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# The *cagA*, *cagE*, *vacA*, *dupA* and *iceA1* genes of *Helicobacter pylori* in Sudanese gastritis patients: Distribution and relationship with clinical outcomes and histological alterations

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## ABSTRACT

**Aims:** *Helicobacter pylori* is a gastrointestinal bacterium that causes peptic ulcers and stomach cancer in nearly half of the world's population. Many virulence factors influence the outcome of *H. pylori* related disorders. The purpose of this study was to see if there was a relationship between *H. pylori* virulence factors and histological and endoscopic findings in stomach biopsy specimens from Sudanese gastritis patients.
**Methodology and results:** In the period between March 2018 and January 2020, a total of 290 gastric biopsies were taken from patients in Khartoum State hospitals. Histopathology and polymerase chain reaction (PCR) assays were performed on all specimens. Histological investigation revealed *H. pylori* in 103/290 (35.5%) samples, while PCR revealed *H. pylori* 16S rRNA positivity in 88/290 (30.3%) samples. Eighty-eight positive PCR specimens were subjected to PCR for genotypic detection of *cagA*, *cagE*, *vacA*, *dupA* and *iceA1* genes. All of strains were *vacA* positive 100% (88/88) followed by *dupA* 50.0% (44/88), *cagA* 40.9% (36/88), *cagE* gene 38.6% (34/88) and *iceA1* gene was detected in only 15.9% (14/88). The *vacA s1/m1* 68.2% (60/88) was the most prevalent *vacA* subtype.
**Conclusion, significance and impact of study:** *Helicobacter pylori* virulence genes were widespread and diversified in Sudanese gastritis patients. *Helicobacter pylori cagA* and *iceA1* were significantly in association with gastric mucosa inflammation degree, whereas the *dupA* gene was found to be associated with the clinical outcomes.

*Keywords: Helicobacter pylori*, peptic ulcer, virulence genes, PCR, Sudan

## INTRODUCTION

*Helicobacter pylori* is gram-negative, spirally curved bacilli that belongs to a microaerophilic bacterium with a single polar, numerous flagellum (Chen *et al.*, 2021; Liu *et al.*, 2021). Infection of *H. pylori* is considered one of the world's most common chronic infections, with a high infection incidence and substantial relation to 95% of stomach disorders, including gastric cancer (Redéen *et al.*, 2011; Hasosah, 2019; Sabbagh *et al.*, 2019; Xu *et al.*, 2021). The colonization of *H. pylori* begins in childhood and persists throughout life, producing sickness in adulthood (Kivi and Tindberg, 2006; Atherton and Blaser, 2009; Kalali *et al.*, 2015; Hasosah, 2019). Many experts have proposed fecal-oral transmission could occur through contaminated water, food and unwashed hands as a possible method of transmission (Aziz *et al.*, 2015).

*Helicobacter pylori* identification is critical for detecting sickness, treating infected individuals and eliminating the bacterium (Kalali *et al.*, 2015). Several diagnostic techniques for epidemiological research have been utilized to identify *H. pylori* infections. It can be diagnosed

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by invasive (endoscopy) and non-invasive techniques. Endoscopy is frequently used to screen for cancer of the stomach and other disorders. It can also be used to examine various epigastric symptoms (Idowu *et al.*, 2019). Histological techniques detect the presence of characteristic spiral motile bacteria accompanied by an inflammatory reaction in histopathological sections of the stomach that are commonly stained with Giemsa or hematoxylin and eosin (H and E) (Kalali *et al.*, 2015). *H. pylori* pathogenesis might be associated with numerous pathogenic conditions (Liu *et al.*, 2021). The degree of *H. pylori* colonization and chronic inflammation may be influenced by a variety of virulence characteristics, the host response and factors associated with the environment (Camorlinga-Ponce *et al.*, 2004; El-Khlousy *et al.*, 2016; Akeel *et al.*, 2019). The most prevalent virulence factors in *H. pylori* are vacuolating cytotoxin A (*vacA*), cytotoxin associated antigen A (*cagA*), cytotoxin associated antigen E (*cagE*), duodenal ulcer promoting gene A (*dupA*) and induced by contact with epithelium A (*iceA*) (Akeel *et al.*, 2019; Idowu *et al.*, 2019). *H. pylori* 16S rRNA is a ribosomal gene which present in all bacteria and it comprises a nucleotide sequences that are specific to a given genus of bacteria. *H. pylori vacA* gene is associated with greater gastric epithelial damage. *vacA* gene is a pore-forming cytotoxin which induces *in vitro* vacuolization in stomach epithelial cells, inhibition of T cell activation proliferation and apoptosis. Most strains of *H. pylori* contain a *vacA* gene with several alleles (*s1*, *s2*, *m1* and *m2*) (Archampong *et al.*, 2017; Chang *et al.*, 2018). *Helicobacter pylori* strain positive for *vacA s1* or *m1* were documented more in patients with peptic ulcer disease (PUD) and gastric cancer (GC) (Akeel *et al.*, 2019).

