Original Article

Analysis of Correlation between the Important *Helicobacter pylori* Virulence Genes (CagA, SabA and Oip) and Gastric Epithelial Stem Cells (LGR5) in Patients with Gastric Disease

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Abstract

Background: Gastric diseases are commonly caused by *Helicobacter pylori*, by colonizing the mucosa of the gastric epithelium in more than half of the population worldwide. It has the ability for adhering to gastric epithelium, with an important aspect of the pathogenicity of the microorganism facilitating the transport of certain proteins such as CagA, sialic acid-binding adhesion (SabA), and outer inflammatory protein (Oip) into that epithelium. The LGR5 are markers that were recognized as cancer stem cells marker that have prognostic value in some disorders such as gastric cancers (GCs), metaplasia, and inflammation of gastric mucosa. This study aimed for determining the association between expression of LGR5 in patients with gastric diseases and the presence of some virulence genes (CagA, SabA and Oip). **Patients, Material and Methods:** For the 140 patients enrolled *H. pylori* virulence factors including (CagA, SabA and Oip) was detected using Multiplex real-time polymerase chain reaction, while LGR5 expressions were carried out via immunohistochemistry. **Results:** SabA and Oip was significant difference with gastric disease such as GC, gastric metaplasia and gastritis with and without *H. pylori*. While no significant difference Cag gene with gastric disease due to all patient have Cag gene. LGR5 stem cell marker expression has significant difference with GC and gastric metaplasia. **Conclusion:** There was a significant increase in *H. pylori* Ag (SabA and Oip) expression among patients with gastric disease compared to the apparently normal individuals this might be able to be that *H. pylori* as one of the risk factor for developing cancer. However, no significant between *H. pylori* Cag Ag expression among patients with gastric disease due to all the patients infected with *H. pylori* has Cag gene (100%). It is suggested that LGR5 may play an important role in gastric carcinoma formation and may be used as a potential marker for the progression in patients with gastric disease.

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

Introduction

Helicobacter pylori is a Gram-negative, spiral shaped, 4–6-flagellated mobile bacterium growing in the gastrointestinal tract in microaerophilic surroundings at 37°C. Flagellae, urease enzyme, and adherence ability are the pivotal elements for *H. pylori* colonizing the gastric mucosa, and its prevalence is estimated as involving nearly 50% of the world population. Pathogenicity of *H. pylori* is significantly related to its virulence factors including CagA, Lewis blood group antigen-binding adhesion (*babA*) and *Helicobacter* outer membrane homB. Initially to colonize the mucosa *H. pylori* take advantage of the adhesion proteins *babA*, sialic acid-binding adhesion (*sabA*) and outer inflammatory protein (Oip) which have significant effect on these first steps of infection.[1]

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H. pylori adhesions such as the Lewis blood group antigen-binding adhesion (babA) and the sabA are believed to play an important role in *H. pylori* colonization. The *H. pylori* outer-membrane proteins family is a minor protein family that consists of the C-terminal hydrophobic motif and signal sequences of outer membrane proteins.[1] Recent research on

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the adherence characteristics of *H. pylori* have shown that babA increases *H. pylori* adhesion to gastric epithelial cells. babA supports the entrance of virulence factors cagA and vacA into host cells. The second adhesion is sabA, which was initially found in the babA mutant strain of *H. pylori*. sabA binds to sialylated carbohydrates on the neutrophil surface.

According to this theory, sabA promotes immunological response.[2] One of the primary issues is that, particularly in the early stages of gastric cancer (GC), the majority of current progression, metastatic disease at diagnosis, and prognostic signs continue to be the cornerstone for patients. Therefore, it is essential to create new biomarkers that might be used to gauge the effectiveness of various therapy approaches and implement upbeat management.[3]

Oip A (OipA) has been connected to the progression of gastrointestinal conditions, bacterial colonization, and clinical outcomes in people with *H. pylori* infection. *H. pylori* strains that are positive for OipA are more likely to result in noticeably more severe irritation of the stomach mucosa than OipA-negative strains. OipA's adherence to stomach epithelial cells causes the production of more Bax/Bcl-2 and intracellular cleaved caspase-3 in the host cells, which mostly initiates the apoptotic cascade through the Bcl-2 family pathway caspases 3.[4]

