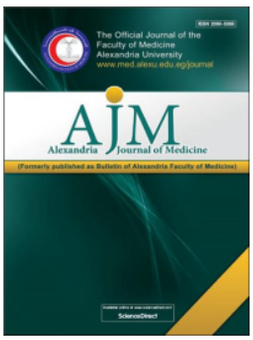

Alexandria Journal of Medicine

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tajm20

# Detection of *Helicobacter pylori oipA* and *dupA* genes among dyspeptic patients with chronic gastritis

**Marwa Shabban El-Sayed, Nevine Musa, Mohamed Eltabbakh, Dalia Hosni Abdelhamid, Shimaa Mostafa Ismail Mostafa, Manar Mohamed Salah, Heba Ahmed Faheem Faheem & Rania Ahmed Hassan**

**To cite this article:** Marwa Shabban El-Sayed, Nevine Musa, Mohamed Eltabbakh, Dalia Hosni Abdelhamid, Shimaa Mostafa Ismail Mostafa, Manar Mohamed Salah, Heba Ahmed Faheem Faheem & Rania Ahmed Hassan (2020) Detection of *Helicobacter pylori oipA* and *dupA* genes among dyspeptic patients with chronic gastritis, Alexandria Journal of Medicine, 56:1, 105-110, DOI: 10.1080/20905068.2020.1780675

**To link to this article:** https://doi.org/10.1080/20905068.2020.1780675



Published online: 30 Jun 2020.

ALEXANDRIA JOURNAL OF MEDICINE
2020, VOL. 56, NO. 1, 105–110
https://doi.org/10.1080/20905068.2020.1780675



# Detection of *Helicobacter pylori oipA* and *dupA* genes among dyspeptic patients with chronic gastritis

Marwa Shabban El-Sayed[a], Nevine Musa[b], Mohamed Eltabbakh[c], Dalia Hosni Abdelhamid[d], Shimaa Mostafa Ismail Mostafa[d], Manar Mohamed Salah[c], Heba Ahmed Faheem Faheem[b] and Rania Ahmed Hassan[a]

[a]Department of Medical Microbiology and Immunology, Faculty of Medicine-Ain Sham University, Cairo, Egypt; [b]Department of Internal Medicine, Faculty of Medicine-Ain Sham University, Cairo, Egypt; [c]Department of Tropical Medicine, Faculty of Medicine-Ain Sham University, Cairo, Egypt; [d]Department of Clinical Pathology, Faculty of Medicine-Ain Sham University, Cairo, Egypt

**ABSTRACT**

**Helicobacter pylori (H. pylori):** is a microbe with wide genetic diversity that infects the stomach of most people in developing countries, leading to several clinical outcomes among different individuals such as gastritis, ulcers, or gastric cancer. Outer inflammatory protein A (*oipA*) and duodenal ulcer promoting (*dupA*) genes are among the possible virulence factors which determine the patient outcome.
**Aim:** To detect *oipA and dupA* genes of *H. pylori* among dyspeptic Egyptian patients, and to investigate their correlation with the varying degrees of the associated chronic gastritis.
**Methods:** The study enrolled 50 patients with dyspepsia, attending the Gastrointestinal Endoscopy unit of the Gastroenterology and Tropical Departments at Ain Shams University Hospital for upper gastrointestinal endoscopy, in the period between, June and, December 2019. Four antral gastric biopsies were taken from each patient for polymerase chain reaction assay to detect the virulence genes *oipA, dupA*, and *cagA* and for histopathological assessment.
**Results:** Forty patients were *H. pylori* positive by histopathology and PCR. *cagA, oipA, and dupA* were identified in 6 (15%), 13 (32.5%), 9 (22.5%) of biopsies, respectively. Both *cagA and oipA* genes were highly significantly associated with increasing the severity of gastritis. Only *oipA* virulence gene showed a highly significant association with gastroduodenitis. There was a highly significant moderate association between *cagA and oipA* genes.
**Conclusion:** *oipA* could be a virulence biomarker that serves a great value in predicting the progress of gastric mucosal damage in patients with chronic gastritis, and targeting antimicrobial therapy in those patients to prevent severe gastroduodenal diseases.

**ARTICLE HISTORY**
Received 31 March 2020
Accepted 3 June 2020

**KEYWORDS**
Chronic gastritis; *H. pylori*; *oipA* gene; *dupA* gene

## 1. Introduction

*H. pylori* colonizes the stomach of around 50% of the world's population, leading to acute and chronic gastritis, gastric, and duodenal ulcers when patients are not treated appropriately [1]. It has been classified by the World Health Organization as Class I Carcinogen and a predisposing factor for gastric cancer, which is recognized worldwide as the second among cancers causing deaths [2].

