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The Relation Between Host TLR9 -1486T/C, rs187084 Gene Polymorphisms and *Helicobacter pylori cagA*, *sodB*, *hsp60*, and *vacA* Virulence Genes among Gastric Cancer Patients

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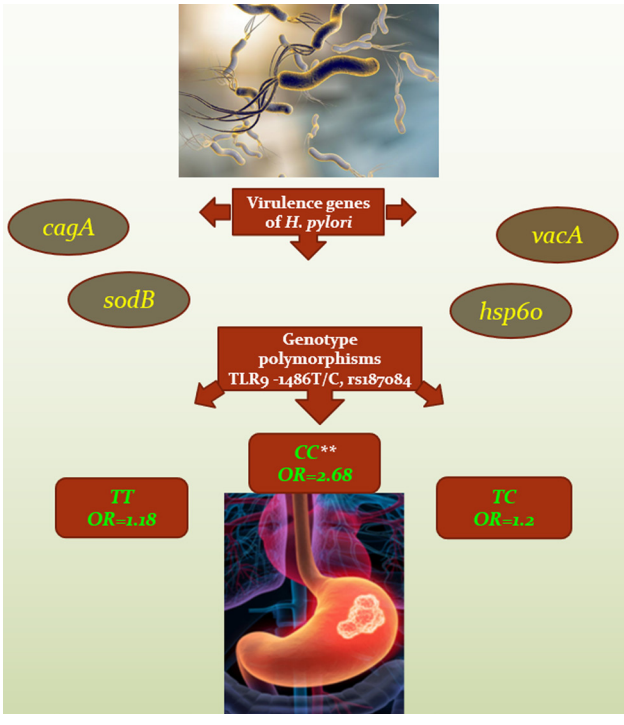
Abstract

To identify the associations between different genotypes of TLR9 -1486T/C (rs187084) with gastric cancer patients and reveal their relation to *Helicobacter pylori* virulence genes (*cagA*, *sodB*, *hsp60* and *vacA)*. Patients with gastric cancer were recruited to our study, diagnosed both endoscopically and histopathologically. *H. pylori* were isolated from gastric samples by culture and PCR amplification of the *glmM* gene. Virulence genes *cagA*, *sodB*, *hsp60*, and *vacA* were detected by multiplex PCR. Blood samples were used for genotyping of TLR9 -1486T/C (rs187084) by PCR-RFLP. Out of 132 patients with gastric cancer, 106 (80.3%) were positive for *H. pylori*. A similar number of healthy participants was recruited as controls. The prevalence of *cagA, sodB, hsp60*, and *vacA* genes among *H. pylori* was 90.6%, 70.8%, 83.0%, and 95.3%, respectively. The *vacA* gene alleles had a prevalence of 95.3% for *vacAs1/s2*, 52.8% for *vacAm1*, and 42.5% for *vacAm2*. The CC genotype of TLR9 -1486T/C had a significantly higher frequency in gastric cancer patients when compared to healthy participants ($p = 0.045$). Furthermore, the CC genotype demonstrated a significant association with *H. pylori* strains carrying *sodB*, *hsp60*, and *vacAm1* virulence genes ($p = 0.021$, $p = 0.049$, and $p = 0.048$ respectively). Patients with CC genotype of TLR9 -1486T/C (rs187084) might be at higher risk for the development of gastric cancer, and its co-existence with *H. pylori* strains carrying *sodB*, *hsp60*, or *vacAm1* virulence genes might have a synergistic effect in the development of gastric cancer. Further studies on a wider scale are recommended.



K e y w o r d s: *Helicobacter pylori*, gastric cancer, gene polymorphism, Toll-like receptor-9, virulence genes

Introduction

Helicobacter pylori, described as spiral-shaped Gram-negative bacteria, can infect gastric mucosa in more than half of the population all over the world (Trindade et al. 2017). Such infection disturbs the homeostasis of the gastric mucosa and induces the release of inflammatory cytokines (Cadamuro et al. 2014). Subsequently, the high association was described between *H. pylori* infection and several gastric pathologies as chronic gastritis and gastric cancer (Polk and Peek 2010; Kao et al. 2016). Moreover, the WHO has classified *H. pylori* as a type one carcinogen because of its high association with gastric cancer (Santos et al.

