The expression of genes *TLR2* and *TLR10* in the gastric tissue of patients with gastroduodenal disorders caused by *Helicobacter pylori*

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Abstract

The bacterium *Helicobacter pylori (H. pylori)* is the key etiological agent in gastritis, peptic ulcer, and gastric cancer disease. Toll-like receptors (TLRs) contribute to the innate immune response through pathogen-associated molecular patterns (PAMPs) derived from *H. pylori* and other microorganisms as well as damage-associated molecular patterns (DAMPs) derived from host cells, such as tumour cells, dead or dying cells, or products released from cells in response to signals such as hypoxia. The study involved gastric biopsies taken from 88 gastroduodenal disorder patients and 21 healthy individuals. The clinical diagnoses of these groups after endoscopy examination included gastritis, peptic ulcers, and gastric cancer. The detection of *H. pylori* infection was performed using PCR to amplify *ureC* and *ureA* genes, then the bands were visualized by gel electrophoresis. The expression of *TLR2* and *TLR10* genes in gastric biopsies was evaluated using the relative method. The current findings indicate that susceptibility to gastroduodenal disorder increases with age, as most of the patients were over 30 years old (68 out of 88 patients) representing 77.2% of patients. The bacterium induced the expression of *TLR2* and *TLR10* genes, the gene *TLR2* was recorded 11.11-fold in patients with gastritis, 7. 65-fold in those with peptic ulcer, and 6.25-fold in patients suffering from gastric cancer, the folding decreased with the prognosis of the disease. On the other hand, the *TLR10* gene recorded the highest value 5.01-fold in peptic ulcer patients, followed by 4.73-fold with gastritis, and 3.10-fold with gastric cancer. The expression of the *TLR2* and *TLR10* genes was significantly higher (p ≤ 0.01) in the patients' group compared with the control one. In gastritis, peptic ulcer, and gastric cancer patients, the *TLR2* and *TLR10* gene expression was higher than in the healthy group, and *TLR2* was higher than *TLR10*.

Keywords: *H. pylori*, Gastritis, Gastric cancer, TLR2, TLR10 genes, Peptic ulcer.

Introduction

In 1982, the bacterium *H. pylori* was identified as a gram-negative, spiral-shaped, microaerophilic bacillus and 5–15% of those infected probably develop gastroduodenal disorders[1]. Recently, *H. pylori* is regarded as the key etiological agent in gastritis, chronic gastritis, peptic ulcer, and gastric cancer disease[2]. It is crucial to understand how the bacterium causes the progression from acute mucosa infection to gastric cancer since it is considered responsible for the chronic inflammation that produces duodenal ulcers and gastric problems[3]. The innate immune response to the

shape of a bacterium and two pathogen-associated molecular patterns (PAMPs) antigens, lipopolysaccharide (LPS) and flagellin[4] were investigated.

The response occurs through Toll-like receptors (TLRs) which are well-studied transmembrane protein receptors. The TLR family has ten members in humans (TLR1–TLR10). They contribute to the innate immune system by PAMPs derived from *H. pylori* and other microorganisms[5] as well as damage-associated molecular patterns (DAMPs) derived from host cells, such as tumour cells, dead or dying cells, or products released from cells in response to signals such as hypoxia[6]. TLRs are relevant not only in the early line of defence against *H. pylori* but also in the progression of gastric cancer. Their signalling induced by PAMPs of *H. pylori* helps to generate antigen-specific acquired immune responses. The ability of *H. pylori* to adhere to specific receptors on the surface of human epithelial cells was investigated, and it was found

that this is the first step in the pathogenic process[7]. The DAMPs produced by persistent inflammation may also contribute to the development of stomach cancer. Understanding TLRs will lead to the development of new diagnostic and predictive biomarkers as well as therapy options for *H. pylori*-associated gastric cancer[8]. The association of potent inflammatory cytokine and the possible oxidative DNA damage biomarkers was recently studied in stomach cancer[6]. They revealed a strong link between oxidative stress and chronic inflammation in gastric cancer.

Thus, the main objective of the present study was to assess if there is a link between the presence of *H. pylori* infection and the expression of *TLR2* and *TLR10* genes in three groups of gastroduodenal disorders including gastritis, peptic ulcer, and gastric cancer, and then investigating the effect of disease progression on gene expression in order to target it as a future treatment.

Materials and Methods

This study involved gastric biopsies from the antrum region, in which 88 gastroduodenal disorder patients were random including 21 healthy individuals. All the samples were frozen immediately with liquid nitrogen and transferred to an ultra-freezer (-80 ◦C).

DNA Extraction and Detection of *H. pylori*

The DNA was extracted from gastric mucosa tissue biopsies using WisPrep™ gDNA mini kit (cat. No. # W71060-100) purchased from Wizbio Solutions (Korea). The extraction was done according to the manufacturer's instructions to obtain purified and intact DNA from tissue and bacterium. The tissue was ground with liquid nitrogen, lysed with lysis buffer and proteinase K, precipitated by absolute methanol, purified by spin column, and eluted by TE buffer to obtain (30.9–276.1 ng /μl) and purity (1.69-2.08). The concentrations were diluted to 10 ng /μl before they were used. The DNA yield was different among the studied samples according to concentration and purity. This may depend on tissue freshness, storage, and the number of bacteria in the tissue samples.

