Loss of p53 expression in the gastric epithelial cells of Helicobacter pylori infected Jordanian patients

Mohammad A. Abu-Lubad
Mu'tah University Faculty of Medicine
Ghada F. Helaly Helaly
Mu'tah University Faculty of Medicine
Weliam J. Haddadin
Jordanian Royal Medical Services
Dua'a AK. Jarajreh
Mu'tah University Faculty of Medicine
Amin A. Aqel
Mu'tah University Faculty of Medicine
Munir A. Al-Zeer (al-zeer@tu-berlin.de)
Technische Universitat Berlin https://orcid.org/0000-0002-1700-9920

Research article

Keywords: Helicobacter pylori, p53, Immunohistochemistry, Gastritis, Jordan

Posted Date: April 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-22599/v1

Version of Record: A version of this preprint was published at International Journal of Microbiology on March 23rd, 2022. See the published version at https://doi.org/10.1155/2022/7779770.

Loss of p53 expression in the gastric epithelial cells of *Helicobacter pylori* infected Jordanian patients

Mohammad A. Abu-Lubad[a], Ghada F. Helaly[a,b], Weliam J. Haddadin[c],Dua'a AK. Jarajreh[a], Amin A. Aqel[a], Munir A. Al-Zeer[d*]

[a]*Department of Medical Microbiology and Pathology, Faculty of Medicine, Mutah University, Al-Karak, Jordan.*

[b]*Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria-Egypt.*

[c]*Royal Medical Services, Amman, Jordan.*

[d]*Department of Applied Biochemistry, Institute of Biotechnology, Technical University of Berlin, Berlin, Germany.*

***Corresponding author**

Dr. Munir A. Al-Zeer, PhD

Address: Dept. of Applied Biochemistry, Institute of Biotechnology,

Technical University of Berlin, Gustav-Meyer-Alle 25, 13355 Berlin, Germany.

E-mails: al-zeer@tu-berlin.de or munir_alzeer@hotmail.com

Abstract

Background: *Helicobacter pylori* infection is one of the most common chronic infections worldwide. Around half of the global population is chronically infected with this stomach bacterium. *H. pylori* infection is a strong risk factor for gastric cancer development. It is well-established that infection of the gastric epithelium with *H. pylori* induces the production of reactive oxygen species, DNA damage and accelerates the degradation of the tumor suppressor protein p53. This p53 dysregulation induced by *H. pylori* infection contributes to gastric carcinogenesis through complex processes including but not limited to cell proliferation and apoptosis.

Methods: In the current study, we examined whether the epithelium of the gastric glands express p53 in subjects infected, chronically, with *H. pylori*. Seventy-five samples from Jordanian patients were analyzed for the presence of *H. pylori* as well as the p53 expression levels in the mucosa and submucosa by immunohistochemical analyses.

Results: In *H. pylori* positive-specimens, p53-positive cells in the gastric mucosa were found significantly lower than in *H. pylori* negative-specimens.

Conclusion: We demonstrated that p53 expression level is downregulated in gastric mucosa of patients from Jordan infected with *H. pylori* and this alteration may predispose individuals for possible tumor initiation in individuals chronically infected with *H. pylori*.

Keywords: *Helicobacter pylori;* p53*;* Immunohistochemistry*;* Gastritis; Jordan.

Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the human stomach (1). *H. pylori* infection is acquired early in life, and it is the strongest identified bacterial risk factor for the development of antral gastritis, peptic ulcer and gastric cancer (2). More than 4.4 billion individuals are infected globally with *H. pylori* in 2015 (3). *H. pylori* is classified as a group 1 carcinogen according to the International Agency for Research on Cancer (IARC). Infection with *H. pylori* is recognized as a necessary but insufficient cause of gastric carcinoma (1, 4). This is due to the complex interplay between bacterial virulence factors and host factors that determine the progression and chronicity of infection (4). The most studied *H. pylori* virulence genes are located in a40kb region of DNA called cytotoxin-associated gene Pathogenicity Island (cagPAI) (5). One of the most distinctive virulent gene of the cagPAI is the cytotoxin-associated gene A (*CagA*) which is delivered into epithelial cells by cagPAI encoded type IV secretion system after bacterial attachment to the host. In turn, CagA as an oncoprotein can disrupt several essential signaling pathways of the host into oncogenic ones (5).

Development of gastric cancer is anticipated to be a multistep and a multifactorial process, including activation of oncogenes, inhibition of tumor suppressor genes, related to oxidative stress and damage induced by pathogen (6). Creating a sequence of events starting from gastritis, atrophy, intestinal metaplasia, dysplasia, and finally gastric cancer; this sequence of events can take several decades to develop (5). It has been reported that, in gastric metaplasia and adenocarcinoma, *H. pylori* cagA positive strain contribute to the loss of function of p53 (7, 8). While other studies have shown that p53 is expressed in the mucosa of patients with pre-neoplastic lesions infected with *H. pylori* (9, 10). In contrast, the inflammatory response was found to induce the expression of the wild type p53during chronic gastritis and gastric ulcer (11). Similarly, *in vitro*

studies revealed the same effect of *H. pylori* infection on the expression level of wild type p53 (12, 13). In contrast, it was shown that *H. pylori* infection is able to activate Akt1 kinase. In turn Akt1 leads to the activation of HDM2 and subsequent degradation of p53 in gastric epithelial cells (14).