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2012). The development of such different gastric diseases has been linked to the interaction between *H. pylori* virulence factors, host genetics, immune responses, and environmental factors (Wroblewski et al. 2010; Bagheri et al. 2018).

Alterations in the host immune components, including Toll-like receptors (TLRs), through their prominent role in activating the innate and adaptive arms following infection, may influence the progress of the disease elicited by *H. pylori* (Wang et al. 2014; Song et al. 2018). These receptors are known as pattern recognition receptors as they identify the pathogen-associated molecular patterns (PAMPs) present in most pathogens (Varga and Peek 2017; Susi et al. 2019). During *H. pylori* infection, TLRs located on immune cells and local gastric epithelium identify various PAMPs present on that pathogen.

TLR9 detects unmethylated CpG oligonucleotides present abundantly in bacterial DNA (Fukata and Abreu 2008). As TLR9 is located inside the intracellular endosomes, its activation requires intracellular transfer of unmethylated CpG oligonucleotides through endocytosis (Fűri et al. 2013). They are expressed by gastric epithelial cells and contribute considerably to immune recognition and signaling following *H. pylori* infection. Moreover, the proper TLRs activation is vital for gut protection and recovery from injury (Wang et al. 2013). Single nucleotide polymorphism (SNP) of TLR9 genes, such as TLR9 -1486 TC (rs187084), can result in altered expression along with dysregulation of TLR9 signaling leading to unbalanced production of inflammatory cytokines with subsequent chronic inflammation, which promotes the development of gastric cancer (Wang et al. 2013). Such associations between TLR9 SNPs and increased risk to develop gastric cancer were previously reported (Wang et al. 2013; Susi et al. 2019).

Different virulence agents of *H. pylori* can be used as tools to predict the clinical outcomes of the infection (Polk and Peek 2010, Yamaoka and Graham 2014). The cytotoxin-associated gene A (CagA) is considered one of the main toxins of *H. pylori* (Ayala et al. 2014) that can cause morphological alterations in the host cell triggering cell differentiation and multiplication, which can help in the development of gastric cancer (Yong et al. 2015). The C-terminal region of CagA protein contains different Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs that serve as phosphorylation sites for the protein. These EPIYA segments were classified into four types according to their flanking sequences: EPIYA-A, B, C, and D (Cristancho Liévano et al. 2018). The CagA oncogenic potential has been linked to the EPIYA motifs C and D (Ofori et al. 2019). This could be related to the affinity of the SHP2 phosphatase protein to join the EPIYA C and D motifs, which affect the carcinogenic ability of *Helicobacter* strains (Cristancho Liévano et al. 2018).

The vacuolating cytotoxin (VacA) is another effective toxin produced by *H. pylori* strains and encoded by the *vacA* gene. It is a pore-inducing toxin that triggers apoptosis by inducing epithelial cellular vacuolation of the stomach (Palframan et al. 2012). The *vacA* gene has a mosaic structure with two main variation regions; the signal (s1 and s2), and the middle regions (m1 and m2), which determine the toxin vacuolating activity (Ofori et al. 2019). The s1/m1 and s1/m2 genotypes are associated with high and moderate vacuolating activity, respectively. On the other hand, the vacuolating activity is absent in the s2/m2 genotypes (Ofori et al. 2019). It was also reported that patients infected with *vacAs1m1* positive *H. pylori* strains are more likely of developing the clinical disease (Miernyk et al. 2011; Ofori et al. 2019).

The superoxide dismutase enzyme (SOD), encoded by the *sodB* gene is an important bacterial enzyme that helps *H. pylori* strains to survive (Seyler et al. 2001; Ryberg et al. 2008). Furthermore, heat shock protein 60 (Hsp60) is a protein expressed abundantly by *H. pylori* that acts as a molecular chaperone, which guards unfolded proteins against acid accumulation (Mendoza et al. 2017).

The TLR9 plays a significant role in initiating the immune response following *H. pylori* infection. Hence, genetic variability in its promoter region as TLR9 -1486T/C (rs187084) SNP may alter the expression of this receptor, modify the response to *H. pylori* infection, and increase the risk of gastric cancer. The outcome of *H. pylori* infection is considerably affected by the interactions between bacterial virulence factors and the host immune responses. Therefore, the aim of this study was to identify the associations between different genotypes of TLR9 -1486T/C (rs187084) with gastric cancer patients and reveal their relation to *H. pylori* virulence genes (*cagA*, *sodB, hsp60*, and *vacA*).