One of the markers represent human intestinal stem cells that has the potential to be another cancer stem cell (CSC) marker is called LGR5 or G protein-coupled receptor 49 (GPR49) which stands for Leucine-rich repeat-containing G protein-coupled receptor5. Precursors and progressed CSC express this marker of their crypts, in addition it is expressed at the basal prospective corpus and pyloric glands in neonatal gastric tissue, mainly confined basally at mature pyloric glands in adults.[5] Its gene is approximately 144 kb long and its location on chromosome 12 positioned at 12q22–q23. Interestingly, LGR5 has been found to be upregulated in different malignancies including small bowl, colonic cancer, and hair follicles.[6]

It was recognized on crypt stem cells (precursor cells) in addition to precancerous lesions. Usually found basally in corpus and pyloric glands in stomachs of neonates and predominantly confined basally in adults' mature pyloric glands.[7]

Aim of the study

The aim of this study is to determine and assessment the distribution of *H. pylori* outer membrane genes (CagA, SabA and Oip) to examine the frequency of virulence genes in the gastric tissue sample and to determine whether; the relationship between these virulence genes and gastrointestinal disease development or associated with different clinical outcomes. Furthermore, investigate the clinical significance of CSCs markers (LGR5) and establish a new model based on these markers to accurately predict prognosis of GC and evaluate if it is related to clinical/pathological features.

Patients, Material And Methods

Specimens' collection

The tissue samples were obtained retrospectively from 140 archived blocks embedded in paraffin during January 2021 to December 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 sample included 68 males and 72 females (mean age 51.49 years ranging from 20 to 86 years) divided into four groups; 30 GCs (mean age 52.66 years ranging from 20 to 85 years), 30 gastric metaplasia tissues (mean age of 53.5 years ranging from of 25 to 86 years), 60 gastritis with (mean age of 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 controls. After obtaining approval of each center for using the specimens, the diagnosis was reconfirmed histopathologically through reviewing freshly prepared hematoxylin and eosin-stained slides.

Material and method of immune staining

Each paraffin block was sectioned into 5 μm. From each block, three sections were taken, one for hematoxylin and eosin (H and E stain) for histopathology revision, two were used on positively charged slides (Fis herbrand) for immunohistochemical staining with LGR5 Rabbit polyclonal Immunoglobulin G (IgG) antibody.

The hematoxylin and eosin (H and E-stained sections) had been reassessed for the morphological types of the tumor, grade, and other parameters by two independent pathologists for LGR5 immunohistochemical detection, Rabbit against human LGR5: Rabbit polyclonal IgG, H-100 and SC-9048, lot # D2613 (Santa Cruz Biotechnology), at 1:50 dilution with an overnight incubation at 4°C for both.

After the primary antibody reacted with tissue antigen, a biotinlabeled secondary antibody added to bind to the primary antibody. After that, this conjugate was added and the biotinylated secondary antibody to form a complex with the peroxidase-conjugated streptavidin. The substrate then added, which contained 3, 3 diaminobenzidines in a chromogen solution which results in the formation of a brown-colored precipitates at the antigen site.

Appearance of reacting brown products at the site of the targeted antigen is associated with a positive reaction in the peroxidase secondary detection system. Counterstain was then used as blue staining of the cell. Appropriate positive control slide (human placenta tissue for LGR5 as indicated by manufacturer instructions) and negative control slide (technically negative by omitting the primary antibody) were included in each run of immunohistochemical staining. Immunostaining evaluation was done by two independent histopathologists who did not know the clinical diagnosis each tissue beforehand.