H. pylori infection remains highly prevalent in most developing countries due to socioeconomic status and hygienic conditions (15).The prevalence of *H. pylori* infection is 79.1%, 63.4%, and 54.7%in Africa, Latin America, and Asia respectively, while it has been reported around 37.1%in Northern America (3). In Jordan, the nationwide seroprevalence of *H. pylori i*s around 88.6%in 2018 (16). Among Jordanian patients, the prevalence of *H. pylori* in gastritis and peptic ulcer, intestinal-type adenocarcinomas, and adenocarcinomas cases were 82%, 55.6%, and 48.8%, respectively (17, 18). A recent study showed that all individuals tested for *H. pylori* in the dental plaque were positive among Jordanian people (19). Even though the prevalence of *H. pylori* in Jordan is high no previous studies were carried out to study the expression levels of p53 in the gastric mucosa from patients infected with *H. pylori*. P53 degradation induction is considered the most common phenotypes related to oxidative stress, and DNA damage during infection which is accompanied by altered cell survival and apoptosis (6). Therefore, we aim in this study is to dissect the p53 expression in gastric tissues from Jordanian patients infected with *H. pylori*.

Materials and Methods

Sample collection

Retrospective case-control study conducted on 75 formaldehyde-fixed paraffin-embedded (FFPE) gastric biopsies from *H. pylori* negative (n=25) and *H. pylori* positive (chronic *H. pylori* pangastritis) (n=50). Samples were collected between 2015 to2017 from the archives of the pathology departments from two selected referral medical centers in the middle (King Hussein Medical City) and Northern of Jordan (Al-Karak Governmental Hospital). In this retrospective analysis in archival material, the inclusion criteria were the age range (15-80 years) and the histopathology analysis for the presence of *H. pylori* in the gastric glands of the patients. As mentioned in the records, the participants were clinically suffering from abdominal pain, weight loss, epigastric pain, vomiting, and dyspepsia. Gastric samples that were histopathologically diagnosed of having no *H. pylori* infection and any other abnormalities were included in this study, as negative controls. The exclusion criteria were *H. pylori* eradication therapy during the last three months before the study as well as a history of gastric surgery or gastric cancer.

Immunohistochemistry

Formalin-fixed, paraffin-embedded gastric biopsies were subjected to immunohistochemical staining as described before (20). Five µm block sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. Then, samples were heated at 95ºC for 15 min using antigen retrieval buffer for antigen retrieval (Dako, USA). Samples were then incubated with 3% H_2O_2 for 30 min at room temperature to block the endogenous peroxidase activity. The samples were blocked 1% bovine serum albumin in PBS for one hour at room temperature. Samples were then stained for p53, *H. pylori*, and DNA using the monoclonal mouse anti-p53 DO-1 antibody from Santa Cruz (sc-126) (1:100 dilutions in blocking buffer), polyclonal

rabbit anti-Urease antibody from Santa Cruz (sc-21016) (1:100 dilution) and DAPI for DNA staining (Sigma-Aldrich) for two hours at RT. Secondary labeled antibodies for immunofluorescence and Western immunoblot analyses were purchased from Jackson Immuno Research Laboratories as published before (21).

Western blotting

Total protein was extracted from the paraffinized tissues according to the manufacturer's instructions (Qproteome FFPE Tissue, Qiagen). Briefly, using xylene the deparaffinized sections were incubated in 100 µl extraction buffer. After centrifugation, extracted proteins were recovered in the supernatant. Recovered proteins were mixed directly with sample buffers and boiled at 95°C for 10 min. Then, gel electrophoresis was carried out using the vertical Biorad Mini-Protean II electrophoresis system, USA. Equal amounts of proteins were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).Proteins were separated under reducing conditions for two hours at 120 V. Protein bands were transferred electrophoretically onto Immobilon-P polyvinylidene difluoridemembranes (Millipore, MA, USA) using a Biorad electroblotting system (Biorad MiniTrans-Blot Electrophoretic Transfer Cell, USA) (22). Finally, the transfer was carried out for three hours using 250 mA at 4°C. The membranes were blocked with5% fat-skim milk in Tris-buffered saline (TBS) pH 7.5, containing 0.05% Tween-20 for one h at RT. Next, membranes were incubated with the anti-p53, anti-cagA, and anti-β-actin (one hour at room temperature) diluted in TBS-0.05% Tween-20. The membranes were washed 3 times for 20 min with TBS-0.05% Tween-20 and then incubated with secondary antibodies conjugated with horseradish peroxidase. Signal detection was performed with the enhanced chemiluminescence system (ECL, Amersham), by mixing chemiluminescence solutions oneand two in 1:1 ratio.

RESULTS:

Detection of *H. pylori* in gastric biopsy specimens using immunohistochemistry

The presence of *H. pylori* in the gastric biopsies from Jordanian patients (75 samples) was confirmed using immunohistochemistry (IHC). *H. pylori* was detected in the gastric biopsies using specific antibody against the urease enzyme of *H. pylori*. Fifty biopsies were diagnosed histologically for the presence of *H. pylori* infection (Figure 1). The bacterium, red fluorescent signal, was detected at the mucosal surface, and in the lumen of the gastric glands (Figure 1). No signal for *H. pylori* was detected in all negative controls samples.