Experimental

Materials and Methods

Patients selection. This study was conducted over 15 months (from December 2019 to February 2021). We have recruited 132 patients with gastric cancer at the Gastro-Enterology Surgical Center, Mansoura University, Egypt. They initially presented with gastric symptoms confirmed endoscopically and histopathologically to be gastric cancer. The control group included healthy participants who presented with gastric symptoms and proved to be free of any gastric pathology by histopathology and negative for *H. pylori* by microbiological processing of the biopsy samples. All the participants' epidemiological and clinical data were gathered from medical records. In addition, clinicians have medically interviewed all subjects.

We have excluded any participant who fulfilled one or more of the set exclusion criteria: the previous gastric surgery and the use of anti-*H. pylori* eradication therapy, antibiotics, anti-inflammatory agents, proton pump inhibitors, chemotherapeutic drugs, or radiotherapy within one month before the endoscopy procedure.

Samples collection. A total of 238 stomach biopsies (each of size of 5 mm × 5 mm) were collected by clinicians during the performance of diagnostic gastric endoscopy procedures. The obtained biopsies were stored on ice and immediately transferred to the Medical Microbiology and Immunology Department, Mansoura University, Egypt, for further processing.

A peripheral blood sample of 10 ml was collected under complete aseptic precautions from each study participant for investigating TLR9 gene polymorphisms (TLR9 -1486T/C, rs187084 SNPs).

Isolation of *H. pylori* from gastric tissue samples. The collected biopsies were inoculated in sterile tubes with brain heart infusion (BHI) broth (Oxoid-UK), and then homogenized by a scalpel on a sterile slide. Homogenized samples were cultured on Colombia agar (Oxoid-UK) plates containing 10% of freshly defibrinated sheep's blood. Besides, plates were supplemented with amphotericin B (4 mg/l), vancomycin (10 mg/l), polymyxin B (10 mg/l), and trimethoprim (5 mg/l) antibiotics (Oxoid-UK). Cultured plates were incubated under microaerophilic circumstances (Campy pack systems, BBL, Cockeysville, Maryland, USA) at 37°C for three days (Adinortey et al. 2018).

After three days, culture plates were examined for colonies where *H. pylori* isolates were identified by having small, translucent, and round colonies. Further recognition of *H. pylori* isolates was conducted by microscopic examination by seeing curved-shaped bacteria along with Gram-stained films followed by biochemical reactions (positive catalase, urease, and oxidase). Suspensions of *H. pylori* strains were prepared using BHI broth supplemented with 20% glycerol and then kept at –20°C for further analysis (Idowu et al. 2019).

Molecular confirmation of isolated *H. pylori* strains through amplification of the *glmM* gene. Whole genomic DNA was obtained from cultured isolates using (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany), in line with the provider's rules, then the resulted DNA was kept –20°C until further completing of laboratory work.

The set of primers used to amplify the targeted gene was mentioned in Table I (Santos et al. 2012). The PCR was carried out using a master mix (Fermentas, Germany) that included 4 mM $MgCl_2$, 0.4 mM of each dNTP, and 0.05 U/µl *Taq* DNA polymerase. In addition, 10 pmol of each primer and 0.5 µg of template DNA were included in the reaction mixture (25 µl) (Menoni et al. 2013).

For amplification of the *glmM* gene, the PCR program started by an initial denaturation at 94°C for five minutes, then 40 cycles of denaturation for sixty seconds at 94°C, annealing for ninety seconds at 55°C, and extension for 120 seconds at 72°C. The final extension was performed at 72°C for seven minutes (Menoni et al. 2013).

Molecular detection of *cagA*, *sodB*, *hsp60*, and *vacA* virulence genes of *H. pylori* using multiplex PCR.

Table I
Sequences of primer sets used.