The cag pathogenicity island (*PAI*) is dominated by *cagA* and *cagE* gene. The *cagA* gene is crucial in determining the clinical outcome of *H. pylori* infection, which might result in gastritis, PUD or GC. Translocated *cagA* alters the cell-cell junctions, motility and cytoskeleton arrangement and induces a proinflammatory and antiapoptotic gene expression profile. In addition to the *cag*PAI, other genes are relevant for colonization, persistence and damage to the gastric mucosa (El-Khlousy *et al.*, 2016; Akeel *et al*., 2019; Chung *et al.*, 2019). A higher risk of GC is also associated with the presence of the *cagE* gene (Bakhti *et al.*, 2020; dos Santos Pereira *et al.*, 2020). Duodenal ulcers are connected to the existence of the functional duodenal ulcer promoting gene *A* (*dupA*) (Yamaoka, 2010; Oktem-Okullu *et al.*, 2020). The function of *dupA* gene is not fully understood. This gene increases the survival of the microorganism at low pH values. It is possible that *dupA* gene acts in the plasticity region to form a type IV secretion system also associated with increased IL-8 production from the antral gastric mucosa *in vivo* as well as from gastric epithelial cells *in vitro*. The presence *dupA* gene is thought to be also involved in DNA uptake/DNA transfer and protein transfer (Roesler *et al.*, 2014).

However, some report has revealed that the *iceA* gene is associated with enhanced mucosal interleukin (IL)-8 expression and acute antral inflammation which led to peptic ulcer illness and gastritis. There are two alleles of *iceA* gene, *iceA1* and *iceA2*. The relationship between *H. pylori iceA* gene and clinical outcomes of disease is controversial (Dabiri *et al.*, 2017; Kamarehei *et al.*, 2020).

The main objectives of this research were to study *H. pylori* virulence factors among Sudanese gastritis patients and to study if there was any link between them and histopathological alterations and endoscopic findings in gastric biopsies.

## MATERIALS AND METHODS

### Clinical specimens and patients

In the period between March 2018 and January 2020, a total of gastric biopsies have been obtained from 290 Sudanese patients who attended gastroenterology clinics at various Khartoum State's public institutions. Gastric tissue samples were collected by physicians from patients (both gender of different age groups) undergoing endoscopic examination and suffering from dyspepsia and other gastritis-related symptoms. Patients who had received antibiotics, proton pump inhibitors, H2 blockers or colloidal bismuth sulfate within the previous two months of endoscopy, patients with a history of gastric resection and patients with complicated peptic ulcer disease, i.e., hemorrhage, were excluded from this study.

Patients were placed in the left lateral decubitus position after a 6-8 h fast and upper endoscopy was performed with a typical forward-viewing endoscope. Topical lidocaine was used to anesthetize the patient's oropharynx. Under direct vision, the esophagus was intubated and subsequently, the esophagus, stomach and duodenum were examined. By retroverting the tip of the gastroscope, the gastric fundus could also be viewed. Each patient had two stomach biopsies obtained from the antrum and corpus. The examined samples revealed gastritis, gastric ulcer, duodenal ulcer, esophagitis and normal stomach mucosa. For the biopsies, histology and PCR were used.

### DNA extraction

For DNA extraction, newly obtained stomach biopsy tissues were collected in tubes with normal saline and processed for DNA extraction immediately using the guanidine chloride technique according to protocol mentioned by Abd Al Rahem and Elhag (2018).

### PCR analysis for *H. pylori* 16S rRNA and virulence genes

*Helicobacter pylori* 16S rRNA (*Hp*16S), *cagA*, *cagE*, *vacA*, *dupA* and *iceA1* genes were detected using PCR for extracted DNA. PCR was performed in the total reaction of a 25 µL including 5 µL of PCR mixture (Intron Biotechnology, Korea), 2 µL of sample DNA, 1 µL of each forward (F) and reverse (R) primer, then with the addition of 16 µL of distilled water (DW) the final volume was completed to 25 µL. Instead of genomic DNA templates,

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**Table 1:** Primers used in *Hp*16S rRNA, *cagA*, *cagE*, *vacA*, *dupA* and *iceA1* sequence amplification assays.

| Primer name | Primer sequence (5′-3′) | Amplicon size (bp) | Reference |
|---|---|---|---|
| 16S rRNA | F´GCTAAGAGATCAGCCTATGTCC<br>R´TGGCAATCAGCGTCAGGTAAT | 532 | (Ye, 2004) |
| *cagA* | F´ATAATGCTAAATTAGACAACTTGAGCGA<br>R´AGAAACAAAAGCAATACGATCATTC | 128 | (Tomasini *et al.*, 2003) |
| *cagE* | F´TTGAAAACTTCAAGGATAGGATAGAGC<br>R´GCCTAGCGTAATATCACCATTACCC | 508 | (Tomasini *et al.*, 2003) |
| *vacA (s1/s2)* | F´ATG GAA ATA CAA CAA ACA CAC<br>R´CTG CTT GAA TGC GCC AAA C | 259/286 | (El-Shenawy *et al.*, 2017) |
| *vacA (m1/m2)* | F´CAA TCT GTC CAA TCA AGC GAG<br>R´GCG TCT AAA TAA TTC CAAGG | 570/642 | |
| *dupA (jhp0917)* | F´TGGTTTCTACTGACAGAGCGC<br>R´AACACGCTGACAGGACAATCTCCC | 307 | (Lu *et al.*, 2005) |
| *iceA1* | F´GTG TTT TTA ACC AAAGTATC<br>R´CTA TAG CCA STY TCT TTG CA | 246 | (El-Shenawy *et al.*, 2017) |

DW was utilized as a negative control in each batch of PCR assays. An automated thermocycler was used to run the reaction mixtures. The PCR condition for *Hp*16S rRNA, *cagA* and *cagE* was as follows: Initial denaturation step at 94 °C for 3 min, 35 cycles (including 30 sec for denaturation at 94 °C, 30 sec for annealing at 53 °C and 45 sec at 72 °C for extension), finally at 72 °C a step of an extension was set at 5 min (Tomasini *et al.*, 2003; Ye, 2004) (Table 1).