Variable clinical outcomes among different individuals can be attributed to host, environmental, dietary factors, as well as virulence factors of different strains [3]. Certain virulence factors possessed by strains might be recognized as a selective advantage for these strains, and a biomarker that aids in predicting the outcome of infection and preventing the resulting damage to gastric mucosa [4].

The cytotoxin-associated gene (*cagA*) exists in nearly half of *H. pylori* strains and is a common marker for the presence of the cag-pathogenicity island [5]. The strains positive for this gene are frequently associated with the pathogenesis of gastric cancer as a result of excessive mucosal inflammation and production of interleukin-8 [6].

Outer inflammatory protein A (*oipA*) is one of the outer membrane proteins, its presence results in a higher probability of gastric cancer and peptic ulcers than gastritis and functional dyspepsia [7]. The possible mechanism of action of *oipA* is through accomplishing *H. pylori* adherence, potentiating the activity of the cag-pathogenicity island, as well as inducing proinflammatory immune responses [8].

The duodenal ulcer promoting gene (*dupA*) can result in duodenal ulceration and/or reduction of risk of gastric cancer in some populations. The *dupA* protein induces the secretion of IL-8 and −12 by antral gastric mucosa in vivo as well as by gastric epithelial cells in vitro [9]. In addition, a significant association was observed with treatment failure [10].

In Egypt, previous studies were conducted to determine some virulence genes such as *cagA, vac A, iceA1, and babA2* and assess their relation to clinical presentation [11–13]; however, no enough reports are available about *oipA* and *dupA* genes. Therefore, this study aimed to detect *oipA and dupA* genes among dyspeptic Egyptian patients and to investigate their correlation with the varying degrees of the associated chronic gastritis.

**CONTACT** Rania Ahmed Hassan rania_ahmedhasan@yahoo.com; rania_ahmedhasan@med.asu.edu.eg Department of Medical Microbiology and Immunology, Faculty of Medicine-Ain Sham University, Cairo, Egypt

## 2. Materials and methods

### 2.1. Study population

The present cross-sectional study was conducted on 50 dyspeptic patients, referred for upper gastro-duodenoscopy at the Gastrointestinal Endoscopy unit of the Gastroenterology and Tropical Departments at Ain Shams University Hospital, in the period between, June and, December 2019. Patients were selected according to the following criteria: being more than 18 years, of either sex, suffering from various dyspepsia symptoms, e.g. nausea, vomiting, epigastric pain, heartburn, and hematemesis, and not responding to proton pump inhibitors. Patients were excluded from the study if they received nonsteroidal anti-inflammatory drugs, H2 receptor antagonists, proton pump inhibitors, or antibiotics 2 to 4 weeks before the procedure, also if they suffered inflammation caused by other bacteria or GIT cancers.

### 2.2. Specimen collection and processing

Four antral gastric biopsies were taken from each patient, divided into two tubes. One of them contained 0.9% of sterile saline solution and was stored in −80°C for DNA extraction and polymerase chain reaction (PCR) for detection of *H. pylori* and the virulence genes *cagA, oipA and dupA*. The other one was sent for histopathological assessment. An informed consent was taken from the patients or their relatives for sample collection, according to the regulations of the ethical committee of scientific research (Faculty of Medicine – Ain Shams University). Diagnosis of *H. pylori* was done according to histopathological findings and then confirmed by PCR amplification of the *ureC* gene in gastric biopsies.

### 2.3. Histopathological examination of H. pylori

Pathologic sections stained with Hematoxylin-Eosin were examined and assessed for the following criteria according to updated Sydney Classification: presence of *H. pylori*, degree of gastritis, and presence of gastric atrophy, intestinal metaplasia, dysplasia, or malignancy [14].

### 2.4. DNA extraction and H. pylori detection

DNA was extracted from biopsy specimens, using Qiagen DNA tissue kit (Qiagen, Germany) according to the manufacturer's instructions. Eluted DNA was stored at −20°C until used. Samples positive for *H. pylori* by histopathology were examined for the presence of *ureC* gene by PCR assay according to Kargar et al. [15].

The target genes *cagA, oipA*, and *dupA* were analyzed through PCR, using one set of oligonucleotides for each gene fragment. Table 1 summarizes the primers' sequences, PCR conditions, and amplicon sizes according to Oktem-Okullu et al. [16]. DNA samples from *H. pylori* strain D0008 (Genekam, Germany) were used as a positive control, and sterile distilled water was used as a negative control. Detection of amplicons was done by gel electrophoresis with ethidium bromide 2% agarose gel.