Conventional PCR for *ureC* and *ureA* Genes

The bacterium was detected by using two specific primers, forward (5'-AAATGATATGCCCGCTTTGC-3'), reverse, (5-ACCAACTAAGCCAGGAAGTG-3') for *ureC*, and forward (5-TCGTTGCTTGCCTATC-3'), reverse (5'-TCGACTCACACTTCCATTTC-3') for *ureA*. The sequences were designated using NCBI/Primer 3 by the researchers. The PCR mixture contained 8 μl of DNA samples, 12.5 μl of Master Mix (Cat. No. # M7122) provided by Promega Company (USA), 1 μl of forward primer (IDT, Belgium), 1 μl of reverse primer (IDT, Belgium), and 2.5 μl of nuclease-free water. Initial denaturing, denaturing, annealing, and extension steps were carried out for a 37-cycle thermocycler (Eppendorf, Germany) at 95 ℃ for 5 minutes, 94 ℃ for 1 minute, 53 ℃ for 45 seconds, and 72 ℃ for 1 minute, respectively. A 2% agarose gel was prepared, stained with RedSafe nucleic acid dye in a 2 μl/100 ml concentration (Cat. No. # 21141) purchased from iNtRON Company (USA), and then used to observe the PCR product.

Evaluating Gene Expression for *TLR2* and *TLR10* Genes

RNA Extraction

Total RNA was extracted from gastric mucosa tissue samples using a standard RNA extraction kit (Cat. No. R1051) provided by Genome Company (China). Tissue biopsies were grounded with liquid nitrogen, then homogenized with trizol, and incubated for 30 minutes at RT. The chloroform was added, and the homogenate was separated into three phases by centrifugation, the clear upper aqueous layer which contained the RNA was transferred to a new microtube, and isopropanol was added to precipitate the RNA. The mixture was transferred to a silica membrane and washed two times. The RNA was eluted with RNase-free water. The concentration and purity were measured by nanodrop (341.8–668.5 ng/μl) and (1.79–2.15). This may depend on tissue freshness, storage, the number of cells in the tissue samples, and preparation technique.

cDNA Synthesis

The tissue-extracted RNA was converted to cDNA by an All-In-One 5X RT MasterMix kit (Cat. No. G592) provided by Applied Biological Materials Inc. (Canada). The components of the reaction were 1000 ng on RNA at a concentration of 250 ng/μl and volume of 4 μl, 4 μl of All-In-One 5X RT MasterMix, and 12 μl of Nuclease-free water (total volume was 20 μl). The mixture was centrifuged and incubated in a gradient thermocycler at 37 ℃ for 15 min, followed by 60 ℃ for 10 minutes, and stopped by heating at 95 ℃ for 3 minutes. Finally, kept in an ultra-freezing at -80 ℃ for qPCR work.

qPCR for *TLR2* and *TLR10* Genes

Gene expression of *TLR2*, *TLR10*, and *β-Actin* (housekeeping) genes was evaluated by using Rotor-Gene Q 6 plex platform (Qiagen, USA) for all study groups. The amplification was performed by using specific primers, forward (5'-ACCAGATGCCTCCCTCTTACC-3'), reverse (5'-TGCCACCAGCTTCCAAAGTC-3') for *TLR2* gene, forward (5'-AACAACACACCCTTGGAACAC-3'), reverse (5'-GCAAGCACCTGAAGACAGAATC-3') for *TLR10* gene, and forward (5'-AAACTGGAACGGTGAAGGTGAC-3') (5'-CTCGGCCACATTGTGAACTTTG-3') for *β-Actin* gene. The designed primer sequences relied on NCBI/Primer 3. The qPCR amplification mixture and program were done according to GoTaq® qPCR Master Mix manufacture company (Cat. No. A6001) by including 4 μl of DNA samples, 10 μl of GoTaq® qPCR Master Mix (Promega, USA), 1 μl of forward primer (IDT, Belgium), 1 μl of reverse primer (IDT, Belgium), and 4 μl of nuclease-free water, and programmed by (standard cycling conditions) GoTaq® Hot Start Polymerase activation at 95 ℃ for 2 minutes and denaturing, annealing, and extension steps were carried out for a 40-cycles at 95 ℃ for 15 seconds, 58 ℃ for 30 seconds, and 72 ℃ for 30 seconds, respectively.

Relative Quantification of the Expression

The cycle threshold (Ct) values were obtained from Rotor-Gene for both the target and reference genes, the values above 35 were regarded as non-specific and excluded from the study. The obtained Ct values were normalized to the Ct value of the reference gene as internal control relation (ΔCt = Ct gene of interest – Ct reference gene) for each sample and normalized to the calibrator sample ΔCt calibrator = The three highest Cts (target gene in the control group)- mean Ct (reference gene in the control). Then, $2^{-\Delta\Delta Ct}$ value was calculated according to Schmittgen and Livak, 2008[9], and the results were expressed as relative expression of *TLR2* and *TLR10* genes to *β-Actin* gene.