PCR condition for *vacA* gene: Initial denaturation step at 94 °C for 5 min, 35 cycles (including one min for denaturation at 94 °C, one min for annealing at 60 °C, and one min at 72 °C for extension), finally at 72 °C a step of a final extension was set at 5 min (El-Shenawy *et al.*, 2017). For the *iceA1* gene: Initial denaturation step at 94 °C for 5 min, 37 cycles (including one min for denaturation at 94 °C, 50 sec for annealing at 55 °C and one min at 72 °C for extension), finally at 72 °C a step of a final extension was set at 5 min (El-Shenawy *et al.*, 2017). For the *dupA* gene: Initial denaturation step at 94 °C for 5 min, 35 cycles (including 30 sec for denaturation at 94 °C, 30 sec for annealing at 54 °C and 45 sec at 72 °C for extension), finally at 72 °C a step of a final extension was set at 5 min (Lu *et al.*, 2005). The primers used are listed in Table 1.

After amplification, five microliters of the product were electrophoresed in agarose gel (1.5%) with 0.5 g/mL ethidium bromide then photographed with a UV illuminator. Size markers of 100-bp and 50-bp DNA ladder were used. Negative control was included in each PCR test (DNA-free).

### Histopathological identification of *Helicobacter pylori*

To explore the inflammatory tissue changes associated with *H. pylori* infection, 290 gastric biopsies specimens were histologically analyzed. After being soaked in 10% formalin, the biopsy specimens were embedded in paraffin wax. The pathologist evaluated four micron-thick tissue portions of two slides that were prepared and stained using hematoxylin and eosin (H and E) stain as well as the modified Giemsa stain. The degree of mononuclear inflammatory cellular infiltrates, inflammatory activity (neutrophilic infiltrations) and the amount of bacterial colonization was assessed on a four-point scale using the criteria outlined in the revised Sydney system: Changes range from 0 to 3, with 0 being no, 1 being mild, 2 being moderate and 3 being severe (Hassan *et al.*, 2016; Domșa *et al.*, 2020; Taşçı and Akbaş, 2020).

### Statistical analysis

The data was analyzed using the IBM Statistical Package for Social Sciences (SPSS) computer program (version 20). The Chi-square test and Spearman's correlation test were used to compare categorical data. A *p*-value of less than 0.05 was considered significant.

## RESULTS

A total of two hundred and ninety stomach biopsy specimens were examined for the presence of *H. pylori*; one hundred and fifty-nine (54.8%) were male, one hundred and thirty-one (45.1%) were female. Moreover, one hundred and ninety-four patients (66.9%) had gastritis, twenty-nine (10%) had a gastric ulcer (GU), twenty-seven (9.3%) had a duodenal ulcer (DU), thirteen (4.5%) had esophagitis and twenty-seven (10%) had normal stomach mucosa, according to gastrointestinal endoscopic findings by a pathologist.

Histopathological evaluation of the gastric biopsies revealed 99 (34.1%) biopsy specimens with normal histology (grade 0). While 191 patients (65.9%) with mononuclear inflammatory cellular infiltrates. The intensity of this chronic inflammation was recorded as; 75 (25.9%) cases were G1 (Grade 1) gastritis, 68 (23.4%) were G2 (Grade 2) gastritis and 48 (16.6%) were G3 (Grade 3) gastritis (Figure 1).

The neutrophilic activity of inflammation was detected in 27 (9.3%) cases with mild activity, 14 (4.8%) cases with moderate activity and 10 (3.4%) cases with severe activity. *H. pylori* colonization was found in 103 (35.5%) specimens when H and E and modified Giemsa stained