### 2.5. Statistical analysis

All statistical procedures were carried out using SPSS version 20 for Windows (SPSS Inc, Chicago, IL, USA). Fisher's exact test was used to examine the relationship between *H. pylori* genotypes and disease severity. Kappa statistics was determined to measure the degree of agreement between two genes.

## 3. Results

Among 50 dyspeptic patients, *H. pylori* was detected in 40 (80%) antral gastric biopsies by histopathological assessment and by PCR amplification of the *ureC* gene. *H. pylori* infected patients were 19 (47.5%) males and 21 (52.2%) females with mean age ±SD (36.20 ± 13.78).

Based on the histopathological examination, the enrolled *H. pylori* positive patients had varying degrees of non-ulcerative chronic gastritis, 22 (55%) had mild gastritis, 13 (32.5%), and 5 (12.5%) showed moderate and severe gastritis, respectively. Gastroduodenitis was observed in 8 (20%) cases. No ulcerative or neoplastic lesions were diagnosed in our patient group. The main complaint was epigastric pain (55%) followed by heartburn and hematemesis (20%).

### 3.1. Detection of virulence genes and their relation to histopathological intensity of gastritis

The virulence genes were recognized in the gastric biopsy samples with different rates and their PCR products are shown in Figures 1–3. Tables 2 & 3 summarize the relation between the target genes and histopathological intensity of gastritis as well as gastroduodenitis among the

**Table 1.** Primers' sequences, PCR conditions, and amplicon sizes for detection of target genes.

| Genes | Primers | Primers' sequence | PCR conditions | Amplicon size |
|---|---|---|---|---|
| *ureC* | ureC-F | AAGCTTTTAGGGGTGTTAGGGGTTT | 35 cycles at 39°C for 1 min, 55°C for 1 min, and 72°C for 1 min | 295 bp |
| | ureC-R | AAGCTTATTTCTAACGC | | |
| *cagA* | cagA-F | AGAGCAAGCGTTAGCCGATCTCAA | • Initial denaturation at 95°C, for 3 min followed by 45 cycles of: | 415 bp |
| | cagA-R | TTTCCCTACACCACCCAAACCACT | • Denaturation at 95°C, for 45 sec | |
| *dupA* | dupA-F | TGAGCGTGGTAGCTCTTGAC | • Annealing at 60°C, for 45 sec | 584 bp |
| | dupA-R | GAGCGCGTTAGCGATATAGG | • Extension at 72°C, for 2 min | |
| *oipA* | oipA-F | GTTTTTGATGCATGGGATTT | | 401 bp |
| | oipA-R | GTGCATCTCTTATGGCTTT | | |

studied patients. *CagA* gene was identified in 6(15%) cases. Its presence was highly significantly associated with increasing intensity of gastritis (p = 0.001); as it was positive in 4 out of 5 cases of severe gastritis (80%), as well as 2 out of 13 cases of moderate gastritis.

The *oipA* gene was found in 13 (32.5%) cases. This gene was highly significantly associated with increasing intensity of gastritis (p = 0.001); as it was positive in all cases of severe gastritis (100%) and 8 (61.5%) out of 13 cases of moderate gastritis. However, both *cagA and oipA* genes were not detected in any case of mild gastritis. Also, *OipA* gene showed a highly significant association with gastroduodenitis being positive in 6(75%) out of 8 cases.

The *dupA* gene was positive in 9 (22.5%) cases distributed as; 3 mild cases, 3 moderate, as well as 3 cases of severe gastritis. However, no significant association was observed for *dupA* and intensity of gastritis. Neither the presence of *cagA* nor *dupA* genes had a statistically significant association with gastroduodenitis.

### 3.2. *Association between* cagA, oipA, *and* dupA *genes*

Table 4 shows there was a highly significant moderate association between *cagA and oipA* genes (kappa = 0.536); *oipA* was present in all *cagA* positive cases. On the other hand, *oipA* was present in 7 cases out of 34 *cagA* negative cases (20.6%). There was a non-significant fair association (kappa = 0.268) between both *cagA and dupA*, and non-significant slight association between *dupA and oipA* (kappa = 0.133).

## 4. Discussion

All patients participated in this study suffered from varying degree of chronic gastritis; 87.5% had mild and moderate degree, while 12.5% had severe degree of gastritis. Nearly similar to our results, Diab et al. [3] in their study reported the predominance of mild and moderate gastritis (82.4%) over severe degree (17.7%).