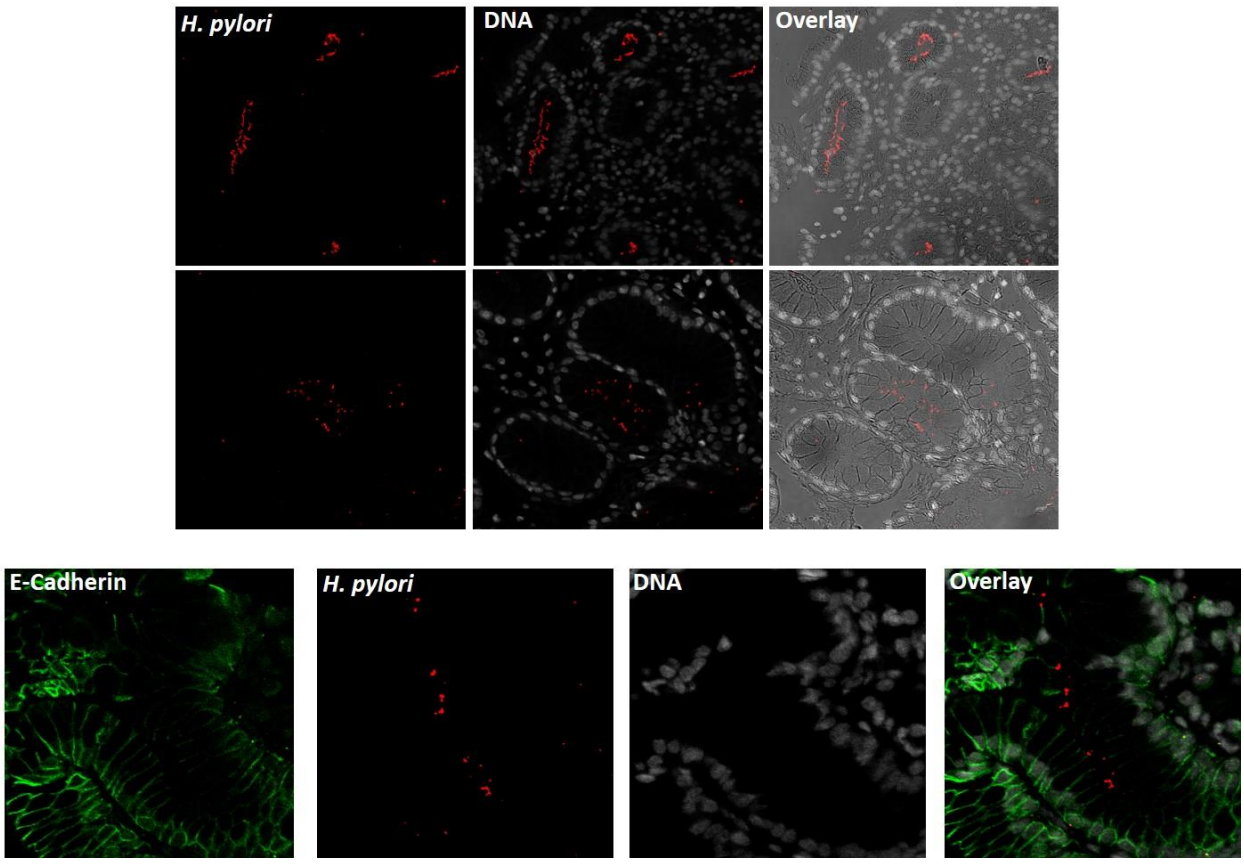

Figure 1: Detection of *H. pylori* in gastric biopsy specimens. An immunohistochemical staining of gastric glands obtained from *H. pylori*-positive patients stained for epithelial marker E-Cadherin (green), *H. pylori* (red), and nuclear DNA using DAPI. Results show that most of the gastric glands are heavily infected with *H. pylori.*

Expression level of p53 in gastric biopsies infected with *H. pylori*

Immunohistochemical staining was used to analyze the expression level of p53 in *H. pylori*-positive and negative gastric biopsy specimens. In control samples, were no *H. pylori* is detectable, the p53 fluorescence signal was strong and detected in all samples irrespective of sectioning sites. Further, the p53 signal was localized to the nucleus of the epithelium of the gastric glands (Figure 2). In contrast, patients harboring *H. pylori* in their gastric glands did not express p53in the nucleus. Rather, expression levels of p53 in infected patients were significantly low or even undetectable compared to control, *H. pylori* negative biopsy specimens (Figure 2). Interestingly, in the stromal cells p53 expression was devoid from the nucleus and localized in the cytoplasm of the *H. pylori* infected mucosa.