Scoring

Examination under the light microscope was carried out to estimate the cell number that gave a positive (brown cytoplasm

staining) reaction. Estimation of the immunohistochemical staining-intensity was estimated by counting the number of cells in each of the 10 fields (×40 magnification), then this score was divided by the number of all cells per each field, so the positively stained gastric cells' percent (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 LGR4 was defined as follows: (0%) no expression, (1%–25%) weak expression, (26%–50%) moderate expression, and the extensive/high expression (≥75%) of neoplastic.[8,9]

Genomic DNA extraction from bacterial isolates

Extraction of genomic DNA (gDNA) from the gastric tissue biopsy by specific Kit based on MagPurix technology (Zinexts Life Science Crop, Taiwan); a fully automated, highly innovative system for rapid molecular biology sample extraction is a cutting-edge platform that uses magnetic beads to extract nucleic acids from samples. From samples to outcomes, the platform provides true walk-away automation in nucleic acid purification. Matrix technology provides high-quality results in three simple steps: load samples, execute the preprogrammed protocol and collect findings. The purification procedure includes stages like lysis, binding, washing, and elution. MagPurix Formalin-Fixed, Paraffin-Embedded (FFPE) DNA Extraction Kit is intended to use with MagPurix® Instruments for the extraction of gDNA from FFPE tissue samples.

Polymerase chain reaction detection of *Helicobacter pylori* gene (CagA gene, sialic acid-binding adhesion gene and outer inflammatory protein gene)

They were prepared for amplifying specific regions of Cag, SabA and Oip genes. Beacon Designer V: 8.21 Software (Macrogen, Korea) was used to design the primers. Alpha DNA Company supplied primers and used according to manufacturer instruction to yield a Primer with concentration of 10 pmol/µl, as shown in Table 1. They were used the detection of CagA for *H. pylori* and SabAgene and Oip genes, as shown in Table 2. The polymerase chain reaction (PCR) Amplification Program is shown in Table 3.

Statistical analysis

Analysis of data was carried out using the available Statistical package for the Social Sciences(SPSS-28). 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. Analysis of variances test was used for variances among more than two numerical variables. The Chi-square test (χ^2-test) with application of Yate's correction or Fisher exact test whenever applicable was used to test the significance of association between the categorical variables. P <0.05 was considered statistically significant throughout the study.

Results

The immunohistochemical expression for marker (LGR4) was cytoplasmic with different intensity as demonstrated and Multiplex real-time PCR for diagnosis of CagA, SabA, and Oip gene. The study sample was divided into four groups, in which gastric carcinoma 30 (21.4%), gastric metaplasia 30 (21.4%), gastritis with and without *H. pylori* 60 (42, 8%) and apparently normal tissue controls samples 20 (14.2). Cag gene was expressed in all gastric tissue sample (100%) this means there is no significant difference between gastric carcinoma, gastric metaplasia, *H. pylori*-positive gastritis, *H. pylori* negative gastritis, and healthy controls in Table 4. The level of Oip gene was significantly different in gastric carcinoma, gastric metaplasia, gastritis caused by the presence of *H. pylori*, gastritis caused by the absence of *H. pylori*, and healthy controls ($P = 0.000$*1*). That's a statistically significant between healthy controls and gastric carcinoma ($P = 0.000$*1*). There was a significant difference with ($P = 0.004$) when comparing healthy controls to those with gastric metaplasia. There was a significant difference with ($P = 0.013$) when comparing healthy controls to those with positive *H. pylori* gastritis. However, there was no significant difference between healthy controls and those with negative *H. pylori* gastritis. When negative *H. pylori* gastritis was compared to gastric carcinoma, there was a significant difference with ($P = 0.0001$). *H. pylori* negative gastritis in compared with gastric metaplasia there was a significant difference with ($P = 0.001$). When compared to negative *H. pylori* with positive *H. pylori* gastritis, there was significant difference with of ($P = 0.006$). However, there was no statistically significant difference between negative *H. pylori* and healthy controls. In addition, there was no statistically significant difference between gastric metaplasia and positive *H. pylori* gastritis. across all study groups [Table 5].