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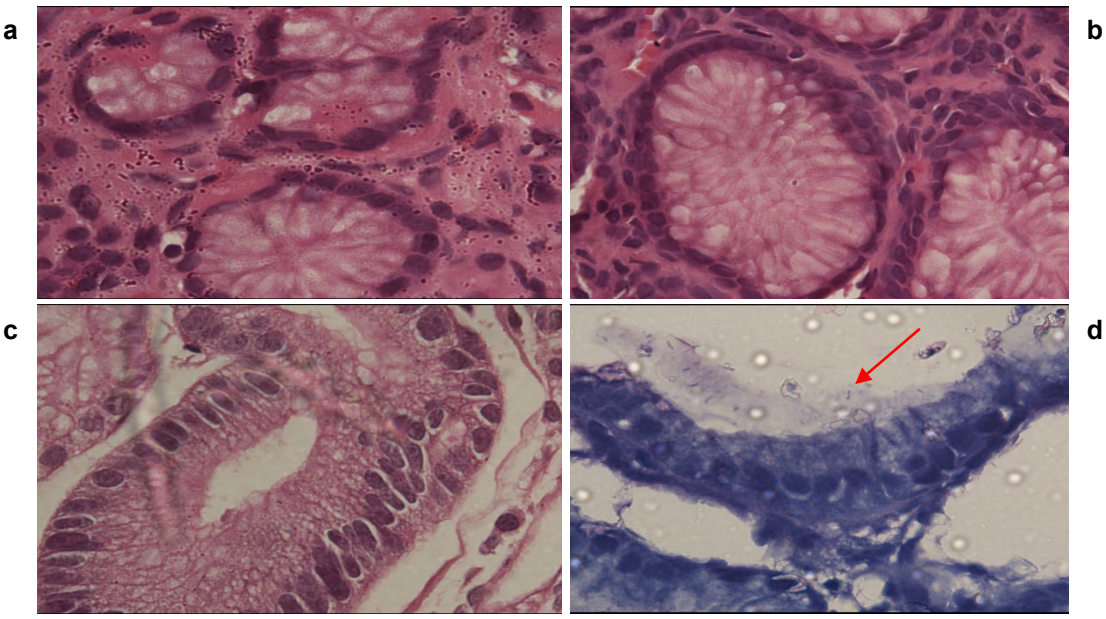


**Figure 1:** a) Mild inflammation detected by H and E stain. b) Moderate inflammation detected by H and E stain. c) Severe inflammation detected by H and E stain. d) *H. pylori* organism detected by modified Giemsa stain. The arrow indicates *H. pylori* curved bacterium at the luminal border of gastric mucous glands.

sections were examined (Figure 1).

With a significant *p*-value of 0.000, Spearman's correlation coefficient showed a significantly strong correlation between the degree of inflammation and *H. pylori* colonization ($r_s$=0.7). Inflammation activity and degree of *H. pylori* concentration also had a moderate positive correlation ($r_s$=0.6) with a significant *p*-value= 0.000.

**Prevalence and distribution of 16S rRNA, *cagA*, *cagE*, *vacA*, *dupA* and *iceA1* genes of *H. pylori***

PCR testing for the *Hp*16S rRNA gene from tissue biopsies showed that 88/290 (30.3%) were positive and 202/290 (69.7%) were negative (Figure 2). For the *cagA* gene, the results revealed that 40.9% (36/88) of tested *H. pylori* were *cagA* gene-positive, 59.1% (52/88) were *cagA* gene-negative. For the *cagE* gene, 38.6% (34/88) were *cagE* positive.

Multiple sets of primers targeted the *H. pylori vacA s1* and *s2* alleles of *s* (signal) regions and *H. pylori vacA m1*, *m2* alleles of *m* (middle) regions genes were used to survey the tested DNA for *vacA* status (genotyping). The *vacA s1* gene was detected in 68/88 (77.3%), while the *vacA s2* gene was found in 20/88 (22.7%); also, the *vacA m1* gene was detected in 71/88 (80.7%), while the *vacA m2* gene was found in 17/88 (19.3%) *H. pylori* strains. Sixty (68.2%) strains of *H. pylori* had *vacA s1/m1* gene, eight (9.1%) strains had *vacA s1/m2* gene, the *vacA s2/m1* was identified in eleven (12.5%) strains, while 9 (10.2%) of *H. pylori* strains had *vacA s2/m2* gene. The *dupA* genotype was detected in 44/88 (50.0%) samples, while the *iceA1* gene was detected in only 14 (15.9%) samples (Figure 2).

**Distribution of *H. pylori* virulence genes according to gender and age of the patients**

*Helicobacter pylori* virulence genes (*cagA*, *cagE*, *vacA*, *dupA* and *iceA1*) distribution according to demographic data (gender and age groups of participant) are shown in Table 2. According to patients' gender, the differences in the distribution of *H. pylori cagA*, *cagE*, *vacA* and *dupA* genes between males and females were not statistically significant, but the difference in the distribution of *H. pylori iceA1* gene there was statistically significant with *p*-value of 0.053.

The *vacA s1* gene of *H. pylori* was positive in 39.8% of males and 37.5% of females. While *vacA m1* gene of *H. pylori* was found in 43.2% of males and 37.5% females. Also, *vacA s1/m1* genotype was higher among males (36.4%), whereas in females, it was (31.8%). Among patient's age groups; the prevalence of *vacA s1* gene was greater (35.2%) in adults (30-49 years old), whereas it was decreased in age groups 14-29 years old and over 50 years old (20.5% and 21.6%, respectively). The *vacA m1* was higher (33.0%) in the adults (30-49 years old). Also, *vacA s1/m1* gene was higher among the patients' age group 30-49 years old (30.7%). The distribution of *H. pylori* virulence genes among patients' age groups was not statistically significant.