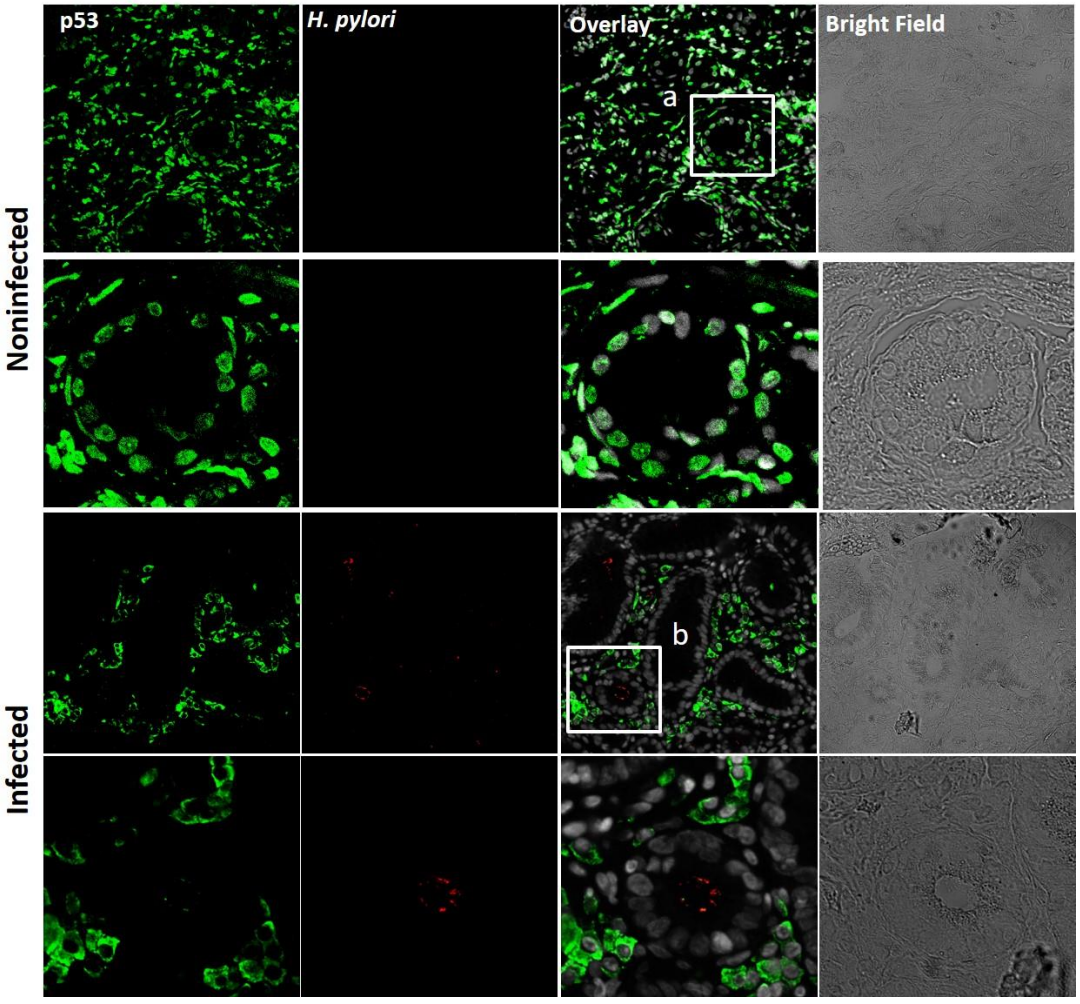

Figure 2: p53 expression in *H. pylori* infected and non-infected gastric glands. a) Control gastric glands (no *H. pylori* infection) exhibit wild-type pattern of p53 (green) expression in the epithelium and the stroma inside the nuclei **b)** p53 staining in gastric glands infected with *H. pylori* (red) exhibit a complete loss of the nuclear p53 staining of the epithelium unlike the stromal cells which display cytoplasmic staining of p53 (green).

To further support our data, we used western blotting to check the expression levels of p53 from infected vs. control uninfected gastric biopsies (Figure 3). Interestingly, results from western blotting showed significant reduction of p53 expression from gastric biopsies infected with *H. pylori* unlike uninfected biopsies confirming our immunohistochemical findings that subject infected with *H. pylori* suffer from loss or significant reduction of the nuclear p53 expression (Figure 3).

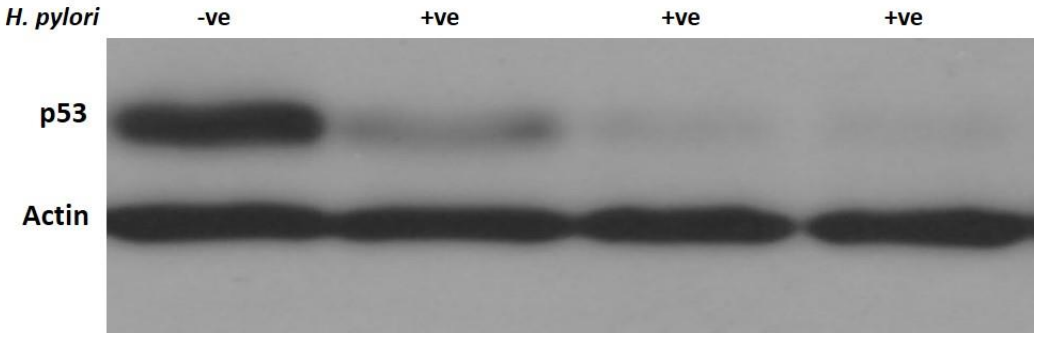

Figure 3: Western blotting analysis of total p53 from paraffinized gastric biopsy specimens. Total protein isolated from gastric tissues infected with *H. pylori* and control non-infected tissues blotted against p53 demonstrated that p53 protein levels were significantly reduced in patients infected with *H. pylori* unlike non-infected patients. Actin was used as a loading control.

Discussion

As in many other infections, *H. pylori* triggers the downregulation of p53 protein by which the bacterium blocks apoptosis one of the main defensive mechanism in infected cells (23, 24). This protective function of p53 is also impaired in many forms of cancer (25, 26). Our present study underpins the direct relationship between chronic infection with *H. pylori* and the loss of p53 expression from the epithelium in the gastric mucosa. Loss of nuclear p53 from the gastric mucosa may render the epithelial cells of the stomach vulnerable for hundreds of mutations that occur every day in almost every cell in our body in individuals infected with *H. pylori*. In contrast, intact p53 protects cells during DNA damage by activating DNA damage response and subsequently DNA repair or apoptosis (25). In this manner, cells are normally sheltered against unwanted changes that might predispose cells for possible transformation or cancer. This scenario is not happening in gastric tissues infected with *H. pylori* which dispose the diseased tissue for cancer development. Further, it has been shown that *H. pylori* ensure the survival of host cells by breaking down p53*in vitro* and *in vivo (8, 23, 24)*, by activating pathways that are already present in cells (14). Yet, this has possibly lethal consequences for the host as the damage of p53, the central "guardian of the genome", upsurges the risk of mutant cells to survive and develop into cancer cells (25, 26)