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

Name of primer	Sequence	Product length	Reference
HP: CagA detection	F: GAGGCTAGTAAGGAAGCA R: GTGGTGTTGGAAGGTTAA	122 bp	Design
G1: Oip	F: GAGGCTAGTAAGGAAGCA R: GCCAAAGTCTTTCAAATTCTTG	111 bp	Design
G2: SabA	F: TCCGCATCCTTAAACAGA R: TGCACAAACGCAAGAAAA	126 bp	Design

Oip: Outer inflammatory protein

Table 6 reveals a significant difference in SabA gene expression in gastric carcinoma, gastric metaplasia, healthy controls, *H. pylori*-positive gastritis, and *H. pylori*-negative gastritis ($P = 0.0001$). Significant differences were found between healthy controls and those with gastric carcinoma ($P = 0.033$). That's a statistically significant between healthy controls and those with positive *H. pylori* gastritis ($P = 0.032$). However, non a statistically significant difference between healthy control and both gastric metaplasia and negative *H. pylori* gastritic. There was a statistically significant difference ($P = 0.018$) when comparing negative *H. pylori* gastritis to gastric carcinoma. A statistically significant difference between the negative and positive results was observed. *H. pylori* ($P = 0.017$). However, The association between negative *H. pylori* gastritis and both gastric metaplasia and healthy controls was not statistically significant. In addition, Across all study groups, there was no statistically significant difference in *H. pylori* positivity and gastric metaplasia.

By observing the results in Table 7, LGR5 stem cell marker The most common scors among gastric carcinoma group (73.3%) was expressed in all gastric tissue, with strong immunostaining reaction. While moderate immunostaining reactions are the most common scors among positive *H. pylori* gastritis patients, (48.6%). In positive *H. pylori* gastritis group mild immunostaining reaction was the most frequent scors (31.4%). While the negative immunostaining reaction observed in healthy controls group (50.0%). LGR5 stem cell marker was signification difference in all study group with ($P = 0.0001$). When comparing healthy controls with patients diagnosed with GC and gastric metaplasia, there was a statistically significant difference ($P = 0.0001$). There was a significant difference between healthy controls with positive *H. pylori* ($P = 0.046$). However, there was no significant difference with negative for *H. pylori* gastritis. In comparing to *H. pylori* negative gastritis

Table 2: Polymerase chain reaction components for amplifying the targeted fragments for detection virulence factors for *Helicobacter pylori* (multiplex real-time polymerase chain reaction by human resource management)

Components	Volume
Forward primer (CagA)	0.5
Reverse primer (CagA)	0.5
Forward primer (Oip)	0.5
Reverse primer (Oip)	0.5
Forward primer (SabA)	0.5
Reverse primer (SabA)	0.5
DNA template	3.5
DW	3.5
PCR Re Mix (ready to use) EVA green	10
Final volume	20

Oip: Outer inflammatory protein, PCR: Polymerase chain reaction, EVA: Economic value added, DW: Distal water

Table 3: Polymerase chain reaction amplification program

Step	Temperature (°C)	Time	Cycle
Initial denaturation	95	5 min	1
Denature	95	10-30 s	30-40
Anneal	55-68	10-60 s	30-40
Melting curve	65-95	2-5 s/step	1

Table 4: Distribution *Helicobacter pylori* cag gene in study groups

Variables	GMP, *n* (%)	Gastric metaplasia, *n* (%)	HP Post, *n* (%)	HP Neg, *n* (%)	HC, *n* (%)	*P*
HP Cag						
Positive	35 (100)	30 (100)	35 (100)	-	-	-
Negative	-	-	-	25 (100)	20 (100)	-
P compared to						
HC		-	-	-	-	-
HP Neg		-	-	-	-	-
HP Pos		-	-	-	-	-
GMP		-	-	-	-	-

*$P<0.05$. HC: Healthy controls, HP Neg: HP negative gastritis, HP Post: HP positive gastritis, GMP: Gastric carcinoma

Table 5: Distribution *Helicobacter pylori* outer inflammatory protein gene in group studies

	GMP, *n* (%)	Gastric metaplasia, *n* (%)	HP Post, *n* (%)	HP Neg, *n* (%)	HC, *n* (%)	*P*
G3 Oip						
Positive	12 (40.0)	10 (33.3)	9 (25.7)	-	-	0.0001*
Negative	18 (60.0)	20 (66.7)	26 (74.3)	25 (100)	20 (100)	
P in comparison to the HC	0.0001*	0.004*	0.013*	-	-	
P in comparison to the HP Neg	0.0001*	0.001*	0.006*	-	-	
P in comparison to HP Post	0.220	0.501	-	-	-	
P in comparison to the GMP	0.592	-	-	-	-	