Statistical Analysis

Data were analysed using IBM SPSS V26, software, and Microsoft Excel with the aid of graphics. The chi-square test of independence was used to describe the demographic characteristics of the study groups. Results were expressed as mean ± SE. The expression of *TLR2* and *TLR10* genes was nonparametric therefore it was analysed by using the Mann–Whitney U test. A P-value of ≤ 0.05 was accepted as statistically significant[10].

Results and Discussion

This study included 88 gastroduodenal disorder patients (54 males and 34 females; mean age 43.5 ± 1.64 SE years; ranging between 15–71 years and the healthy group consisted of 21 (8 males and 13 females with a mean age 45.4 ± 2.95 SE ranging between 26–69 years used for comparison. As shown in Tables 1 and 2, the patient group consisted of three sub-groups, 36 patients suffer from gastritis, 32 with peptic ulcer, and 20 with gastric cancer (Fig. 1). Their mean ages were 38.19 ± 2.56 SE; 46.69 ± 2.17 SE, and 48.10 ± 3.96 SE years old; they ranged between 17-71, 22-66, and 15-69 years, respectively. The incidence of *H. pylori* infection and severity of the gastroduodenal disorder in males were more than in females and increased with age based on the sample groups (Tables 1 and 2).

Table 1. Age distribution in patients suffering from gastroduodenal disorders.

Group		Age group						Total	Means ± SE	
		< 20	20-29	30-39	40-49	50-59	≥ 60			
Gastritis	Count	4	10	7	7	4	4	36	38.19	±
	% within Group	11.1%	27.7%	19.4%	19.4%	11.1%	11.1%	100.0%	2.56	
Peptic ulcer	Count	0	2	8	7	6	9	32	46.69	±
	% within Group	0.0.%	6.25%	25%	21.8%	18.3%	28.1%	100.0%	2.17	
Gastric cancer	Count	3	1	1	1	9	5	20	48.10	±
	% within Group	15.0%	5.0%	5.0%	5.0%	45.0%	25.0%	100.0%	3.96	
Total patients	Count	7	13	16	15	19	18	88	43.5	±
	% within Group	7.95%	14.77%	18.18%	17.04%	21.59%	20.45%	100%	1.64	
Healthy	Count	0	2	8	2	5	4	21	45.4	±
	% within Group	0.0%	9.5%	38.1%	9.5%	23.8%	19%	100.0%	2.95	
Total	Count	7	15	24	17	24	22	109	43.5	±
	% within Group	6.4%	13.8%	22%	15.6%	22%	20.1%	100.0%	1.64	
Chi-Square Tests			P-value			0.001**				

Table 2. Gender distribution in gastroduodenal disorders.

Group			Gender		Total
			Male	Female	
Group	Gastritis	Count	17	19	36
		% within Group	47.2%	52.8%	100.0%
	Peptic ulcer	Count	22	10	32
		% within Group	68.75%	31.25%	100.0%
	Gastric cancer	Count	15	5	20
		% within Group	75%	25%	100.0%
	Healthy	Count	8	13	21

		38.1%	61.9%	100.0%
Total	Count	62	47	109
	% within Group	56.9%	43.1%	100.0%
Chi-Square Tests		P-value		0.001**