Numerous bacterial contagions are now supposed to be a factor in cancer development but their association is not so decisively proven as for *H. pylori*, which can induce chronic gastritis, gastric ulcer and finally lead to cancer development(7) . Consequently, the more verified connection between infection and cancer raises the importance to develop effective vaccines and antibiotics to preclude cancer. With the recognition of *H. pylori's* cagPAI type 4 secretion system (T4SS) as a significant virulence factor, our study did not show whether Cag A is expressed in

patients infected with *H. pylori* and this needs further investigation. CagA is an inflammatory and potentially transforming determinant of *H. pylori (23)*. The translocation of CagA effector protein into host cells and its phosphorylation by Src kinase and uncovered crucial signaling routes induced by CagA, including its function in downregulating innate epithelial defense factors, are responsible for the vast pro-inflammatory activity of the T4SS(27). Our data shows that infected gastric epithelial with *H. pylori* lost the nuclear p53 expression, unlike normal healthy *H. pylori*-negative glands using immunohistochemistry. The loss of p53 expression from the epithelium is correlated with the presence of *H. pylori* within the lumen of the gastric glands. The unique ability of the bacterium to escape the mucosal defense and thereby ensure life-long persistence that could be related to higher colonization grades of bacteria and p53 expression.

To date, all the evidences point towards a role of p53 in tumor suppression as well as its role as a nuclear transcription factor (14). Therefore, p53 interactions, modifications and subcellular localization in the cytoplasm might reserve new unexpected aspects of this extensively studied protein and provide a rationale for therapeutic intervention. Interestingly, our findings suggest that p53 is localized to the cytoplasm of the stromal cells in patients infected with *H. pylori*. Whether, *H. pylori* directly or indirectly influenced the subcellular localization and or posttranslational modification of p53 in the stroma is an open question which needs further investigation. However, this localization may affect signal transduction, metabolism, and apoptosis (28). While several mechanisms of p53 cytoplasmic that lead to the activation of apoptosis have been described to date, most of the mechanisms regulating its cytoplasmic activities remain largely unknown (29). However, possible contribution of cytoplasmic p53 to foster oncogenesis cannot be excluded but needs further studies to understand the exact role of the stromal cells in rendering or enhancing the gastric carcinogenesis.

In conclusion, while it has been widely accepted that *H. pylori* plays a major role in the development of gastric cancer, our results represent an approach that can reveal a causality between a particular bacterial infection and the development of cancer in humans. This bacterium induces the loss of p53 from the epithelium that might enhance cell proliferation and inhibition of apoptosis. Moreover, infected cells may acquire a cancer-related phenotype thus providing deep insight into the cancer-promoting potential of *H. pylori*.

Conclusion

In conclusion, the nuclear expression of p53 is drastically downregulated in gastric mucosa of individuals from Jordan infected with *H. pylori* and this may facilitate tumor initiation. Further studies are needed in order to further correlate the relationship between the bacterium serotype, inflammation and severity of disease development.

Ethical consideration

This project was conducted with approval from the Ethics Committee of faculty of medicine, Mutah University, Al-Karak-Jordan under the number 20170 according to the local institutional ethical considerations and guidelines that was in agreement with the ethical guidelines of the Declaration of Helsinki. Human tissues were obtained from the histopathology department from two selected referral medical centers in the middle (King Hussein Medical City) and Northern of Jordan (Al-Karak Governmental Hospital) as this a retrospective study in compliance with the standards of institutional ethical committee.

Funding

All authors declare no financial support.

Conflict of interest

All authors declare that they have no conflict of interest.

Consent for publication

Not applicable.

Author's contributions

MAL and MAZ: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; MAL, MAZ and WH resources; MAL, MAZ, AA, GH, DJ and WH technical, or material support; study supervision study concept and design; critical revision of the manuscript, administrative, technical, or material support; MAL, MAZ, AA and GH: critical revision of the manuscript for important intellectual content administrative. All authors approved the final version of the manuscript.

Acknowledgment

The authors deeply appreciate and thank Prof. Dr. Thomas F Meyer- a director at Max Planck Institute for Infection Biology/Berlin- for his support to do the immunohistochemical analysis in his lab.