*$P<0.05$. HC: Healthy controls, HP Neg: HP negative gastritis, HP Post: HP positive gastritis, GMP: Gastric carcinoma, Oip: Outer inflammatory protein

with gastric carcinoma there was a significant difference with (P = 0.0001) and there was a significant difference with gastric metaplasia with (P = 0.001*)*, However, there was no statistically significant difference between positive *H. pylori* and healthy controls. When compared positive *H. pylori* with gastric carcinoma indicate a significant difference with (P = 0.0001) and there was a statistically difference with gastric metaplasia with (P = 0.001). However, there was no statistically difference between *H. pylori*-negative gastritis and healthy controls. There was a statistically difference between gastric metaplasia and gastric carcinoma (P = 0.026). However, there is no statistically difference between groups.

Data presented in Table 8 revealed an associated between LGR5 and different types of *H. pylori* gene. In comparing LGR5 with Sab A gene in gastric carcinoma, there was a significant difference with of (P = 0.044). However, nonsignificant difference with other study groups. In comparing LGR5 with Oip gene in all study groups, there was no significant difference.

Discussion

This study revealed all the patients infected with *H. pylori* have Cag gene (100%) with no significant association between gastric disease and CagA gene Table 4. In Egypt, El-Sabbagh *et al*. identified CagA gene in 30 (50%) of *H. pylori* positive patients and was associated with gastritis (51.6%), ulcer (44%), and gastric CA (75%).[10] In another study also in Egypt by Amer, they reported rate of CagA gene was (65%).[11] However, in the same country of Egypt, El-Shenawy *et al.* reported positive CagA gene only among 26.6% of *H. pylori* + ve

Table 6: Distribution *Helicobacter pylori* SabA gene in study

	GMP, *n* (%)	Gastric metaplasia, *n* (%)	Hp Post, *n* (%)	HP Neg, *n* (%)	HC, *n* (%)	*P*
G4 SabA						
Positive	6 (20.0)	2 (6.7)	7 (20.0)	-	-	0.0001*
Negative	24 (80.0)	28 (93.3)	28 (80.0)	25 (100)	20 (100)	
P in comparison to the HC	0.033*	0239	0.032*	-	-	
P in comparison to the HP Neg	0.018*	0.188	0.017*	-	-	
P in comparison to the HP Post	-	0.121	-	-	-	
P in comparison to the GMP	0.129	-	-	-	-	

*P<0.05. HC: Healthy controls, HP Neg: HP negative gastritis, HP Post: HP positive gastritis, GMP: Gastric carcinoma

Table 7: Lgr5 immunohistochemistry among studied groups

Lgr5% stem cell marker	GMP, *n* (%)	Gastric metaplasia, *n* (%)	HP Post, *n* (%)	HP Neg, *n* (%)	HC, *n* (%)	*P*
Negative (0)	1 (3.3)	1 (3.3)	6 (17.1)	9 (36.0)	10 (50.0)	0.0001*
Mild (1-24)	-	5 (16.7)	11 (31.4)	6 (24.0)	6 (30.0)	
Moderate (25-49)	7 (23.3)	12 (40.0)	17 (48.6)	9 (36.0)	4 (20.0)	
Strong (≥50)	22 (73.3)	12 (40.0)	1 (2.9)	1 (4.0)	-	
P in comparison to the HC	0.0001*	0.0001*	0.046*	0.484		
P in comparison to the HP Neg	0.0001*	0.001*	0.400			
P in comparison to the HP Post	0.0001*	0.001*				
P in comparison to the GMP	0.026*					

*Percentages of significant difference using the pearson Chi-square test (at 0.05 level), P<0.05 independent samples-*t*-test, P<0.05 ANOVA-test.
HC: Healthy controls, HP Neg: HP negative gastritis, HP Post: HP positive gastritis, GMP: Gastric carcinoma