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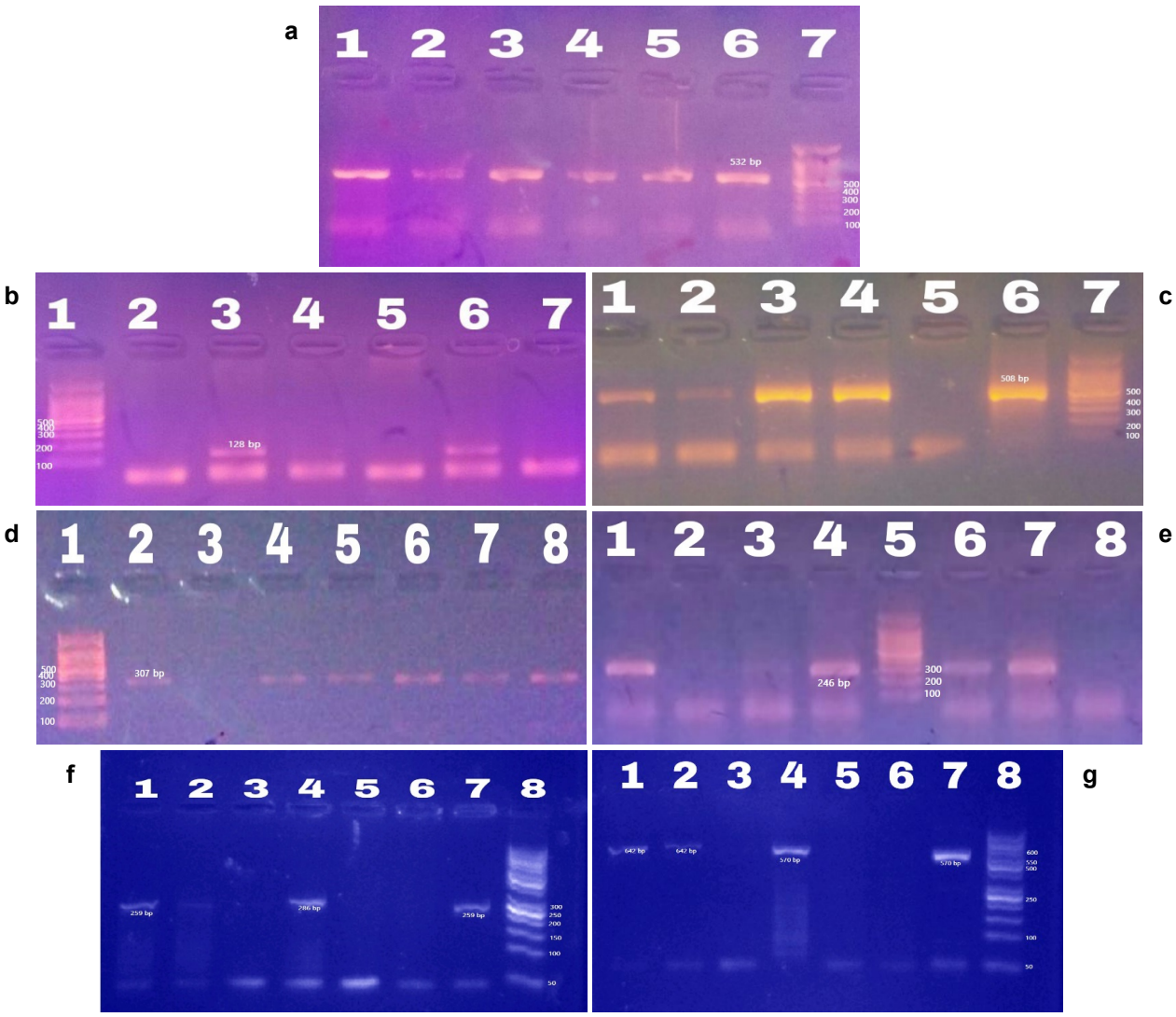


**Figure 2:** PCR amplification of *H. pylori* genes. a) *Hp*16S rRNA gene. Lane 7: Ladder with 100 bp, Lane 1: Positive control, Lanes 2-6 contains a product with 532 bp of positive samples. b) *cagA* gene. Lane 1: Ladder with 100 bp, Lanes 3 and 6 contain positive samples with 128 bp. c) *cagE* gene. Lane 7: Ladder with 100 bp, Lanes 1-4 and 6 contain amplicon of *cagE* gene with 508 bp. d) *dupA* gene. Lane 1: Ladder with 100 bp, Lanes 2, 4-8 contains amplicon of positive samples with 307 bp. e) *iceA1* gene. Lane 5: Ladder with 100 bp, Lanes 1, 4, 6 and 7 contain amplicon of *iceA1* with 246 bp. f) *vacA s* gene. Lane 8: Ladder with 50 bp, Lanes 1 and 7 contain amplicons of *vacA s1* with 259 bp, Lane 4 contains amplicons of *vacA s2* with 286 bp. g) *vacA m* gene. Lane 8: Ladder with 50 bp, Lanes 4 and 7 contain positive sample with *vacA m1* (570 bp), Lanes 1 and 2 contain positive samples with *vacA m2* (642 bp).

**Association of *H. pylori* virulence genes with clinical outcomes and histopathological changes (degree of inflammation)**

A physician performed an endoscopic examination for all patients to evaluate the clinical outcomes, which were classified as normal gastric mucosa, gastritis, GU, DU and esophagitis, while the histological alterations (degree of inflammation) were determined through microscopy (histopathological examination) of stained slides and then were classified into; normal histology, mild inflammation, moderate inflammation or severe inflammation. The authors demonstrate the link between clinical outcomes (endoscopic findings) and histological investigation results and *H. pylori* virulence genes in (Table 3 and Figure 3).