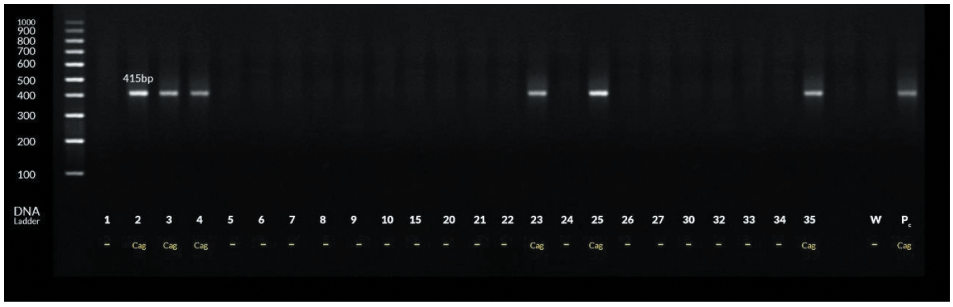


**Figure 1.** PCR products for *H. pylori* with *cagA* gene-based primers. Lanes 2–4 and 23, 25 and 35 are patients' positive biopsy samples. Lanes Pc & W are positive (strain D0008) & negative (sterile distilled water) control, respectively.

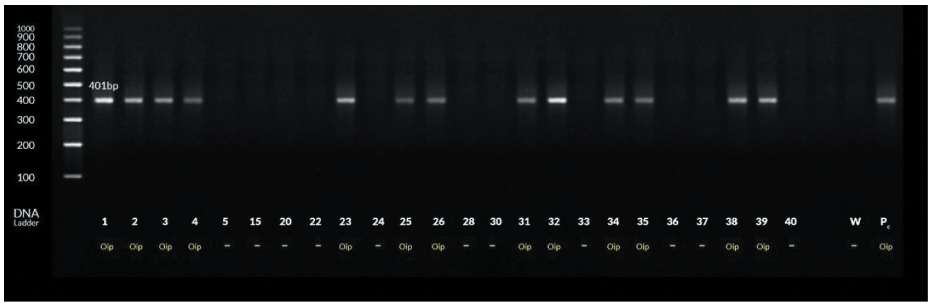


**Figure 2.** PCR products for *H. pylori* with *oipA* gene-based primers. Lanes 1–4 and 23, 25–26, 31–32, 34–35 and 38–39 are patients' positive biopsy samples. Lanes Pc & W are positive (strain D0008) & negative (sterile distilled water) control, respectively.


**Figure 3.** PCR products for *H. pylori* with *dupA* gene-based primers. Lanes 1–4 and 15, 20 and 24–26 are patients' positive biopsy samples. Lanes Pc & W are positive (strain D0008) & negative (sterile distilled water) control, respectively.

**Table 2.** Relation between studied virulence genes and histopathological intensity of gastritis among cases.

| | | Intensity of gastritis | | | | |
|---|---|---|---|---|---|---|
| | | Mild (22 cases) | Moderate (13 cases) | Severe (5 cases) | | |
| | | N (%) | N (%) | N (%) | P | Sig |
| *cagA* | Negative | 22 (100%) | 11 (84.6%) | 1 (20%) | 0.001* | HS |
| | Positive | 0 (0%) | 2 (15.4%) | 4 (80%) | | |
| *oipA* | Negative | 22 (100%) | 5 (38.5%) | 0 (0%) | 0.001* | HS |
| | Positive | 0 (0%) | 8 (61.5%) | 5 (100%) | | |
| *dupA* | Negative | 19 (86.4%) | 10 (76.9%) | 2 (40%) | 0.108* | NS |
| | Positive | 3 (13.6%) | 3 (23.1%) | 3 (60%) | | |

*Fisher's exact test, HS = highly significant, NS = non-significant.

**Table 3.** Relation between studied genes and gastroduodenitis among cases.

| | | Gastroduodenitis (8 cases) | | | |
|---|---|---|---|---|---|
| | | Negative | Positive | | |
| | | N (%) | N (%) | P | Sig |
| *cagA* | Negative | 29 (90.6%) | 5 (62.5%) | 0.082* | NS |
| | Positive | 3 (9.4%) | 3 (37.5%) | | |
| *oipA* | Negative | 25 (78.1%) | 2 (25%) | **0.008*** | **HS** |
| | Positive | 7 (21.9%) | 6 (75%) | | |
| *dupA* | Negative | 25 (78.1%) | 6 (75%) | 1.0* | NS |
| | Positive | 7 (21.9%) | 2 (25%) | | |