Table 8: The association between Lgr5 and several *H. pylori* gene types under investigation in study groups

Lgr 5% Stem cell marker	GMP		Gastric metaplasia		HP Post		HP Neg		HC	
	n	Mean±SD	*n*	Mean±SD	*n*	Mean±SD	*n*	Mean±SD	*n*	Mean±SD
Oip										
Positive	12	52.92±19.59	10	36.50±13.75	9	21.67±6.61	-	-	-	-
Negative	17	62.94±13.24	19	41.32±25.70	20	21.50±14.96	16	22.19±12.64	10	13.00±10.33
P		0.111		0.587		0.975		-		-
SabA										
Positive	6	70.83±10.68	1	50.00±	6	16.67±9.83	-	-	-	-
Negative	23	55.65±16.60	28	39.29±22.47	23	22.83±13.38	16	22.19±12.64	10	13.00±10.33
P		0.044#		0.643		0.303		-		-

#Percentages of significant difference using the pearson Chi-square test (at 0.05 level), ANOVA test with a 0.05 level significant difference between more than two independent means. Oip: Outer inflammatory protein, ANOVA: Analysis of variance, SD: Standard deviation, HC: Healthy controls, HP Neg: HP negative gastritis, HP Post: HP positive gastritis, GMP: Gastric carcinoma

patients.[12] While, Kadi *et al*.[13] reported that CagA gene was predominantly found in patients with gastritis (85%) rather than metaplasia (77%). In Cuba, Feliciano *et al*.[14] found CagA in 56% of their study sample and reported no difference in distribution of this gene between patients with peptic ulcer disease and gastric metaplasia. These difference might be due to individual variability among various races countries, small sample size, and diverse social factors.[1]

OipA Oipis an outer membrane protein that plays a role in both adhesion and increased inflammation. Specifically, we found a correlation between oipA and the prevalence of GC, gastric metaplasia, and gastritis (40.0%, 33.3%, and 25.7% correspondingly) with a significance level of (P = 0.0001) in Table 5. Another research of the oipA gene discovered a link between its presence and an increased risk of chronic gastritis. This finding is consistent with the findings of Souod *et al*., who discovered a higher frequency of the oipA gene and a stronger link between this gene and the development of chronic gastritis. The higher stimulation of Interleukin (IL)-8 production in humans, which results in a severe inflammatory process and, if persistent, can lead to the formation of chronic gastritis, as documented in earlier studies, may help explain the other observation.[15]

Similar findings were made by Zhang J, *et al*.(2018) who found a link between the oipA gene and both increased neutrophil activity and more severe gastritis. However, other research did not identify a link between the oipA gene and chronic gastritis. Instead, they discovered that the gene was exclusively linked to the occurrence of GC[16] and gastric ulcers.[17]

According to other research, the oipA gene is linked to an increased risk of gastritis, gastric metaplasia, and GC. The stimulation of IL-8 synthesis, which results in more serious inflammation and associated gastrointestinal illnesses, is most likely what causes this increase. A deeper comprehension of the activation pathways necessary for IL-8 expression and the emergence of gastrointestinal disorders in which the oipA gene participates is required.

We discovered a highly significant link between the oipA gene and the severity of gastritis and the prevalence of gastric illnesses, with the gene being positive in all instances and 61.5% of patients with severe and moderate gastritis, respectively, and 75% of patients with gastroduodenitis. Similar findings were reported by Tunisian researchers Ben Mansour *et al*.[18] Iranian researchers Souod *et al*.,[19] and Brazilian researchers Sallas *et al*.[20]

Other studies reported no correlation between oipA and disease outcome or increased gastroduodenal damage. The outer membrane protein sabA, which interacts with a specific receptor present in gastric epithelium, can be responsible for facilitating binding between the bacteria and the host.[21] Previous study the high prevalence of sabA genes had an active infuence over the correlation between *H. pylori* and gastric diseases.[22] Yamaoka *et al*.[17] found that sabA was associated with GC and intestinal metaplasia.