According to endoscopic findings, there was no significant association of *H. pylori* virulence genes (*cagA*, *cagE*, *iceA1* and *vacA s/m* genes) with *p*-values of 0.342, 0.821, 0.901 and 0.554, respectively). The *dupA* gene was significantly associated with the clinical outcome with a *p*-value of 0.017. Regarding the histopathological examination findings (degree of inflammation) of all

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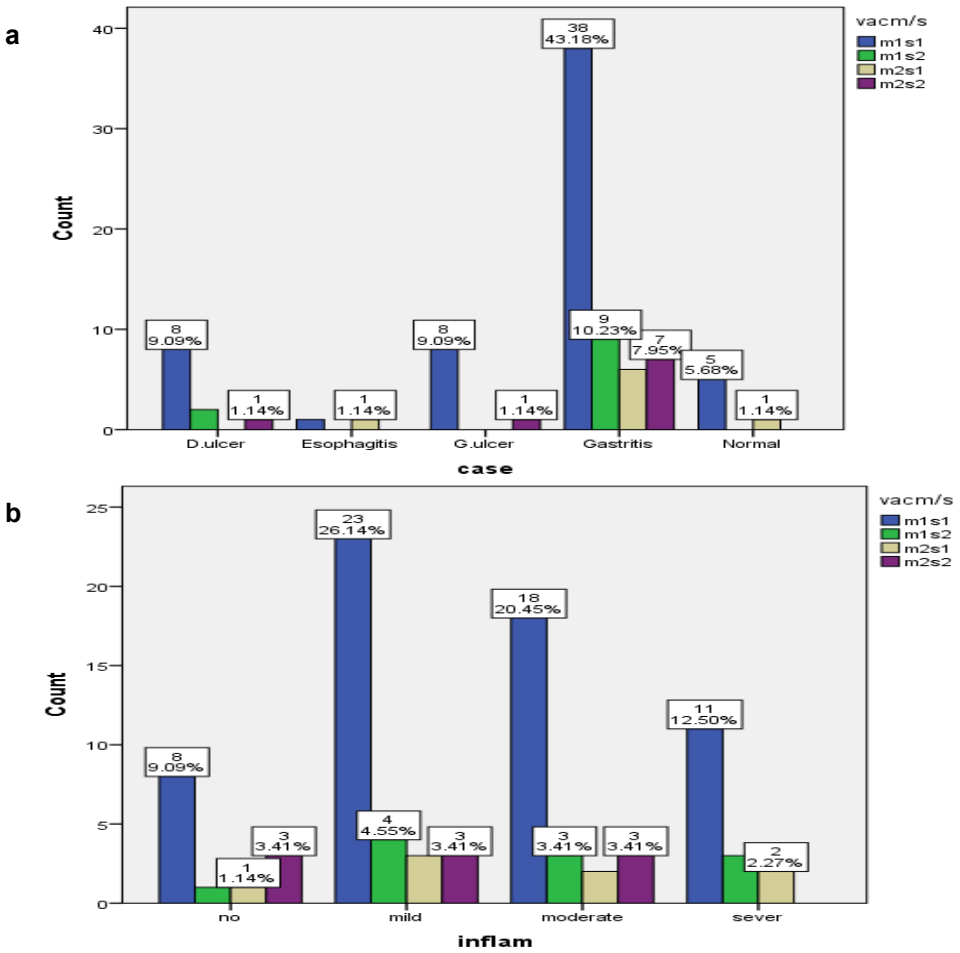


**Figure 3:** *Helicobacter pylori vacA* subtypes in association with a) Clinical outcomes (endoscopic examinations) and b) Degree of inflammation (histopathological examination of stained slides).

**Table 2:** Distribution of *H. pylori* virulence genes according to gender and age of the patients.

| Genotype/allele | | Gender | | *p*-value | Age group | | | *p*-value |
|---|---|---|---|---|---|---|---|---|
| | | Male N (%) | Female N (%) | | 14-29 | 30-49 | 50 and older | |
| *vacA* | *s1/m1* | 32 (36.4) | 28 (31.8) | 0.852 | 16 (18.2) | 27 (30.7) | 17 (19.3) | 0.786 |
| | *s1/m2* | 3 (3.4) | 5 (5.7) | | 2 (2.3) | 4 (4.5) | 2 (2.3) | |
| | *s/2m1* | 6 (6.8) | 5 (5.7) | | 4 (4.5) | 2 (2.3) | 5 (5.7) | |
| | *s2/m2* | 5 (5.7) | 4 (4.5) | | 2 (2.3) | 4 (4.5) | 3 (3.4) | |
| | *s1* | 35 (39.8) | 33 (37.5) | 0.781 | 18 (20.5) | 31 (35.2) | 19 (21.6) | 0.428 |
| | *s2* | 11 (12.5) | 9 (10.2) | | 6 (6.8) | 6 (6.8) | 8 (9.1) | |
| | *m1* | 38 (43.2) | 33 (37.5) | 0.632 | 20 (22.7) | 29 (33.0) | 22 (25.0) | 0.885 |
| | *m2* | 8 (9.1) | 9 (10.2) | | 4 (4.5) | 8 (9.1) | 5 (5.7) | |
| *cagA* | +ve | 21 (23.9) | 15 (17.0) | 0.344 | 11 (12.5) | 16 (18.2) | 9 (10.2) | 0.617 |
| | -ve | 25 (28.4) | 27 (30.7) | | 13 (14.8) | 21 (23.9) | 18 (20.5) | |
| *cagE* | +ve | 21 (23.9) | 13 (14.8) | 0.157 | 9 (10.2) | 16 (18.2) | 9 (10.2) | 0.717 |
| | -ve | 25 (28.4) | 29 (33.0) | | 15 (17.0) | 21 (23.9) | 18 (20.5) | |
| *dupA* | +ve | 22 (25.0) | 22 (25.0) | 0.669 | 13 (14.8) | 14 (15.9) | 17 (19.3) | 0.124 |
| | -ve | 24 (27.3) | 20 (22.7) | | 11 (12.5) | 23 (26.1) | 10 (11.4) | |
| *iceA1* | +ve | 4 (4.5) | 10 (11.4) | 0.053 | 6 (6.8) | 6 (6.8) | 2 (2.3) | 0.229 |
| | -ve | 42 (47.7) | 32 (36.4) | | 18 (20.5) | 31 (35.2) | 25 (28.4) | |
| Total | | 46 (52.3) | 42 (47.7) | | 24 (27.3) | 37 (42.0) | 27 (30.7) | |