*Fisher's exact test

The *cagA* gene was identified in 15% of the cases; 4 cases with severe gastritis and 2 cases with moderate gastritis; however, the gene did not exist in any of mild gastritis cases. Our results agree with those reported by Ezzat et al. [17], they detected *cagA* gene in 13.3% of gastritis patients. Similarly, El Shenawy et al. [13] and Diab et al. [18] found the gene in 18.2% and 18.5% of *H. pylori* strains isolated from gastritis cases, respectively. Other studies reported prevalence about 33–38% [10,12,19]. The variation between different studies could be explained by different number of participants in addition to their living conditions and socioeconomic status [10]. We observed a highly significant association of *cagA* gene with increasing the severity of gastritis but not with the presence of gastroduodenitis. Our study is consistent with previous studies [3,10]. Others [19,20] found no statistically significant association between *cagA* genotype and gastroduodenal diseases.

In the present study *oipA* gene was detected in 32.5% of the cases. A higher prevalence of the gene 52.6% and 57% was found by Saeidi et al. [21] and Dadashzadeh [9], respectively. We found a highly significant association between the *oipA* gene and the intensity of gastritis and presence of gastroduodenitis; being positive in all cases and 61.5%, of severe and moderate gastritis, respectively, as well as 75% of patients with gastroduodenitis. Similar results were reported by Ben Mansour et al. [22] in Tunisia, Souod et al. [23] in the west of Iran, and Sallas et al. [24] in Brazil. Other studies reported no correlation between *oipA* and disease outcome or increased gastroduodenal damage [25,26].

In this work, *dupA* gene was found positive in 22.5% of cases. The prevalence of *dupA* ranged from 6% to 92% as reported by different studies around the world [27]. Our results are consistent with Lu et al. [28], they detected the gene in 21% of the gastritis cases from East Asia and South America, and its presence was associated with more density of neutrophil infiltrate and IL-8 levels. Also, Arachchi et al. [29] showed that *dupA* was present in 22.8% of dyspepsia patients, from north India. A higher prevalence was reported by Nguyen et al. [30], who found 29.5% of *H. pylori* strains isolated from chronic gastritis patients in Japan were positive for this gene. Lower prevalence 12.2% was reported in southeast India [31], 18.8% in Northern Iraq [32], and 13.6% in Okinawan subpopulation, Japan [33]. Differences in results reported by mentioned studies may result from the characteristics of studied dyspeptic population, in addition to regional variability and study methods [9]. In the present study, no significant association was observed between *dupA* gene and gastroduodenal diseases. Our results agree with previous reports [30,34]. On the other hand, another systematic review confirmed the association of *dupA* with gastroduodenal diseases [35]. The conflicting reports of *dupA* association with disease outcomes might be attributed to the location of this gene in the plasticity region of *H. pylori* resulting in variability of its expression or the limitation of applied PCR technique to detect the different forms of the gene [36].

Regarding the association pattern between the *H. pylori* studied virulence genes; we found a highly significant moderate association between *cagA and oipA* genes. Previous studies showed a close association of both genes [37]. They may act in a synergistic manner regulating the signaling pathways that mediate inflammation [38]. On the other hand, we found a non-significant fair association between both *cagA and dupA*, and a non-significant slight association between *dupA and oipA genes*. Similar findings were reported by Zhang et al. [39], they showed that the presence of *dupA* was not associated with any other virulence factors *(cagA, vacA, iceA2, babA2)* for all patient groups enrolled in their study. Also, Matteo et al. [4] reported that *dupA and oipA genes* were present together in a limited number of strains. Other reports found a statistically significant relationship between the presence of *dupA* and *cagA* genes [24], as well as between *dupA* and *oipA* genes in isolated strains [9,24].

Several studies have evaluated the association between different virulence factors of *H. pylori* and clinical manifestations in Egyptian patients. To our knowledge, this study is

**Table 4.** Association between *cagA, oipA*, and *dupA* genes among cases.

| | | *cagA* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Negative | | Positive | | | |
| | | N | % | N | % | KAPPA | p (Sig) |
| *dupA* | Negative | 28 | 82.4% | 3 | 50.0% | 0.268 | 0.115 (NS) |
| | Positive | 6 | 17.6% | 3 | 50.0% | | |
| *oipA* | Negative | 27 | 79.4% | 0 | 0% | 0.536 | **0.001(HS)** |
| | Positive | 7 | 20.6% | 6 | 100% | | |
| | | *oipA* | | | | | |
| | | Negative | | Positive | | | |
| *dupA* | Negative | 22 | 81.5% | 9 | 69.2% | 0.133 | 0.437 (NS) |
| | Positive | 5 | 18.5% | 4 | 30.8% | | |

*Kappa statistics

the first to detect *oipA* and *dupA* genes among dyspeptic Egyptian patients infected with *H. pylori* and investigate their relationship with the degree of associated chronic gastritis. Our results highlight the role of *oipA* as a virulence biomarker and a candidate that could be used for future preparation of vaccines against such pathogen. The limitations of our work were the relatively small sample size of studied patients and they were all from a single endoscopy unit.