Detection of *H. pylori*

The detection of *H. Pylori* was performed depending on the gene's *ureA* and *ureC,* so as to ensure the correct strain detection then compared with strain IRQ19-106 as a positive control, which was isolated, identified strain by cytotoxin-associated gene a (*CagA* gene) and recorded in NCBI by Kalaf *et al*. [11] as shown in Figs. 2 and 3.
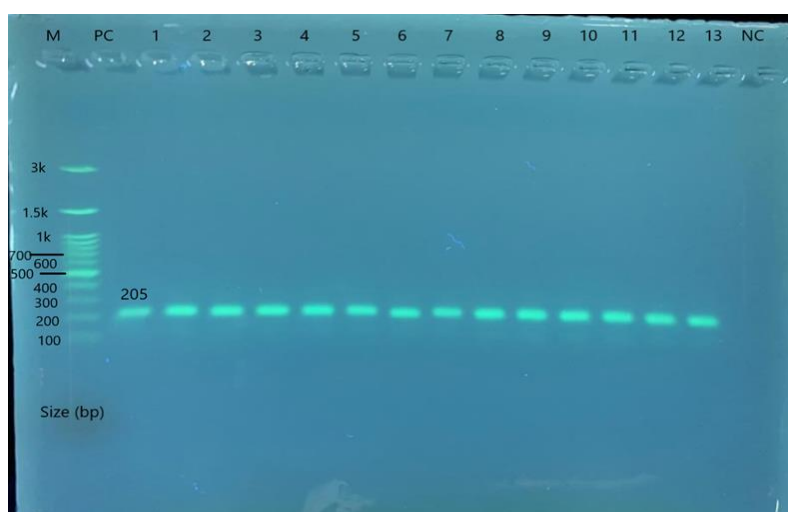
Figure 2. Agarose gel electrophoresis of PCR products of a specific primer for *H. pylori ureA* gene with product size 205 bp. The electrophoresis was conducted on 2% agarose gel at 100 volts for 10 min and 70 volts for 50 min. Representative results are present; Line M is DNA marker (100-3000bp), PC is positive control (strain IRQ19-106), 1-13 patients infected with *H. pylori,* NC is negative control.
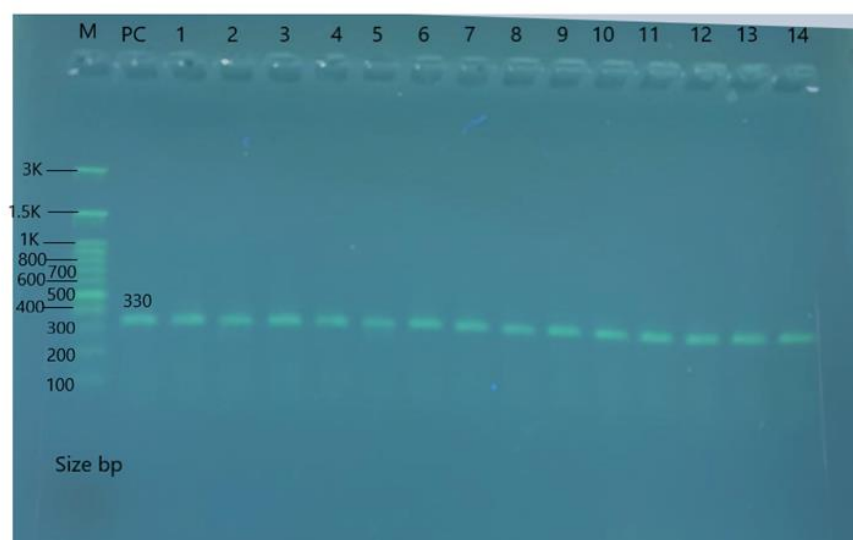

Figure 3. Agarose gel electrophoresis of PCR products of a specific primer for the *H. pylori ureC (glm)* gene with product size 330 bp. The electrophoresis was conducted on 2% agarose gel at 100 volts for 10 min and 70 volts for 50 min. Representative results are present; Line M is DNA marker (100-3000bp), PC is positive control (strain IRQ19-106), 1-13 patients infected with *H. pylori*.

The expression of Genes *TLR2* and *TLR10* in Patients with Gastroduodenal Infection