Gene targeted	Sequence	Size of amplified product (bp)	Ref
glmM	F: 5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3' R: 5'-AAGCTTACTTTCTAACACTAACGC-3'	294	Santos et al. 2012
cagA	F: 5'-GATAACAGGCAAGCTTTTGAGG-3' R: 5'-CTGCAAAAGATTGTTTGGCAG-3'	349	Amin et al. 2019
sodB	F: 5'-GCCCTGTGGCGTTTGATTTCC-3' R: 5'-CATGCTCCCACACATCCACC-3'	425	Ryberg et al. 2008
hsp60	F: 5'-GCTCCAAGCATCACCAAAGACG-3' R: 5'-GCGGTTTGCCCTCTTTCATGG-3'	603	Ryberg et al. 2008
vacA	F: 5'-CAATCGTGTGGGTTCTGGAGC-3' R: 5'-GCCGATATGCAAATGAGCCGC-3'	678	Ryberg et al. 2008
vacAs1/s2	F: 5'-ATGGAAATACAACAAACACAC-3' R: 5'-CTGCTTGAATGCGCCAAAC-3'	259	Harrison et al. 2017
vacAm1	F: 5'-GGTCAAAATGCGGTCATGG-3' R: 5'-CCATTGGTACCTGTAGAAAC-3'	290	Harrison et al. 2017
vacAm2	F: 5'-CATAACTAGCGCCTTGCAC-3' R: 5'-GGAGCCCC*AG*GAAACATTG-3'	352	Harrison et al. 2017
TLR9-1486T/C, rs187084	5'-TTCATTCATTCAGCCTTCACTCA-3' 5'-GAGTCAAAGCCACAGTCCACA-3'	490	Roszak et al. 2012

Multiplex PCR was undertaken following the coming steps simultaneously: beginning with incubation for 5 minutes at 95°C; then 34 cycles consisted of one minute at 94°C, then another one minute for primer annealing at 55°C, followed by extension for 60 seconds at 72°C; to be finished with the final extension step at 72°C for ten minutes (Amin et al. 2019). The reaction volume was 25 µl and consisted of a PCR master mix (Fermentas, Germany) that included 4 mM $MgCl_2$, 0.4 mM of each dNTP, and 0.05 U/µl *Taq* DNA polymerase. Besides, 0.5 µg of DNA and 10 pmol of each primer were added. PCR products were then electrophoresed (Farshad et al. 2007). The reference strain (ATCC26695) was used as a positive control (Ryberg et al. 2008). Primer sets used were supplemented in Table I.

Molecular detection of different *vacA* gene alleles in *vacA*-positive *H. pylori* isolates (*vacAs1*/*s2*, *vacAm1*, and *vacAm2*). PCR was conducted by applying the following cycling parameters – first: 95°C for 5 minutes; second: 35 cycles (95°C for 30 seconds, 54°C for 30 seconds and 72°C for 16, 18, and 21 seconds respectively according to the required allele to be amplified (*vacAs1*/*s2*, *vacAm1* and *vacAm2*)). Then final extension was at 72°C for 10 minutes (Harrison et al. 2017). Sets of primers used were listed in Table I.

Genotyping of TLR9 -1486T/C, rs187084 polymorphisms by PCR-RFLP. Genomic DNA was extracted from the obtained buffy coat (leukocyte-enriched fraction of whole blood) using Gene JET Whole Blood Genomic DNA purification Mini kit (Fermentas Life Sciences, Canada) according to provider's guidelines then exposed to PCR-RFLP. Blood samples were subjected to centrifugation at 2,500 × *g* for 10 minutes to release three layers: the upper transparent layer containing plasma, the intermediate buffy coat, and the bottom layer of concentrated erythrocytes.

PCR reaction was run with thermal cycling conditions of 4 minutes at 95°C then 35 cycles each starting with 30 seconds at 95°C followed by 20 seconds at 60°C, and 30 seconds at 72°C to be ended with final extension for 5 minutes at 72°C to produce a DNA piece of 490 bp (Paradowska et al. 2016). The primers used were illustrated in Table I (Roszak et al. 2012).

Then the DNA products were digested using AflII restriction enzyme (Thermo Scientific, EU, Lithuania) by incubation for three hours at 37°C to yield one of three variants: two fragments of 192 bp, and 327 bp that indicated TT allele, or three fragments of 192 bp, 327 bp, and 490 bp that proved the presence of TC allele or an intact PCR fragment of 490 bp indicating the presence of CC allele (Paradowska et al. 2016).