## 5. Conclusion

*OipA* could be a virulence biomarker that serves a great value in predicting the progress of gastric mucosal damage in patients with chronic gastritis and targeting antimicrobial therapy in those patients to prevent severe gastroduodenal diseases.

## 6. Recommendations

Extended large-scale studies over longer periods include patients with other *H. pylori* related complications such as peptic ulcer disease and gastric cancer are required to confirm these results and distinguish the role of *oipA and dupA genes* in the pathogenesis of *H. pylori* related diseases.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

## Notes on contributors

*Nevine Musa* is a Professor of Internal medicine, Ain Shams University Hospitals, Faculty of Medicine since 30/9/2019. Master of internal medicine in 2003(Ain Shams university). MD degree in Internal Medicine in 2009 (Ain Shams university).Specialist at Endoscopy unit Ain Shams university hospitals. JAG accredited from Hull University in 2012, UK. Master Colonoscopy Class from Hull University, 2014 UK. Professional Diploma in Video Capsule endoscopy, in 2016 from China.One of Committee team of VCE at Kafrawy unit at Endoscopy unit, Ain Shams university hospitals.Member of the Arab society of Infectious diseases and antimicrobials ASIDA since 2005.Member of the Egyptian society of endoscopy and hepatogastroenterology since 2004.Member of the Scientific Society of faculty of medicine, Ain Shams University. Participated in a number of national and international publications in the field of GIT and Hepatology.

*Mohamed Eltabbakh* is a Lecturer of Tropical Medicine, hepatology, gastroenterology and liver transplantation, Ain Shams University, Faculty of Medicine.Fellowship in Advanced Endoscopic Resection (EMR-POEM-ESD) at Zhongshan hospital-Shanghai-China.I am interested in advanced therapeutic endoscopic techniques. I have many research interests in the field of HCV, HBV, HCC, IBD, H.pylori , liver transplantation and advanced endoscopic techniques. I have many international publications in the last 3 years.

*Dalia Hosni Abdelhamid* is a Assistant professor of Clinical Pathology (subspecialty microbiology), Ain Shams Faculty of Medicine since 2017, MD degree in Clinical Pathology 2010 (Ain Shams university), Professional Diploma in Infection control, Jan 2014.

Member of The Society for practitioner of Infection Control Egypt (SPICE) and Egyptian Society of Laboratory Medicine (ESLM). Participated in a number of national and international publications. participating in several conferences , training courses and workshops.

*Shimaa Mostafa Ismail Mostafa* is a Lecturer of Clinical pathology, Faculty of Medicine, Ain Shams University since 2018, MD degree in Clinical pathology in 2018 (Ain Shams university). Clinical pathology consultant at main labs in Eldemerdash hospital and Ain Shams university specialized hospital.

Member of the Egyptian society of Laboratory Medicine. Member of quality control unit of clinical pathology department, Ain shams university. Participated in a number of national conferences in the field of Clinical and chemical pathology.

*Manar Mohamed Salah* is a Lecturer of Tropical Medicine, Gastroenterology and Liver Diseases Faculty of medicine – Ain shams universityConsultant of Hepatology, Gastroenterology & Liver transplantation at Ain Shams Center for Organ Transplantation" ASCOT".Member of ESLC, ETS& EASLGDGastrointestinal Endoscopy Consultant

*Heba Ahmed Faheem Faheem* is a Lecturer of Internal Medicine, Gastroenterology and Hepatology, Ain Shams University, Faculty of Medicine since 2017, MD degree in Internal Medicine in 2017 (Ain Shams university).

Member of ASCOT (Ain Shams Center for Organ Transplantation), and The Egyptian Society of Liver Disease. Participated in a number of national and international publications in the field of Internal Medicine, Hepatology and Gastroenterology

*Rania AhmedHassan* is a Assistant professor of Medical Microbiology and Immunology, Ain Shams Faculty of Medicine since 2016, MD degree in Medical Microbiology and Immunology in 2009 (Ain Shams university), Professional Diploma in Infection control, 2018.