To establish a baseline of immune response to *H. pylori* infection by *TLR2* and *TLR10*, three groups of gastroduodenal disorder were included in this study and compared with the healthy control group. These groups included gastritis, peptic ulcer, and gastric cancer. The level of *TLR2*, *TLR10*, and *β-actin* mRNA was measured by using qPCR, and the melt was performed to evaluate the primers' specificity (Fig 4) and the quantitation data analysis for cycling A. green (Fig 5). The bacterium *H. pylori* induced the expression of *TLR2* and *TLR10* genes, it recorded 11.11-fold for gene *TLR2* in samples with gastritis; 7.65-fold in peptic ulcer, and 6.25 in those infected with gastric cancer. Folding decreased with the prognosis of the disease. On the other hand, the *TLR10* gene resulted in the highest value with peptic ulcer 5.01-fold, followed by 4.78-fold in gastritis, and 3.10-fold in gastric cancer samples (Fig. 6).
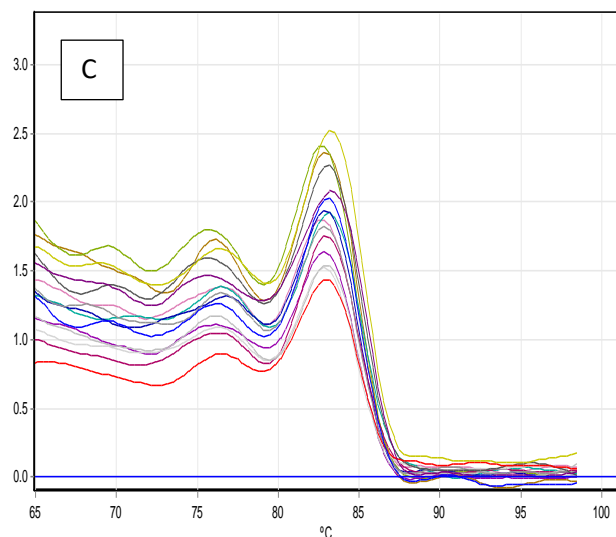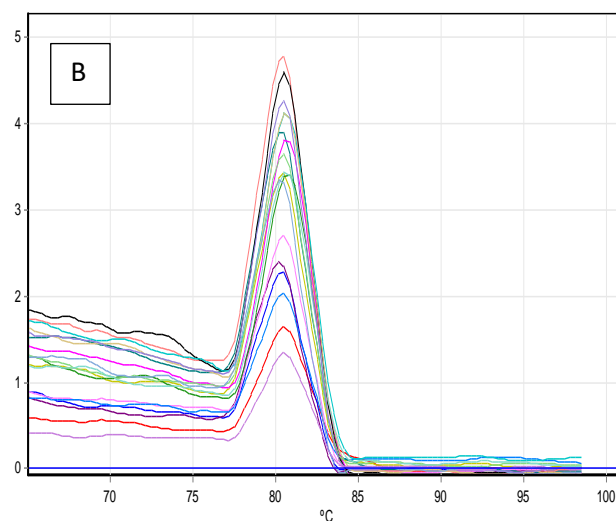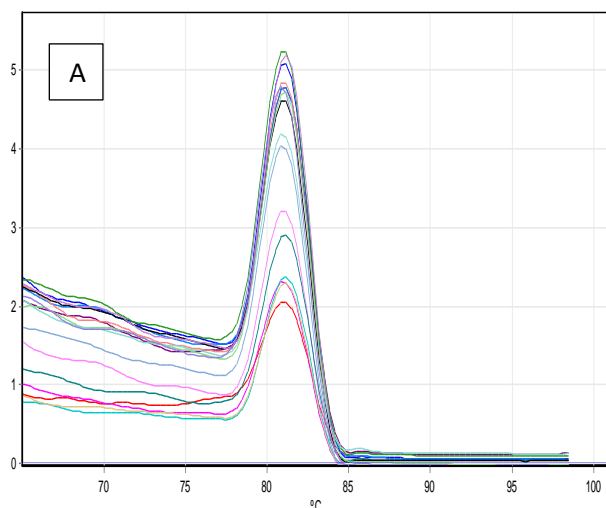
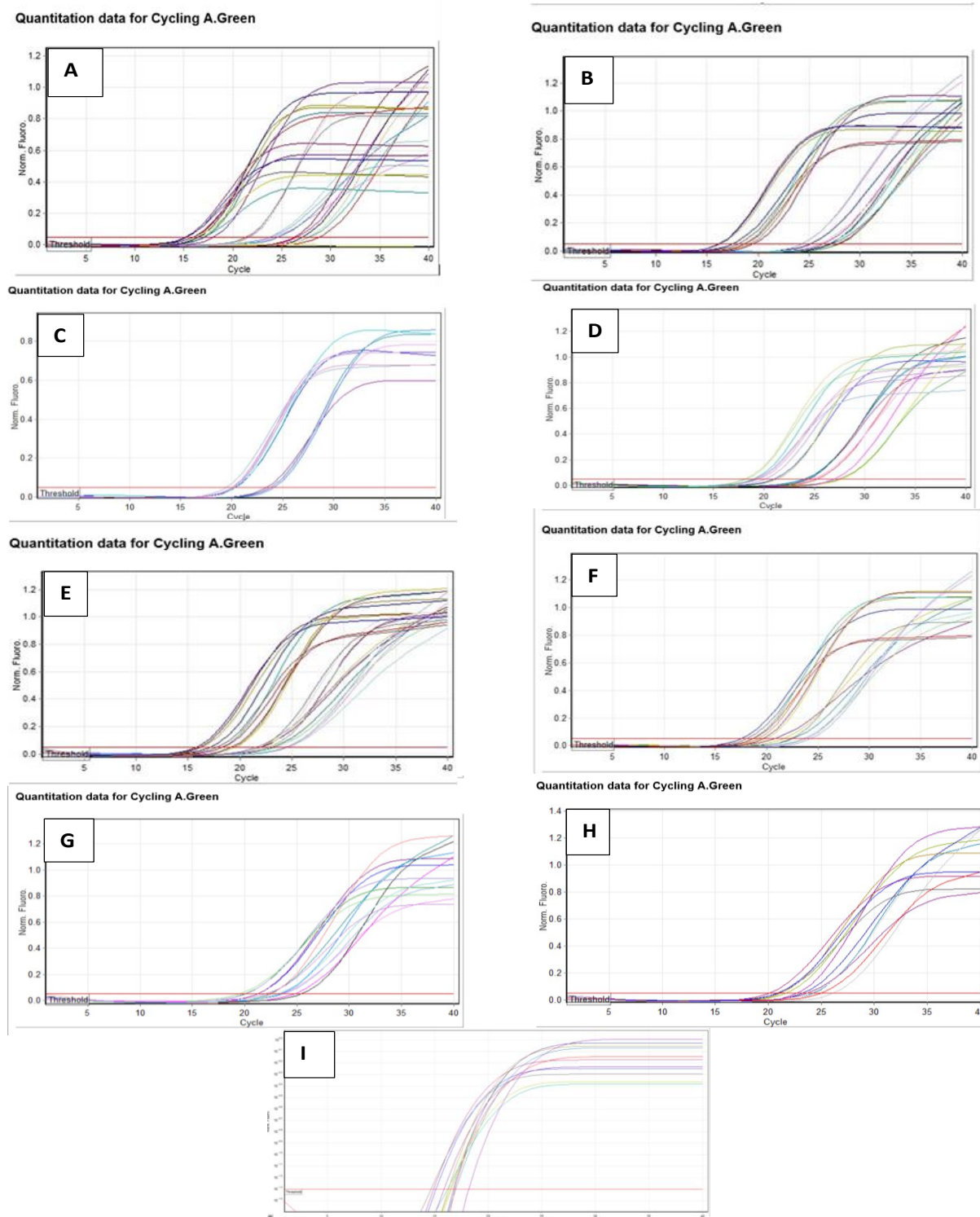**Figure 4. Illustrate Primers melting's (A) *TLR2* melts, (B) *TLR10* melts, (C) *β-Actin* primer melts.**