This work used PCR analysis to evaluate the prevalence of the sabA genes in *H. pylori* isolated from patients with gastrointestinal illnesses and the connection between this virulence factor sabA and gastric disease like GC, gastric metaplasia and gastritis (20.0%, 6.7%, 20.0% respectively) with (P = 0.000*1)* in Table 6. Other findings indicate that *H. pylori* is associated with gastric disorders such as gastritis and increases the risk of GC. Given that this bacteria is capable of causing a severe inflammatory response in the human stomach epithelium, these findings were expected, as other studies have concluded since the discovery of *H. pylori*.[23] While another study gene was found in more than half of the *H. pylori* positive investigated population, no statistically meaningful outcomes were obtained in this experiment.

Other findings concur with those reported by Pakbaz *et al*.[24] however they disagree with those reported by Oleastro *et al*.[25] who discovered a link between the sab gene and GC in the western population. According to Wang *et al.* (2016), the presence of this gene is associated with the development of GC and ulcers because it gives bacteria the ability to control the host's cell metabolism.

LGR5 is G protein even Coupled receptor 49, or leucine rich repeat containing G-protein-coupled receptor 5, is an independent G protein coupled receptor molecule that is a member of the G protein coupled hormone receptor family.[26] (GPR49). The human LGR5 gene has a length of 4208 bp and is found on chromosome 12q;[22,23] it has 22 exons and 21 introns. The human body contains large amounts of LGR5, which is mostly expressed in the gastrointestinal tract, skeletal muscles, brain, spinal cord, and breast tissue.[1]

In our study detection stem cell marker LGR5 by immunohistochemistry revealed variant relationship with gastric diseases the most significant relationship between gastric carcinoma and gastric metaplasia of (P = 0.0001) and relationship between GC and *H. pylori* positive gastritis of (P = 0.046), and absence relationship with *H. pylori* negative gastritis of (P = 0.484) and healthy control as in Table 7 and Figure 1. Another interesting gastric CSC marker is LGR5, and high LGR5 expression in the group with poor prognosis may indicate that CSCs played a role in determining the prognosis. In poorly differentiated GC, migration capacity and epithelial-mesenchymal transition (EMT) are elevated.[27]

As a result, the poor prognosis of patients with high LGR5 expression may be linked to the histological characteristics of poorly differentiated cancer, which include increased ability to migrate, expression of EMT-related proteins, and LGR5 expression. GC prognosis is known to be impacted by cancer cell migration. Additionally, LGR5 expression is associated with EMT and migratory ability, despite not being expressed in the stomach.[28] A correlation between vascular invasion and high LGR5 expression in another study may support the idea that LGR5 expression is related to EMT. According to reports from[29,30] high LGR5 expression and EMT are connected to GC. LGR5 expression in stomach cancer has been studied in a number

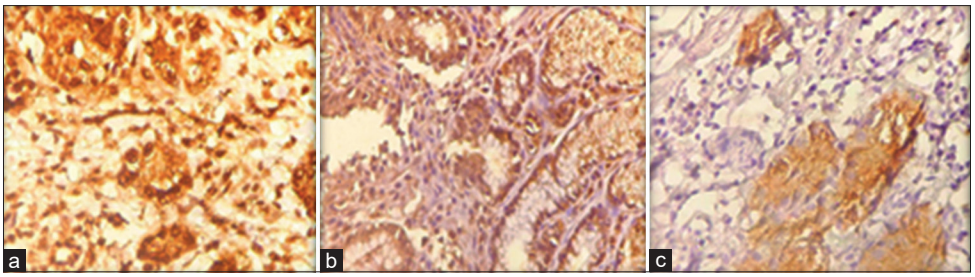

Figure 1: (a) Immunohistochemical staining for LGR5 in gastric carcinoma tissue, strong expression. (b) Immunohistochemical staining for LGR5 in gastric metaplasia tissue, moderate expression, (c) Immunohistochemical staining for LGR5 in gastritis tissue, moderate expression