References

1. Naja F, Kreiger N, Sullivan T. Helicobacter pylori infection in Ontario: prevalence and risk factors. Canadian Journal of Gastroenterology and Hepatology. 2007;21(8):501-6.
2. Kikuchi S. Epidemiology of Helicobacter pylori and gastric cancer. Gastric cancer. 2002;5(1):6-15.
3. Sjomina O, Pavlova J, Niv Y, Leja M. Epidemiology of Helicobacter pylori infection. Helicobacter. 2018;23:e12514.
4. Pellicano R, Ribaldone DG, Fagoonee S, Astegiano M, Saracco GM, Mégraud F. A 2016 panorama of Helicobacter pylori infection: key messages for clinicians. Panminerva medica. 2016;58(4):304-17.
5. Atherton JC, Blaser MJ. Coadaptation of Helicobacter pylori and humans: ancient history, modern implications. The Journal of clinical investigation. 2009;119(9):2475-87.
6. Brüggemann H, Al-Zeer MA. Bacterial signatures and their inflammatory potentials associated with prostate cancer. APMIS. 2020.
7. Shibata A, Parsonnet J, Longacre TA, Garcia MI, Puligandla B, Davis RE, et al. CagA status of Helicobacter pylori infection and p53 gene mutations in gastric adenocarcinoma. Carcinogenesis. 2002;23(3):419-24.
8. Unger Z, Molnár B, Prónai L, Szaleczky E, Zágoni T, Tulassay Z. Mutant p53 expression and apoptotic activity of Helicobacter pylori positive and negative gastritis in correlation with the presence of intestinal metaplasia. European journal of gastroenterology & hepatology. 2003;15(4):389-93.
9. Satoh T, Pandey J, Okazaki Y, Asahi A, Kawakami Y, Ikeda Y, et al. Single nucleotide polymorphism of interleukin-1β associated with Helicobacter pylori infection in immune thrombocytopenic purpura. Tissue Antigens. 2009;73(4):353-7.
10. Shimizu T, Marusawa H, Matsumoto Y, Inuzuka T, Ikeda A, Fujii Y, et al. Accumulation of somatic mutations in TP53 in gastric epithelium with Helicobacter pylori infection. Gastroenterology. 2014;147(2):407-17. e3.
11. César ACG, de Freitas Calmon M, Cury PM, Caetano A, Borim AA, Silva AE. Genetic alterations in benign lesions: chronic gastritis and gastric ulcer. World Journal of Gastroenterology: WJG. 2006;12(4):625.
12. Ahmed A, Smoot D, Littleton G, Tackey R, Walters CS, Kashanchi F, et al. Helicobacter pylori inhibits gastric cell cycle progression. Microbes and infection. 2000;2(10):1159-69.
13. Ashktorab H, Ahmed A, Littleton G, Wang XW, Allen CR, Tackey R, et al. p53 and p14 increase sensitivity of gastric cells to H. pylori-induced apoptosis. Digestive diseases and sciences. 2003;48(7):1284-91.
14. Wei J, Nagy TA, Vilgelm A, Zaika E, Ogden SR, Romero–Gallo J, et al. Regulation of p53 tumor suppressor by Helicobacter pylori in gastric epithelial cells. Gastroenterology. 2010;139(4):1333-43. e4.
15. Yokota Si, Konno M, Fujiwara Si, Toita N, Takahashi M, Yamamoto S, et al. Intrafamilial, preferentially mother-to-child and intraspousal, Helicobacter pylori infection in Japan determined by

mutilocus sequence typing and random amplified polymorphic DNA fingerprinting. Helicobacter. 2015;20(5):334-42.
16. Obaidat MM, Roess AA. First nationwide seroepidemiology and risk factors report of Helicobater pylori in Jordan. Helicobacter. 2019;24(3):e12572.
17. Bani-Hani KE, Hammouri SM. prevalence of Helicobacter pylori in Northern Jordan. Endoscopy based study Saudi Med J. 2001;22(10):843-7.
18. Awad HA, Hajeer MH, Abulihya MW, Al-Chalabi MA, Al Khader AA. Epidemiologic characteristics of gastric malignancies among Jordan University Hospital patients. Saudi medical journal. 2017;38(9):965.
19. Abu-Lubad M, Alzoubi H, Jarajreh Da, Al Sawalqa A, Bruggemann H, Albataineh E, et al. Analysis of Helicobacter pylori Genotypes Amongst Jordanians' Dental Plaque Samples. Gastroenterology research. 2018;11(1):46.
20. Ando K, Oki E, Saeki H, Yan Z, Tsuda Y, Hidaka G, et al. Discrimination of p53 immunohistochemistry-positive tumors by its staining pattern in gastric cancer. Cancer medicine. 2015;4(1):75-83.
21. Al-Zeer MA, Xavier A, Lubad MA, Sigulla J, Kessler M, Hurwitz R, et al. Chlamydia trachomatis prevents apoptosis via activation of PDPK1-MYC and enhanced mitochondrial binding of hexokinase II. EBioMedicine. 2017;23:100-10.
22. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Sciences. 1979;76(9):4350-4.
23. Vallejo-Flores G, Torres J, Sandoval-Montes C, Arévalo-Romero H, Meza I, Camorlinga-Ponce M, et al. Helicobacter pylori CagA suppresses apoptosis through activation of AKT in a nontransformed epithelial cell model of glandular acini formation. BioMed research international. 2015;2015.
24. Alfarouk KO, Bashir AH, Aljarbou AN, Ramadan AM, Muddathir AK, AlHoufie ST, et al. The Possible Role of Helicobacter pylori in Gastric Cancer and Its Management. Frontiers in oncology. 2019;9:75.
25. Muller PA, Vousden KH. Mutant p53 in cancer: new functions and therapeutic opportunities. Cancer cell. 2014;25(3):304-17.
26. Węsierska-Gądek J. Targeting p53 as a promising therapeutic option for cancer by re-activating the wt or mutant p53's tumor suppression. Future medicinal chemistry. 2018;10(7):755-77.
27. Mueller D, Tegtmeyer N, Brandt S, Yamaoka Y, De Poire E, Sgouras D, et al. c-Src and c-Abl kinases control hierarchic phosphorylation and function of the CagA effector protein in Western and East Asian Helicobacter pylori strains. The Journal of clinical investigation. 2012;122(4):1553-66.
28. Harris SL, Levine AJ. The p53 pathway: positive and negative feedback loops. Oncogene. 2005;24(17):2899-908.
29. Comel A, Sorrentino G, Capaci V, Del Sal G. The cytoplasmic side of p53's oncosuppressive activities. FEBS letters. 2014;588(16):2600-9.