Member of the Arab society of Infectious diseases and antimicrobials, the Egyptian Society of Virology, the Egyptian Society for Medical Microbiology, and the Clinical & Scientific Society of faculty of medicine, Ain Shams University. Participated in a number of national and international publications in the field of Medical Microbiology and Immunology

## ORCID

Nevine Musa http://orcid.org/0000-0001-9962-2894
Manar Mohamed Salah http://orcid.org/0000-0001-9909-4016

## References

[1] Malnick SD, Melzer E, Attali M, et al. Helicobacter pylori: friend or foe? World J Gastroenterol. 2014;20:8979–8985.
[2] Hooi JKY, Lai WY, Ng WK, et al. Global prevalence of Helicobacter pylori infection: systematic review and meta analysis. Gastroenterology. 2017;153(2):420–429.
[3] Diab M, Saad El-Dine S, Aboul-Fadl L, et al. *Helicobacter pylori cag* pathogenicity Island genes

among dyspeptic patients with chronic gastritis. Egypt J Med Microbiol. 2009;18(1):43–53.
[4] Matteo MJ, Armitano RI, Granados G, et al. Helicobacter pylori *oipA, vacA* and *dupA* genetic diversity in individual hosts. J Med Microbiol. 2010;59:89–95.
[5] Amer FA, El-Sokkary RH, Elahmady M, et al. Helicobacter pylori genotypes among patients in a university hospital in Egypt: identifying the determinants of disease severity. J Microbiol Infect Dis. 2013;3(3):109–115.
[6] Bibi F, Alvi SA, Sawan SA, et al. Detection and genotyping of *Helicobacter pylori* among gastric ulcer and cancer patients from Saudi Arabia. Pak J Med Sci. 2017;33(2):320–324.
[7] Liu J, He C, Chen M, et al. Association of presence/absence and on/off patterns of *Helicobacter pylori oipA* gene with peptic ulcer disease and gastric cancer risks: a meta-analysis. BMC Infect Dis. 2013;13:555.
[8] Imkamp F, Lauener FN, Pohl D, et al. Rapid characterization of virulence determinants in *Helicobacter pylori* isolated from non-atrophic gastritis patients by next-generation sequencing. J Clin Med. 2019;8(7):1030.
[9] Dadashzadeh K. Relevance of Helicobacter pylori *dupA* and *oipA* genotypes and development of gastric disease. Biomed Res. 2017;28(19):8179–8183.
[10] Abu-Taleb AM, Abdelattef RS, Abdel-Hady AA, et al. Prevalence of *Helicobacter pylori cagA* and *iceA* genes and their association with gastrointestinal diseases. Int J Microbiol. 2018;Article ID 4809093:7.
[11] Zaki ME, Elewa A, Ali MA, et al. Study of virulence genes *Cag A* and *Vac A* in *Helicobacter pylori* Isolated from Mansoura University hospital patients by multiplex PCR. Int J Curr Microbiol App Sci. 2016;5(2):154–160.
[12] Azeem EM, Abdel-Ghaffar AB, Shokaeir MH, et al. Genotyping of *Helicobacter pylori* isolates from Egyptian patients. Int J Biosci. 2017;10(4):121–128.
[13] El-Shenawy A, Diab M, Shemis M, et al. Detection of *Helicobacter pylori vacA, cagA* and *iceA1* virulence genes associated with gastric diseases in Egyptian patients. Egypt J Med Hum Genet. 2017;18(4):365–371.
[14] Stolte M, Meining A. The updated sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. Can J Gastroenterol. 2001;15:591–598.
[15] Kargar M, Baghernejad M, Doosti A. Role of NADPH-insensitive nitroreductase gene to metronidazole resistance of *Helicobacter pylori* strains. Daru. 2010;18(2):137–140.
[16] Oktem-Okullu S, Tiftikci A, Saruc M, et al. Multiplex-PCR-based screening and computational modeling of virulence factors and T-cell mediated immunity in *Helicobacter pylori* infections for accurate clinical diagnosis. PLoS ONE. 2015;10(8):e0136212.
[17] Ezzat AH, Ali MH, El-Seidi EA, et al. Genotypic characterization of *Helicobacter pylori* isolates among Egyptian patients with upper gastrointestinal diseases. Chin Ger J Clin Oncol. 2011. DOI:10.1007/s10330-011-0880-x
[18] Diab M, Shemis M, Gamal D, et al. *Helicobacter pylori* Western *cagA* genotype in Egyptian patients with upper gastrointestinal disease. Egypt J Med Hum Genet. 2018;19(19):297–300.