Statistical analysis. The data obtained were evaluated by the computer program SPSS (Statistical package for social science) version 22.0. Descriptive items were shown as means ± standard deviation (SD) or frequency (number-percent). *p*-Values less than 0.05 were significant. The relation between genotypes and the risk of gastric cancer was detected by calculating the odd ratios (ORs) and 95% confidence interval (CIs). We applied Hardy-Weinberg equilibrium to compare genotype frequencies we observed to the expected ones in studied healthy control. The relation between the virulence genes of *H. pylori* and TLR9 -1486T/C, rs187084 genotypes in gastric cancer patients was identified using chi-square test.

Results

This study included 106 subjects with gastric cancer and positive for *H. pylori* infection. A similar number of 106 subjects free of any gastric pathology and negative for *H. pylori* infection were recruited as a control group. No significant difference was found between both groups in both age and sex, as shown in Table II. Gastric cancer lesions were classified according to the histopathologi-

Table II
Demographic and histopathological data of the subjects included in the study.

Variable	Gastric cancer patients	Healthy study participants	*p*-value
Age			
Years (mean ± SD)	56.55 ± 8.63	53.27 ± 8.55	0.93
Sex			
Male	63 (59.4)	59 (55.7%)	0.76
Female	43 (40.6%)	47 (44.3%)	
Differentiation of the tumor			
Well differentiated tumor	16 (15.1%)	NA	–
Moderately differentiated tumor	35 (33.0%)		
Poorly differentiated tumor	55 (51.9%)		

Values are given as mean ± SD, or number (percentage)
NA – Not applicable

Table III
Distribution of *H. pylori* among gastric tissue samples in studied gastric cancer patients.

	Number	%	*p*-value
Positive	106	80.3	0.000*
Negative	26	19.7	
Total	132	100%	

* – statistically significant

Table IV
Distribution of virulence genes in *H. pylori* strains isolated from gastric cancer patients.

Virulence gene	N = 106	%	*p*-value
cagA			
Positive	96	90.6	0.000*
Negative	10	9.4	
sodB			
Positive	75	70.8	0.000*
Negative	31	29.2	
hsp60			
Positive	88	83.0	0.000*
Negative	18	17.0	
vacA			
Positive	101	95.3	0.000*
Negative	5	4.7	
vacAs1/s2			
Positive	101	95.3	0.000*
Negative	5	4.7	
vacAm1			
Positive	56	52.8	0.56
Negative	50	47.2	
vacAm2			
Positive	45	42.5	0.12
Negative	61	57.5	

* – statistically significant

cal examination into well-differentiated tumors (15.1%), moderately differentiated tumors (33.0%), and poorly differentiated tumors (51.9%) (Table II).

Of the 132 gastric cancer patients, 106 (80.3%) gave positive results for *H. pylori* by both culture and PCR, whereas 26 (19.7%) were negative. The results were statically significant (p-value = 0.000) (Table III). The multiplex PCR revealed that the prevalence of *cagA*, *sodB*, *hsp60*, and *vacA* genes among the isolated *H. pylori* strains were 90.6%, 70.8%, 83.0%, and 95.3%, respectively. Regarding the *vacA* gene alleles, they had a prevalence of 95.3% for *vacAs1*/*s2*, 52.8% for *vacAm1*, and 42.5% for *vacAm2* (Table IV).

We screened both the case and control groups for TLR9 -1486T/C, rs187084 polymorphism by PCR-RFLP. The obtained frequencies of genotypes of TLR9 -1486T/C, rs187084 in the healthy group were all on line with Hardy-Weinberg equilibrium. In gastric cancer patients, the frequencies of T and C alleles were 72 (34.0%) and 140 (66.0%), respectively, whereas in the control group, the T allele frequency was 122 (57.5%), and the C allele frequency was 90 (42.5%). We found that the frequency of the C allele in gastric cancer patients was significantly higher than in the control group ($p = 0.047$). The frequency of CC genotype in gastric cancer patients (52.8%) was significantly higher than the control group (22.6%) with a p-value of 0.045, whereas both TT and TC genotypes were not, as they recorded p-values of 0.73 and 0.68, respectively as demonstrated in Table V.