Figure 5. Illustrate Real-time PCR quantitation data analysis for cycling A. green. (A) *TLR2* **normalized to** *β-actin* **in the healthy group, (B)** *TLR10* **normalized to** *β-actin* **in healthy group, (C)** *TLR2* **normalized to** *β-actin* **in gastritis group, (D)** *TLR10* **normalized to** *β-actin* **in gastritis group, (E)** *TLR2* **normalized to** *β-actin* **in peptic ulcer group, (F)** *TLR10* **normalized to** *β-actin* **in peptic ulcer group, (G)** *TLR2* **in gastric cancer group, (H)** *TLR10* **in gastric cancer group (I)** *β-actin* **in gastric cancer group the normalized gene for G and H.**

Table 3. Fold change expression of *TLR2* gene normalized to *β-Actin* gene in healthy, gastritis, peptic ulcer, and gastric cancer calculated by 2^- ΔΔCT method.

Group	Mean Ct Actin-β ± SD	Mean Ct TLR2 ± SD	Δ Ct	Δ Ct Calibrator	ΔΔCt	$2^{-ΔΔCt}$	experimental/ control group	Fold change (RQ)
Healthy	18.16 ±1.79	25.99 ±3.75	7.83	12.50	-4.67	25.43	25.43/25.43	1.00
Gastritis	18.66 ±1.52	23.02 ±1.63	4.36	12.50	-8.14	282.62	282.62/25.43	11.11
Peptic ulcer	18.47 ±1.96	23.36 ±2.19	4.89	12.50	-7.60	194.55	194.55/25.43	7.65
Gastric cancer	17.82 ±2.53	23.00 ±2.13	5.18	12.50	-7.31	159.11	159.11/25.43	6.25
Mann–Whitney U test			**P-value**			**0.001****		

Relative quantification (RQ) is the fold change compared to the calibrator, ** (P≤0.01) is significant.

Table 4. Fold change expression of *TLR10* gene normalized to *β-Actin* gene in healthy, gastritis, peptic ulcer, and gastric cancer calculated by 2^- ΔΔCT.

Group	Mean Ct Actin-β ± SD	Mean Ct TLR10 ± SD	Δ Ct	Δ Ct Calibrator	ΔΔ Ct	$2^{-ΔΔCt}$	experimental group/ control group	Fold of gene expression (RQ)
Healthy	18.64 ±2.03	26.28 ±3.95	7.65	12.50	-4.85	28.84	28.84/28.84	1.00
Gastritis	18.59 ±1.56	23.98 ±1.92	5.39	12.50	-7.11	137.88	137.88/28.84	4.78
Peptic ulcer	18.16 ±1.88	23.48 ±2.19	5.32	12.50	-7.17	144.44	144.44/28.84	5.01
Gastric cancer	17.82 ±2.53	23.84 ±2.33	6.02	12.50	-6.48	89.32	89.32/28.84	3.10
Mann–Whitney U test			**P-value**					**0.001****