[19] Habib AM, Alam MJ, Rudra B, et al. PCR-based analysis of *Helicobacter pylori* virulent genotypes among dyspeptic patients from Chittagong, Bangladesh. Malaysian J Microbiol. 2017;13(1):33–40.
[20] El-Toukhy N, Saeed AM, Emara NM. Clinical relevance of the *cagA, vacA* and *babA2* virulence factors of *Helicobacter pylori* in Egyptian patients with gastroduodenal diseases. Int J Adv Res. 2016;4(4):1002–1020.
[21] Saeidi Y, Pournajaf A, Gholami M, et al. Determination of *Helicobacter pylori* virulence-associated genes in duodenal ulcer and gastric biopsies. Med J Islam Repub Iran. 2017;31:95.
[22] Ben Mansour K, Fendri C, Zribi M, et al. Prevalence of *Helicobacter pylori vacA, cagA, iceA* and *oipA* genotypes in Tunisian patients. Ann Clin Microbiol Antimicrob. 2010;9:article 10.
[23] Souod N, Sarshar M, Dabiri H, et al. The study of the *oipA* and *dupA* genes in Helicobacter pylori strains and their relationship with different gastroduodenal diseases. Gastroenterol Hepatol Bed Bench. 2015;8:S47–S53.
[24] Sallas ML, Melchiades JL, Zabaglia LM, et al. Prevalence of *Helicobacter pylori vac*A, *cag*A, *dup*A and *oip*A genotypes in patients with gastric disease. Adv Microbiol. 2017;7:1–9.
[25] Torres K, Valderrama E, Sayegh M, et al. Study of the *oipA* genetic diversity and EPIYA motif patterns in *cagA*-positive Helicobacter pylori strains from Venezuelan patients with chronic gastritis. Microb Pathog. 2014;76:26–32.
[26] Farzi N, Yadegar A, Aghdaei HA, et al. Genetic diversity and functional analysis of *oipA* gene in association with other virulence factors among Helicobacter pylori isolates from Iranian patients with different gastric diseases. Infect Genet Evol. 2018;60:26–34.
[27] Shiota S, Matsunari O, Watada M, et al. Systematic review and meta-analysis: the relationship between the *Helicobacter pylori dupA* gene and clinical outcomes. Gut Pathol. 2009;2:1–6.
[28] Lu H, Hsu PI, Graham DY, et al. Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology. 2005;128:833–848.
[29] Arachchi HS, Kalra V, Lal B, et al. Prevalence of duodenal ulcer-promoting gene (*dupA*) of *Helicobacter pylori* in patients with duodenal ulcer in North Indian population. Helicobacter. 2007;12:591–597.
[30] Nguyen LT, Uchida T, Tsukamoto Y, et al. *Helicobacter pylori dupA* gene is not associated with clinical outcomes in the Japanese population. Clin Microbiol Infect. 2010;16:1264–1269.
[31] Alam J, Maiti S, Ghosh P, et al. Significant association of the *dupA* gene of *Helicobacter pylori* with duodenal ulcer development in a South-east Indian population. J Med Microbiol. 2012;61:1295–1302.
[32] Salih AM, Goreal A, Hussein NR, et al. The distribution of *cagA* and *dupA* genes in *Helicobacter pylori* strains in Kurdistan region, northern Iraq. Ann Saudi Med. 2013;33(3):290–293.
[33] Takahashi A, Shiota S, Matsunari O, et al. Intact long-type *dupA* as a marker for gastroduodenal diseases in Okinawan subpopulation, Japan. Helicobacter. 2013;18(1):66–72.
[34] Jung SW, Sugimoto M, Shiot S, et al. The intact *dupa* cluster is a more reliable *Helicobacter pylori* virulence marker than *dupA* alone. Infect Immun. 2012;80(1):381–387.
[35] Hussein NR. The association of *dupA* and *Helicobacter pylori*-related gastroduodenal diseases. Eur J Clin Microbiol Infect Dis. 2010;29:817–821.
[36] Alam J, Ghosh P, Ganguly M, et al. Association of intact *dupA (dupA1)* rather than *dupA1* cluster with duodenal ulcer in Indian population. Gut Pathog. 2015;7:9. PMID: 25829953.
[37] Markovska R, Boyanova L, Yordanov D, et al. *Helicobacter pylori oipA* genetic diversity and its associations with both disease and cagA, vacA s, m, and i alleles among Bulgarian patients. Diagn Microbiol Infect Dis. 2011;71:335–340.
[38] Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol. 2010;7:629–641.
[39] Zhang Z, Zheng Q, Chen X, et al. The *Helicobacter pylori* duodenal ulcer promoting gene, *dupA* in China. BMC Gastroenterol. 2008;8:49.