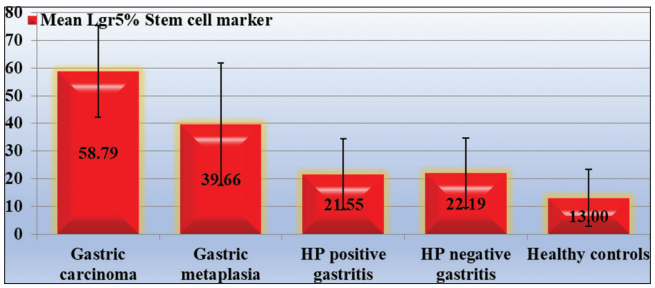

Figure 2: Descriptive differential analysis and mean level differences LGR5 stem cell marker expression between each studied subject

of papers. High immunostaining scores for LGR5 were shown to be strongly related with an elevated risk of death in another research of GC. Together, these findings suggest that the function of LGR5 may be tissue-specific and that the control of its protein and mRNA may be more intricate than first thought. Technical or racial factors could be the cause of this variance. Additionally, LGR5 expression has been linked to a good prognosis, according to Bu *et al*. Furthermore, lymph node metastases and LGR5 expression were linked to advanced stage.[31]

According to Xi *et al.,* 2014 poorly differentiated cancer is related with high LGR5 expression[32] LGR5 is, nevertheless, strongly expressed in well-differentiated malignancy, metaplasia, and gastric intestinal metaplasia all had higher levels of LGR5-immunodetected expression than the normal tissues examined in the current study. All of these GC-related lesions have the potential to show intestinal type differentiation. It has been demonstrated that the isthmus' stem cells, which are abundant in the crypts, are the source of intestinal metaplasia.

Conclusion

In conclusion, there is potential for this protein to serve as a significant biomarker for the early identification of patients at higher risk for gastric tumorigenesis based on the immunodetectable expression pattern of LGR5, a CSC-related gene, which increases from normal tissues to lesions of dysplasia, gastric carcinoma, and finally metastases. Additionally, as an intestinal stem cell marker, differential LGR5 expression in conjunction with intestinal metaplasia development may indicate a precancerous situation rather than a precursor to carcinoma. Therefore, LGR5 may be a novel and sensitive marker of intestinal stem cells and may have a close association with the intestinal GC subtype. A total of 58.7 cases of GC, 39.6 cases of gastric metaplasia, 21.5 cases of HP-positive gastritis, 22.1 cases of HP-negative gastritis and13.0 cases of healthy control were reported in this investigation, mirroring the results of our own analysis [Figure 2].

In this work, we discovered no link between *H. pylori* genes (sabA and oip,) and stem cell marker (LGR5) in gastric illness. We also discovered that *H. pylori* colonizes the surface of gastric stem and progenitor cells. Although *H. pylori* has been shown to adhere to epithelial cells of the stomach mucosa, most investigations show bacteria sticking to superficial pit cells. Because these cells are terminally differentiated and do not live long, it is vital to understand how *H. pylori* infection impacts gland growth.

We discovered that *H. pylori* has the ability to survive deep within the stomach glands, producing gland-associated microcolonies right on the epithelial junctions in areas where immature progenitor and long-lived stem cells are abundant. The alteration of stem cell biology caused by gland-associated *H. pylori* leads to hyperplastic alterations.

These effects do not occur in all glands, but rather are localized in nature and correlate with the location of gland-associated bacteria. We show that colonization of the surface mucus is not sufficient to induce stem cell activation and that contact-dependent effects of the *H. pylori* gene are necessary for these effects using bacterial mutants that colonize the surface mucus but not the glands. this display in Table 8.

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Availability of data and materials

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

Authors' contributions

Safyia Khalid: Conceptualization, Methodology, Data curation, Formal analysis, Writing-original draft. Wasan A Bakir and Mais Ibrahim Alsikafi: Supervision, Validation, Writing – review and editing.

Ethics approval and consent to participate

Already take from Department of pathology/College of medicine/Mustansiriyah University, Baghdad, Iraq.

Patient consent for publication

Authors give permission for the publication to the journal.

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Conflicts of interest

There are no conflicts of interest.

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