Relative quantification (RQ) is the fold change compared to the calibrator, ** (P≤0.01) is significant.
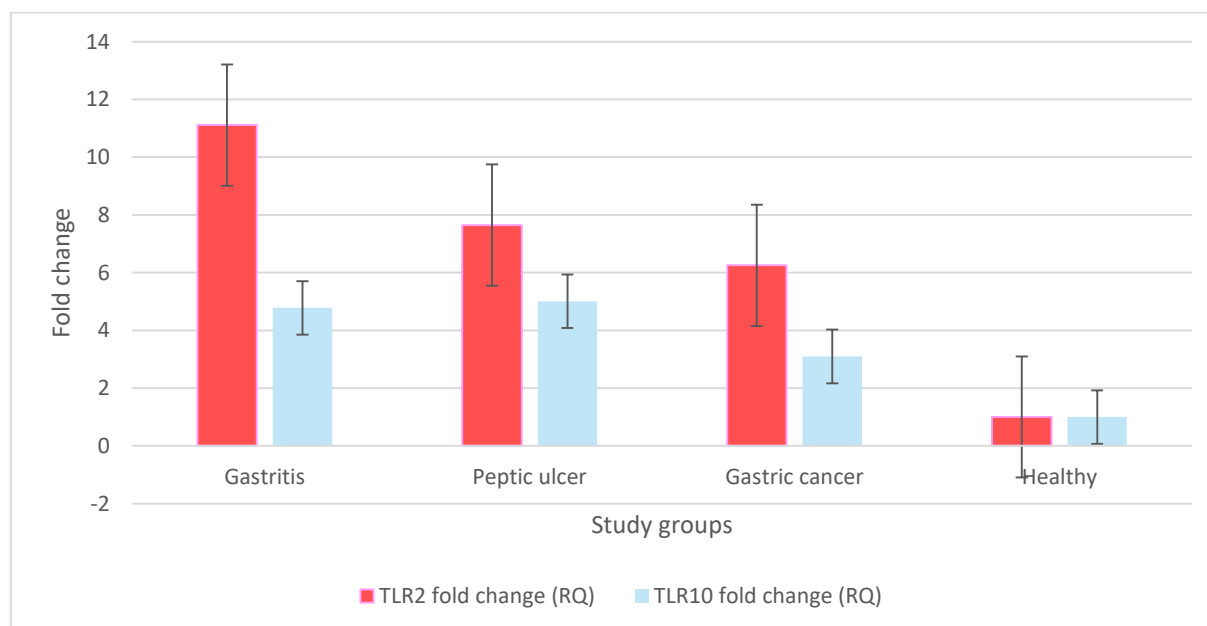
Figure 6. Fold change expression of *TLR2* and *TLR10* genes normalized to *β-Actin* gene in healthy, gastritis, peptic ulcer, and gastric cancer calculated by 2^- ΔΔCT method. Statistical analyses of fold change were calculated with the Mann–Whitney U test. Healthy (n=21), gastritis (n=36), peptic ulcer (n=32), and gastric cancer (n=20). The test revealed significant differences P ≤ 0.001 between the healthy group and patient groups. The comparison between groups gave R-values as follows for the *TLR2* gene 14.42 and 31.1for healthy with gastritis, 16.45and 27.79 for healthy with peptic ulcer, 14.68 and 22.76, and The R-value for *TLR10* gene= 18.42 and 28.15 for healthy with gastritis, 18.11 and 26.96 for healthy with peptic ulcer, 15.5 and 20.65 for healthy and gastric cancer. R (Mean Rank for Mann-Whitney U test).

Discussion

The prevalence of gastroduodenal disorder was significantly (p = 0.001) higher in elderly people; it was noticed that 21.59% of patients ranged from 50 to 59 years, followed by 20.45% who were ≥60 years, 18.18% who were 30-39 years, 15.6% who were 40-49, 13.8% who were 20 to 29 years, and 7.95% who were less than 20 years. Similar findings were recorded by Salman *et al*.[12] and Shehab *et al*.[13].

It is concluded from the present study that gastroduodenal disorder is more common in elderly individuals than others which may be due to decreasing the ability of protection and reparative capacity for the gastric mucosa layer which has two different repair mechanisms. The first mechanism is mucosal restitution by migration of viable epithelial cells from gastric pits and glands and the second mechanism is cell division replacement of lost cells. These mechanisms are affected by gastrointestinal

hormones and growth factors which regulate the reparation of mucosa membranes like EGF (epidermal growth factor) and TGF-α (Transforming growth factor alpha) activating the intrinsic tyrosine kinase (Tyr-k) of their epidermal growth factor receptor (EGF-R) and its activation decreases with age. In addition, polyamines and prostaglandins are also thought to be involved in gastric mucosal reparative processes which decreased with aging[14]. In this study, the incidence of gastroduodenal disorder was recorded in males more than in females. The female has a stronger immune response and antibody production due to additional protection from the extra copy of chromosome X. Although this chromosome is exposed to X chromosome inactivation mechanisms, there are genes that escape from silencing and skewed inactivation, which may contribute to an immunological advantage[15]. Several X-linked genes have roles in both the innate

and adaptive immune systems like TLR7 and TLR8, furthermore, Estrogen stimulates the immune system, whereas testosterone suppresses it.

These results agree with the finding of Kalaf et al.[11] and Zhu et al.[16] who reported that the infection rate in men is significantly higher than that in women while disagreeing with those of Majeed and Khoshnaw[17], and Lafta and AL-Faisal[18] who reported a high prevalence of H. pylori in women.

All patients were subjected to H. pylori detection by the ureA gene and ureC (glm) relying on the ability of the bacterium to produce urease, which hydrolyses the urea into ammonia and carbon dioxide, a process by which the bacterium can normalize the acidity of gastric juice and persist strong colonization in the mucosa of the stomach[18]. The urease is encoded by four genes (ureA, ureB, ureC, and ureD). PCR amplification of H. pylori urease genes (ureA and ureC) was used for ensuring the presence or absence of H. pylori in the stomach.

Any infection in the mucosa membrane induces cell apoptosis, oxidative stress, cell migration, and proliferation, especially with H. pylori infection. The apoptosis protects mucosa against inflammation and neoplasia however, the upregulation of cell migration and proliferation in response to injury by CagA or UreA facilitates tissue regeneration and increases the risk of neoplasia. By comparison, the downregulation of cell regeneration by H. pylori LPS may promote chronic inflammation[19]. The ureC gene encodes a phosphoglucosamine mutase since this gene is not associated with urease synthesis which was renamed as glmM. This gene is classified as a "housekeeping" gene since it directly participates in cell wall formation[20].