Figures

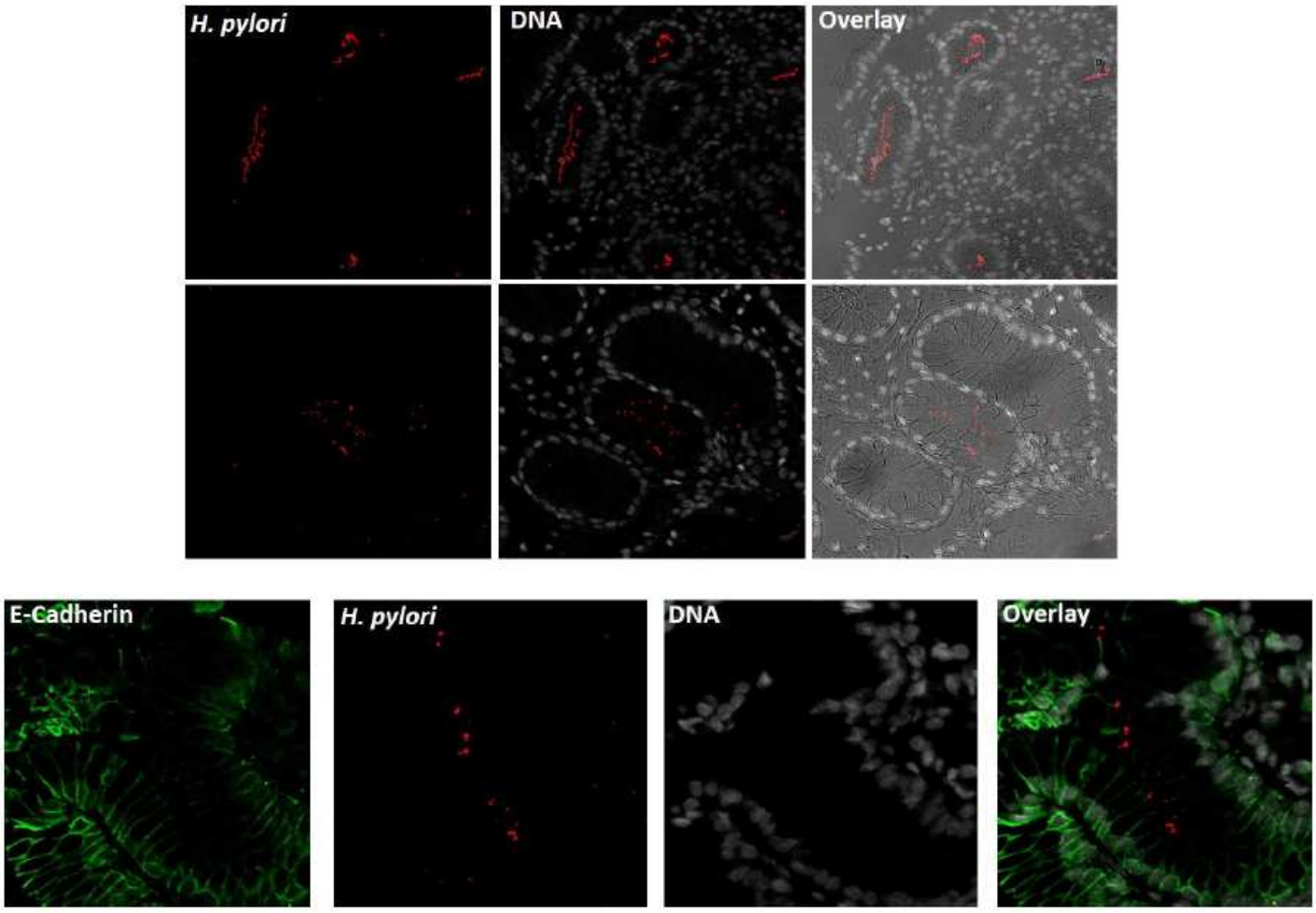

Figure 1

Detection of H. pylori in gastric biopsy specimens. An immunohistochemical staining of gastric glands obtained from H. pylori-positive patients stained for epithelial marker E-Cadherin (green), H. pylori (red), and nuclear DNA using DAPI. Results show that most of the gastric glands are heavily infected with H. pylori.

