather than cagA or babA2 was associated with increased risk for gastric ulcers and CA.

The current study interestingly found a significant difference between homB gene concentrations in different gastric diseases such as gastric cancer (30.0%), gastric metaplasia (16.7%), and gastritis (17.1%). These results were consistent with the findings by Kpoghomou *et al.*[14] who reported that the presence of the homB gene significantly increased the odds ratio for gastric CA by 2.16 times, and they reported that in some studies done in Western countries, the homB gene was associated with peptic ulcer disease in the pediatric population, in contrast to studies done in our country, Turkey, and South Korea that reported no such results.

Another study conducted by Zhao *et al.*[17] in China reported that in United States and Colombian people, homB status was associated with increased risk of developing gastric cancer and might even function as

a marker to differentiate cancer from ulceration only, and the proposed mechanisms included induction of inflammation, persistence of infection, and gastric atrophy.[18]

Similarly, Talebi Bezmin Abadi *et al.*[19] in Iran reported that more than three-quarters of the strains isolated from cases with gastric cancer were positive for the homB gene. When reviewing the aforementioned studies, it was clear that geographically dependent differences may explain the association between gastric cancer and homB gene of *H. pylori*; for example, Keikha and Karbalaei[20] in their meta-analysis reported that the Asian population, unlike the Western population, had increased risk for duodenal ulceration and had increased risk of gastric cancer.

The present study detected the stem cell marker CD133 by immunohistochemistry, revealed a variant relationship with gastric diseases, the most significant relationship with gastric carcinoma and gastric metaplasia ($P = 0.0001$), and a less relationship with *H. pylori*-positive gastritis ($P = 0.017$), but no relationship with *H. pylori*-negative gastritis and healthy controls. It is reported that increased expression of CD133 is associated with advanced-staging and worse outcomes. However, the interesting point is that it is a step-wise process during the course of illness, which can be used as a marker for both diagnosis and follow-up.[21] Similarly, Morales-Guerrero *et al.*[22] in Mexico reported the upregulation of stem cell marker-encoding genes, especially PROM1 and LGR5. In addition, it was suggested that gastric CA originates from stem cells, which also contribute in maintaining the tumor cells,[23] and since *H. pylori* has the ability to invade stem cells through its glycan receptors,[24] altering its criteria leads to metaplasia.[25]

CD133 was shown to be a potent CSC marker,[26] and that $CD133^+$ positive is associated with cancer recurrence and distant metastasis. This concept was supported by the results of Zhao *et al.*[27] who studied 336 patients with gastric cancer and reported that 38 out of 46 recurrence cases had upregulation of CD133, and the results of Fu[28] who carried out a meta-analysis indicated that 26 studies showed that CD133 upregulation was associated with metastasis of gastric cancer.

The study detected the stem cell marker LGR5 by immunohistochemistry, revealed variant relationship with gastric diseases, the most significant relationship between gastric carcinoma and gastric metaplasia ($P = 0.0001$) , but and relationship between gastric cancer and no relationship with *H. pylori*-positive gastritis ($P = 0.161$) or *H. pylori*-negative gastritis ($P = 0.170$) and healthy control. LGR5 also functions as a stem-cell marker,[26] that when overexpressed, leads to a worse prognosis, suggesting that, like CD133, CSCs affects the prognosis, which could be due to its role in enhancing gastric cancer migration,[29] proliferation, resistance to chemotherapy, stemness, and epithelial–mesenchymal transition induction.[30] In addition, Huang *et al.*[31] reported that overexpression of LGR5 increased the risk of mortality.

In contrast to the previously mentioned works, Bu *et al.*[32] reported that LGR5 positive in the early stage of gastric cancer had better prognosis and Jang *et al.*[33] used a relatively new technique in RNA *in situ* hybridization, named RNAscope, to identify LGR5 positive cells. They reported that stem cells were found at the basal glands and were associated with the early stages of the carcinogenic process, which is why they were found more frequently in low-grade gastric cancers. These differences could be attributed to the complexity of the mRNA regulation, also in addition to the ethnicity background and the technique used to identify LGR5 expression.[29]

Conclusion

The co-expression of LGR5 and CD133 is a prognostic marker for patients with gastric disease, and a survival analysis of overall survival was conducted. Therefore, it is of great importance to identify more effective biomarkers for gastric cancer in order to develop personalized treatment approaches and improve outcomes for gastric cancer patients.

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Conflicts of interest

The are no conflicts of interest.

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