The TLRs are innate immune system sensors that identify conserved microbial components (for example, LPS, lipoteichoic acid, and flagellin), causing macrophages, neutrophils, dendritic cells, and epithelial cells to produce more inflammatory mediators[21]. In this study, the TLR2 and TLR10 gene expression was higher in gastroduodenal disorder compared with healthy subjects. These results agree with those of Nagashima et al.[22]. The highest value in TLR2 gene expression was in gastritis, followed by a peptic ulcer, then gastric cancer at 14.76-fold, 13.26-fold, and 10.04-fold respectively. This plays a significant role in the immune response to H. pylori infection, activation of NF-κB, and induction of cytokine expression in epithelial cells. Furthermore, it forms a complex with TLR1, 6, and 10 to form a heterodimer[23]. Therefore, there is a great need to increase the expression of its gene. While the highest value in TLR10 gene expression was recorded in peptic ulcer, followed by gastritis, then gastric cancer at 7.93-fold, 8.11-fold, and 6.16-fold. That means it is involved in the immune response to H. pylori and identifies the (PAMPs) and (DAMPs).

Gastritis for a long time leads to ulceration, therefore it recorded the highest value among other groups. In this study's comparison of TLR gene expression, TLR2 was shown to be more expressed than TLR10. This finding contrasts the findings of Nagashima et al.[22], who found the TLR10 gene 15.43-fold and the TLR2 gene 3.10-fold.

Conclusion

It is concluded from the current study that TLR2 and TLR10 genes are expressed in both uninfected and infected tissues with H. pylori; the bacterium elevated the expression. The TLR2 gene expression has the highest value in gastritis, followed by peptic ulcer, then gastric cancer. On the other hand, the TLR10 gene has the highest value in peptic ulcers, followed by gastritis, then gastric cancer.

Acknowledgment

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' Contribution Statement

This work was carried out in collaboration between all authors and the manuscript was revised and approved by all authors.

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التعبير عن الجينات *TLR2* و*TLR10* في خزعات النسيج المعدي لمرضى مصابون باضطرابات المعدة والاثني عشري التي سببها *Helicobacter pylori*

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الخلاصة

تعد بكتيريا *Helicobacter pylori* (*H. pylori*) المسبب الرئيسي في التهاب المعدة والقرحة الهضمية وأمراض سرطان المعدة. تساهم مستقبلات Toll-like receptors (TLRs) في الاستجابة المناعية الذاتية من خلال المسبب المرضي المرتبط بالأشكال الجزيئية Pathogen-associated molecular patterns (PAMPs) المشتقة من *H. pylori* والكائنات الدقيقة الأخرى بالإضافة إلى التلف المرتبط بالأشكال الجزيئية damage-associated molecular patterns (DAMPs) المشتقة من الخلايا المضيفة، مثل الخلايا السرطانية أو الخلايا الميتة أو المحتضرة أو المنتجات المنبعثة من الخلايا استجابة لبعض الإشارات منها نقص الأوكسجين. اشتملت الدراسة الحالية على خزعات معوية مأخوذة من 88 مريضًا مصابون باضطراب المعدة والأمعاء اضافة الى 21 مجموعة لا تعاني من تلك الاعراض. وشملت التشخيصات السريرية لهذه المجموعات بعد الفحص بالمنظار التهاب المعدة والقرحة الهضمية وسرطان المعدة. تم إجراء الكشف عن بكتيريا *H. pylori* باستخدام PCR لتضخيم جينات *ureC* و*ureA* بمساعدة بادئات محددة. صورت الحزم بعد الترحيل الكهربائي للهلام وتم تقدير التعبير عن جينات *TLR2* و*TLR10* في عينات خزعات المعدة بالطريقة النسبية. تشير النتائج الحالية إلى أن القابلية للإصابة باضطراب المعدة والأمعاء تزداد مع تقدم العمر، اذ كان عمر معظم المرضى أكثر من 30 عامًا (68 من أصل 88 مريضًا) ويمثلون 77.2٪ من المرضى. تحفز البكتيريا التعبير للجيني*TLR2* و*TLR10* وسجل الجين TLR2 11.11-fold في مرضى التهاب المعدة، و7.65-fold في مرضى القرحة الهضمية، بينما انخفض الى6.25-fold في المرضى الذين يعانون من سرطان المعدة، وانخفض folding مع تقدم المرض. من ناحية أخرى، سجل جين TLR10 أعلى 5.01-fold في مرضى القرحة الهضمية، تلتها 4.78-fold في مرضى التهاب المعدة، و3.10-fold في سرطان المعدة. سجل التعبير عن جيني *TLR2* و*TLR10* قيمة معنوية اعلى ($p \leq 0.01$) في مجموعة المرضى مقارنة بمجموعة السيطرة. في مرضى التهاب المعدة والقرحة الهضمية وسرطان المعدة، كان التعبير الجيني *TLR2* و*TLR10* أعلى من المجموعة السليمة، في الوقت الذي سجل جين *TLR2* أعلى من جين *TLR10*.

الكلمات المفتاحية: بكتريا *H. pylori* ، التهاب المعدة، قرحة المعدة، سرطان المعدة، جينات *TLR2*،*TLR10* .