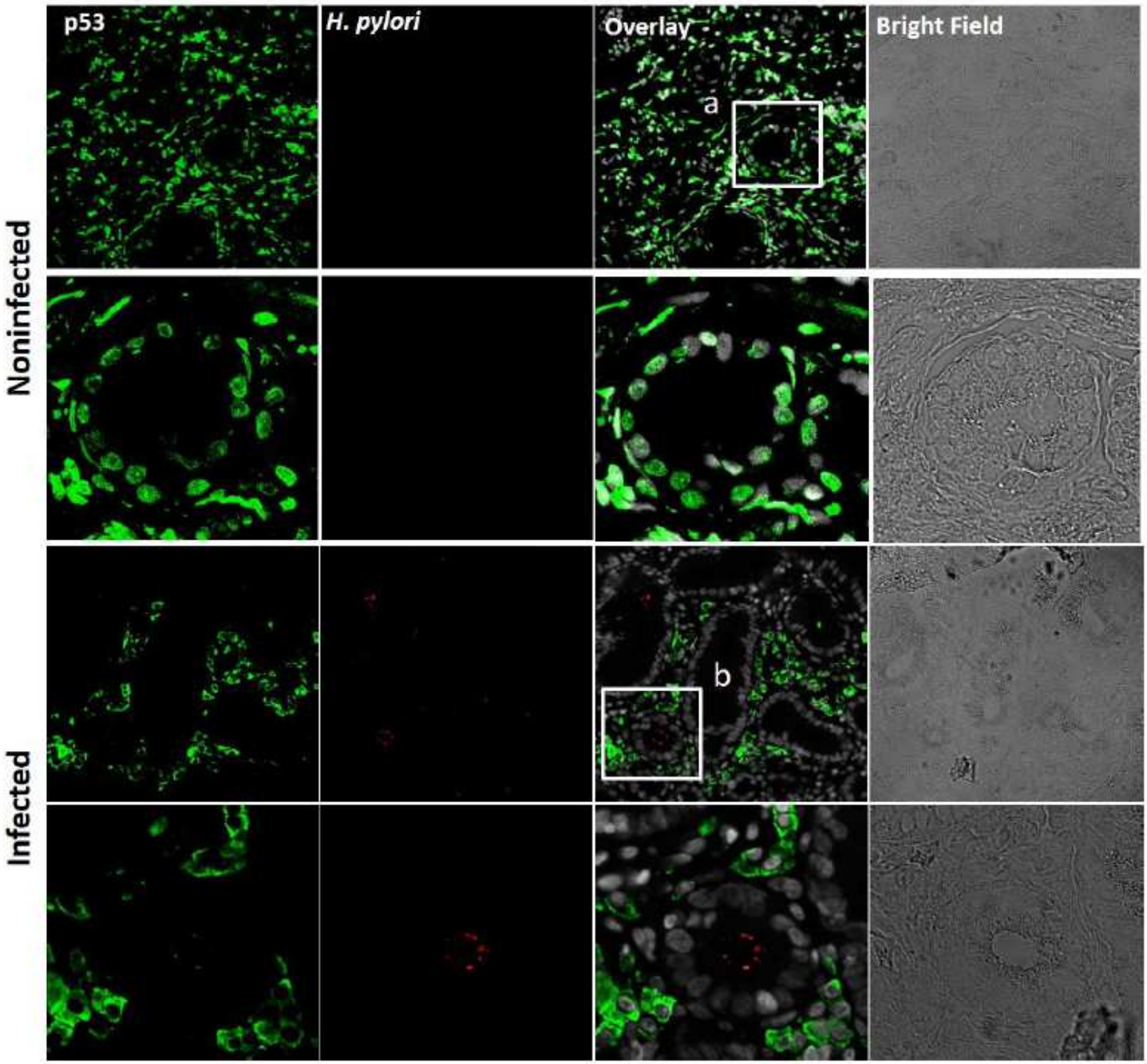

Figure 2

p53 expression in H. pylori infected and non-infected gastric glands. a) Control gastric glands (no H. pylori infection) exhibit wild-type pattern of p53 (green) expression in the epithelium and the stroma inside the nuclei b) p53 staining in gastric glands infected with H. pylori (red) exhibit a complete loss of the nuclear p53 staining of the epithelium unlike the stromal cells which display cytoplasmic staining of p53 (green).

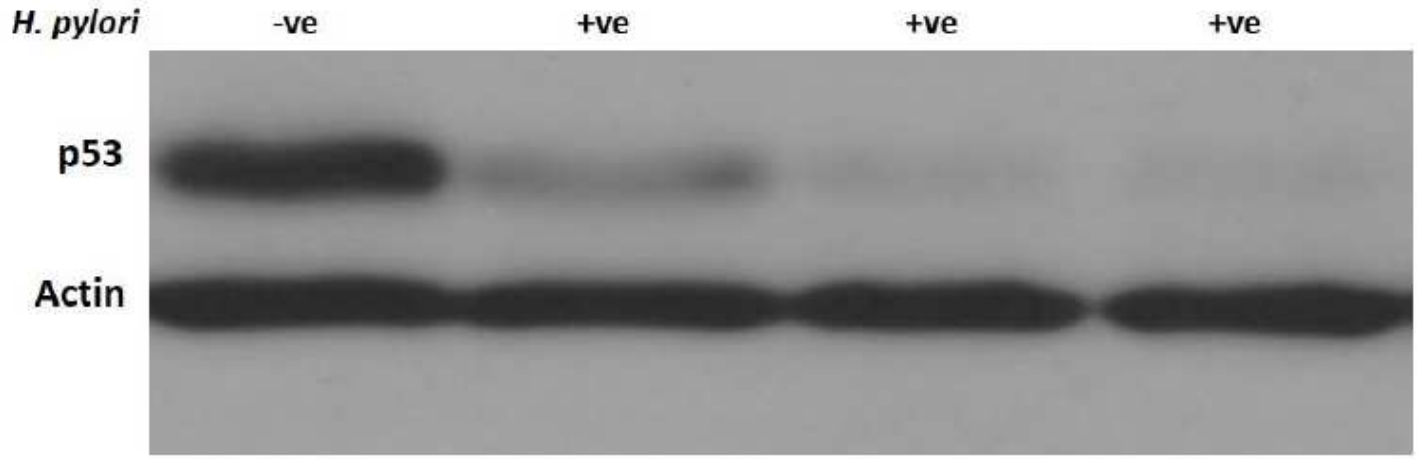

Figure 3

Western blotting analysis of total p53 from paraffinized gastric biopsy specimens. Total protein isolated from gastric tissues infected with H. pylori and control non-infected tissues blotted against p53 demonstrated that p53 protein levels were significantly reduced in patients infected with H. pylori unlike non-infected patients. Actin was used as a loading control.