The CC genotype of TLR9 -1486T/C, rs187084, when compared to TT + TC genotypes, demonstrated a significant relation with *H. pylori* strains carrying the *sodB*, *hsp60* or *vacAm1* virulence genes ($p = 0.021$, $p = 0.049$ and $p = 0.048$ respectively). None of the *cagA*, *vacA, vacAs1*/*s2,* or *vacAm2* genes showed a significant association to CC genotype ($p = 0.075$, $p = 0.88$, $p = 0.88$ and $p = 0.81$, respectively) (Table VI).

Discussion

We have reported a prevalence of *H. pylori* of 80.3% in patients with gastric cancer that was in agreement with previous studies (Ang and Fock 2014; Park et al.

Table V
Distribution of genotypes of TLR9 -1486T/C, rs187084 polymorphism in studied groups.

Genotype frequency	Healthy study participants N = 106		Gastric cancer patients N = 106		OR	95% CI	X^2	*p*-value
	N	%	N	%				
TT	40	37.7	22	20.8	1.18	0.45–3.1	0.12	0.73
TC	42	39.6	28	26.4	1.2	0.49–2.88	0.16	0.68
CC	24	22.6	56	52.8	2.68	1.0–7.14	4.03	0.045*

Genotype frequencies are presented in the form of absolute numbers with percentages
OR – Odds ratio; CI – Confidence interval; * – statistically significant

Table VI
The relation between virulence genes of *H. pylori* and TLR9 -1486T/C, rs187084 genotypes in gastric cancer patients.

TLR9-1486T/C, rs187084 genotype	CC N = 56		TT + TC N = 50		*p*-value
Virulence gene	N	%	N	%	
cagA	55	98.2	41	82.0	0.075
sodB	53	94.6	22	44.0	0.021*
hsp60	53	94.6	35	70.0	0.049*
vacA	54	96.4	47	94.0	0.88
vacAs1/s2	54	96.4	47	94.0	0.88
vacAm1	35	62.5	21	42.0	0.048*
vacAm2	19	33.9	26	52.0	0.81

* – statistically significant

2018). A slightly lower prevalence of *H. pylori* at 74.2% has been reported by Wang and his colleagues (2013). Nevertheless, Ezzat et al. (2012) reported a 100% prevalence of *H. pylori* infection in gastric cancer patients. Such differences in *H. pylori* prevalence can be attributed to the host immune response, bacterial virulence factors, and environmental elements (Ofori et al. 2019).

In our study, the prevalence of *cagA* in *H. pylori* strains was 90.6% that was nearly similar to previous reports from Vietnam at 95% (Uchida et al. 2009), North America at 88% (Yamaoka et al. 1999), and Sweden at 82% (Ryberg et al. 2008). In our study, the *vacA* gene was detected in most of the isolated *H. pylori* strains (95.3%) consistent with other reports (Ezzat et al. 2012; Amin et al. 2019). Also, we have reported the prevalence of the *vacAm1* allele at 52.8%, which was higher than that of *vacAm2* (42.5%). In line with our results, the *vacAm1* allele is commoner in Northern Asia while the *vacAm2* allele predominates in Southeast Asia, which has a lower incidence of gastric cancer (Yamaoka and Graham 2014). Similarly, it was suggested that patients infected with the *vacAm1*-positive *H. pylori* strains are at more risk for severe outcomes as gastric cancer than *vacAm2* genotype-positive *H. pylori* strains (Yamaoka and Graham 2014; Idowu et al. 2019). We detected the *sodB* and *hsp60* genes in 70.8% and 83.0% of *H. pylori* strains, respectively, which was in line with Ryberg and his colleagues (2008).

TLR9 plays a vital role in recognizing *H. pylori* DNA and initiating immune responses (Wang et al. 2013). Rad and his colleagues (2009) reported that TLR9 recognition of *H. pylori* has resulted in proinflammatory responses. It was recently suggested that *H. pylori* could reduce the inflammatory response through TLR9, leading to persistent infection (Varga and Peek 2017). Besides, it was reported that *H. pylori* DNA triggered TLR9-dependent activation of NF-kB in human neutrophils that increased IL-8 production, which can participate in gastric cancer development (Alvarez-Arellano et al. 2014). Interestingly, some studies have reported that *H. pylori* can induce TLR9 expression, resulting in stimulating cellular mitogen-activated protein kinases, which trigger angiogenesis and cellular invasion (Chang et al. 2005). Furthermore, TLR9 has been associated with the up-regulation of cyclooxygenase-2 in *H. pylori*-infected gastric mucosa, which was linked to gastric cancer (Fukata and Abreu 2008).