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**Table 3:** Association of *H. pylori* virulence genes with clinical outcomes and histopathological changes (degree of inflammation).

| | *cagA* status N (%) | | | *cagE* status N (%) | | | *dupA* N (%) | | | *iceA1* N (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | +ve | -ve | *p*-value | +ve | -ve | *p*-value | +ve | -ve | *p*-value | +ve | -ve | *p*-value |
| Endoscopic findings | | | | | | | | | | | | |
| Normal | 2 (2.3) | 4 (4.5) | 0.342 | 3 (3.4) | 3 (3.4) | 0.821 | 5 (5.7) | 1(1.1) | 0.017 | 1 (1.1) | 5 (5.7) | 0.901 |
| Gastritis | 26 (29.5) | 34 (38.6) | | 24 (27.3) | 36 (40.9) | | 24 (27.3) | 36 (40.9) | | 10 (11.4) | 50 (56.8) | |
| G. ulcer | 1 (1.1) | 8 (9.1) | | 2 (2.3) | 7 (8.0) | | 7 (8.0) | 2 (2.3) | | 2 (2.3) | 7 (8.0) | |
| Esophagitis | 1 (1.1) | 1 (1.1) | | 1 (1.1) | 1 (1.1) | | 0 (0.0) | 2 (2.3) | | 0 (0.0) | 2 (2.3) | |
| D. ulcer | 6 (6.8) | 5 (5.7) | | 4 (4.5) | 7 (8.0) | | 8 (9.1) | 3 (3.4) | | 1 (1.1) | 10 (11.4) | |
| Histopathological changes | | | | | | | | | | | | |
| Grade 0 | 7 (8.0) | 6 (6.8) | 0.020 | 5 (5.7) | 8 (9.1) | 0.382 | 7 (8.0) | 6 (6.8) | | 0 (0.0) | 13 (14.8) | 0.006 |
| Grade 1 | 8 (9.1) | 25 (28.4) | | 10 (11.4) | 23 (26.1) | | 13 (14.8) | 20 (22.7) | | 4 (4.5) | 29 (33.0) | |
| Grade 2 | 10 (11.4) | 16 (18.2) | | 10 (11.4) | 16 (18.2) | | 18 (20.5) | 8 (9.1) | 0.093 | 3 (3.4) | 23 (26.1) | |
| Grade 3 | 11 (12.5) | 5(5.7) | | 9 (10.2) | 7 (8.0) | | 6 (6.8) | 10 (11.4) | | 7 (8.0) | 9 (10.2) | |
| Total | 36 (40.9) | 52 (59.1) | | 34 (38.6) | 54 (61.4) | | 44 (50.0) | 44 (50.0) | | 14 (15.9) | 74 (84.1) | |

slides, there was a statistically significant association with *H. pylori cagA* and *iceA1* genes with *p*-value=0.020 and 0.006, respectively. But *cagE*, *dupA* and *vacA s/m* genotype of *H. pylori* showed no statistically significant association (*p*-value=0.382, 0.093 and 0.837, respectively).

## DISCUSSION

Several diagnostic approaches for *Helicobacter* infections have been proposed. The availability of resources, the sample population, the status of the patients and the investigator's competence or experience are usually used to select an appropriate diagnostic approach (Suerbaum and Josenhans, 2007). There is a lot of genomic and allelic variation in the *Helicobacter* bacterium. This unique property allows the bacterium to actively participate in various gastrointestinal problems in infected people all over the world (Suerbaum and Josenhans, 2007).

Among 290 cases, 40.9% were positive for *cagA* gene, 50.0% for *dupA*, 38.6% for *cagE* gene and 15.9% for *iceA1* gene. Previously, similar data for *cagA* gene-positive (45.9%) were reported in Egypt (El-Khlousy *et al.*, 2016). Also in Saudi Arabia the prevalence of *H. pylori* virulence genes (*cagA*, *vacA*, *iceA1* and *iceA2*) were 49.2%, 100%, 42.2% and 32.8%, respectively (Akeel *et al.*, 2019). According to a recent South African study, *cagA* 62% (145/234) and *dupA* 53.4% (125/234) were detected in most *H. pylori* strains (Idowu *et al.*, 2019). Another study reported that the distribution of virulence genes in 160 *H. pylori* strains were 69% *cagA,* 51% *cagE* and 26% *iceA1* genotypes (Dabiri *et al.*, 2017).