For TLR9 -1486T/C gene polymorphisms, we found that the frequency of CC genotype was significantly higher in gastric cancer patients than the control group (p-value = 0.045). Also, the frequency of the C allele in gastric cancer patients was significantly higher than in the control group ($p = 0.047$). Our findings suggested that C allele might have a role in modifying the immune response to *H. pylori* infection and subsequently promoting gastric carcinogenesis. In line with our findings, Wang and his colleagues (2013) reported that CC and TC genotypes of TLR9 -1486 and the C allele carriers were associated with a higher gastric cancer risk among the Chinese population. Meanwhile, Susi et al. (2019) reported an association between CT and TT genotypes and increased risk of gastric cancer among the Brazilian population. On the other hand, Liu et al. (2015) found no association between TLR9 -1486 T/C polymorphism and gastric cancer risk. These different findings can be attributed to racial differences, environmental factors, and diverse genetic backgrounds in various populations.

In a gene assay conducted by Tao et al. (2007), the C allele of TLR9 -1486T/C down-regulates the expression of TLR9, which leads to deficient immune recognition and signaling in response to *H. pylori*. Consequently, both innate and adaptive immune responses will be reduced, which favors the development of chronic gastritis. This state of chronic gastritis can promote the development of gastric cancer, which can explain our findings. In a more recent study, Xu and his colleagues (2018) have reported a significant association between the CC genotype of TLR9 -1486T/C and increased expression of TNF-α and IL-1β cytokines, affecting the infection outcome. Therefore, such SNP can modify the TLR9 expression, affect the pathogenesis of *H. pylori* infection and increase the risk of gastric cancer (Wang et al. 2013). Still, the precise mechanism by which this SNP affects the risk of *H. pylori*-induced gastric cancer has to be clarified in future studies.

Most studies have focused on either host or bacterial risk factors for developing gastric cancer; however, we explored the relationship between the studied virulence genes of *H. pylori* and genotypes of TLR9 -1486T/C (rs187084) polymorphism in gastric cancer patients. Interestingly, our findings have shown that

the CC genotype had a significantly higher frequency in gastric cancer patients than the control group and that the CC genotype is significantly associated with *H. pylori* strains to harbor the *sodB*, *hsp60* or *vacAm1* virulence genes. Our findings can help clinicians stratify patients according to their tendency to develop gastric cancer and plan strategies for eradicating *H. pylori* infection.

Conclusions

This study suggested that patients with CC genotype of TLR9 -1486T/C (rs187084) might be at higher risk for the development of gastric cancer. In addition, our results offered some evidence that the co-existence of CC genotype of TLR9 -1486T/C and *H. pylori* strains to carry the *sodB*, *hsp60* or *vacAm1* virulence genes might have a synergistic effect promoting the development of gastric cancer. Our findings support the link between host genetics, bacterial virulence genes, and gastric cancer. However, further studies are recommended on a larger number of cases and in more diverse populations.

Abbreviations

BHI – brain heart infusion broth
CagA – cytotoxin-associated gene A
Hsp60 – heat shock protein 60
PAMPs – pathogen-associated molecular patterns
PCR-RFLP – PCR-based restriction fragment length polymorphism
TLRs – Toll-like receptors
SOD – superoxide dismutase enzyme
VacA – vacuolating cytotoxin
SNP – single nucleotide polymorphism

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki, besides the national and institutional standards. Its protocol was approved by the Mansoura Faculty of Medicine Research Board (R.21.01.1165). Informed written consent was received from all participants included in the study.

Author contributions

Both Yasmin Nabiel and Amira Sultan were the creators of the research idea. They both shared in designing the protocol to be carried out, conducting the sample procedures, writing the manuscript, and analyzing the resulting data. Ragy Shenouda shared in processing the samples besides writing and revising the manuscript. Both Ahmed M. Sultan and Ahmed Shehata shared in performing surgical maneuvers and collecting required samples from included study members.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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