The *vacA* genotype was found in all of the isolates in our research (100%). Dabiri *et al*. (2017) and Akeel *et al*. (2019) revealed a 100% *vacA* detection rate in Iran and SouthWestern Saudi Arabia, respectively, which was similar to our findings. Also, a study was conducted in Morocco to detect the distribution of *H. pylori vacA* gene, which was determined to be 99% of isolated strains (El Khadir *et al.*, 2017). El Khadir *et al.* (2017) and Essawi *et al.* (2013) reported that low rates of *H. pylori vacA* genotype distribution were observed in Ethiopia, Netherlands and Palestine (90, 93%, and 43.2%, respectively).

The most common *vacA* subtypes in the current investigation were *s1/m1* (68.2%), followed by *s2/m1* (12.5%), *s2/m2* (10.2%) and *s1/m2* (9.1%). Similar to our finding, many authors reported that *vacA* gene subtype *s1/m1* is the most predominant *vacA* genotype (Basso *et al.*, 2008; Román-Román *et al.*, 2017; Sallas *et al*., 2017; Idowu *et al.*, 2019). In addition, most investigations reported that *H. pylori* strain with *vacA s1/m2* subtype was the most common and prevalent genotype (Marie, 2012; Essawi *et al.*, 2013; Keikha *et al.*, 2020). Many researchers have found a


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significant association between the pathogenicity of the disease caused by *H. pylori* and vacuolating toxin activity. The highest toxin activity is caused by *vacA s1/m1* genotype, the moderately toxin activity caused by *vacA s1/m2* genotype, while the lowest toxin activity is caused by *vacA s2/m2* genotype (El-Khlousy *et al.*, 2016). Numerous studies reported that *H. pylori* strains with *vacA s1/m1* subtype was present in 24-84 percent of people worldwide (Kim *et al.*, 2001; Akeel *et al.*, 2019). In this study, we found that *H. pylori cagA* gene was found in 30.7% of the *H. pylori* strains with the *vacA s1/m1* genotype. This relationship has previously been observed in other investigations (Marie, 2012). This variation in genotypes prevalence rates could be related to differences in the study population, ages and sample collection site (Akeel *et al.*, 2019).

Several researchers around the world reported that the degree of gastric mucosa inflammation was influenced by the presence of *cagA* gene in *H. pylori* strains. Furthermore, the *iceA1* gene was associated with increased stomach inflammation, which may lead to GU and GC (Ladeira *et al.*, 2004). These findings were in agreement with our findings, as we discovered a statistically significant relationship between the presence of *cagA* and *iceA1* genes in *H. pylori* strains and the degree of stomach inflammation (*p*-value=0.020 and 0.006, respectively). A study conducted in Northern Brazil and the Middle East observed that; *H. pylori* strains that carry *cagA* and *vacA s1/m1* genes were associated with increased stomach inflammation, the degree of neutrophilic activity and the occurrence of intestinal metaplasia (Siddique *et al.*, 2014; Vinagre *et al.*, 2015). According to Akeel *et al*. (2009), *iceA* genes and *vacA* subtypes were associated with histological changes.

However, multiple investigations in Seoul, Korea, found that *H. pylori* strain carrying the *cagA*, *iceA* and *vacA* genes were not associated with a higher degree of stomach inflammation (Ko *et al.*, 2008). Investigations conducted in Brazil, as well as in South-eastern Europe, found that *iceA1* was not linked to a greater level of inflammation (Ashour *et al.*, 2001; Ribeiro *et al.*, 2003; Homan *et al.*, 2009).

According to this study, only *H. pylori dupA* gene showed statistical significance (*p*-value=0.017) with endoscopic results (clinical outcome). Furthermore, several researchers from all around the world have found a link between the existence of *H. pylori* strains that carry *dupA* gene and disease outcomes (Lu *et al*., 2005; Argent *et al.*, 2007; Hussein, 2010). The severity of gastritis and clinical consequences may be influenced by many factors, i.e., host factors and/or environmental factors (Akeel *et al.*, 2019). A study conducted by Akeel *et al*. (2019) reported that *H. pylori cagA* gene was not associated with clinical outcomes of disease, which agrees with our findings. Also, our results contrast with those of a study in Saudi Arabia which reported that *H. pylori cagA* and *vacA* gene were associated with disease outcomes (Marie, 2012). Akeel *et al*. (2019) also revealed that *iceA* genes and *vacA* subtypes were linked to clinical outcomes. In our investigation, the relationship between endoscopic examination of patients' gastric mucosa and histopathological examination of stained sections was statistically significant (*p*-value=0.000). This finding harmonized with a similar study conducted in Saudi Arabia (Hassan *et al.*, 2016).

## CONCLUSION

*Helicobacter pylori* virulence genes were highly prevalent and more diverse among Sudanese gastritis patients. *Helicobacter pylori* strains that carry *cagA* and *iceA1* genes were significantly associated with histopathological changes in gastric mucosa. Regarding clinical outcomes of the disease caused by *H. pylori*, there was a significant association with *H. pylori* strains that carried *dupA* gene.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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