

Clarithromycin and Amoxicillin Effect on Helicobacter Pylori-Infected Oxidative DNA Damage

Zinah Shakir Shallal

Department of Pathological Analysis, College of Science, Wasit University, Iraq

zalsoza@uowasit.edu.iq

Received: March 22, 2024; Accepted: Apr 29, 2024; Published: May 10, 2024;

Abstract: Epidemiological studies reveal that the risk of developing stomach cancer increase by Helicobacter pylori infection, and scientists assume that oxidative DNA damage caused by the bacteria is to blame. Oxidative DNA damage in Helicobacter pylori-infected cases was measured before and after two weeks of clarithromycin and amoxicillin (1 g/day) +amoxicillin (1 g/day) treatment using DNA chain fractures and form amidoprimidine DNA glycosylase (fpg)-sensitive regions. After treatment, it was found that the Comet method's measurement of the frequency of fpg-sensitive regions decreased (P0.001), while the frequency of DNA chain breaks remained unchanged. A favorable connection between age and prevalence off fpg-sensitive areas was found ($r = 0.59$, $P 0.05$). The results suggest that lowering oxidative DNA damage through treatment with clarithromycin + amoxicillin in helicobacter pylori-infected individuals may lessen the risk of helicobacter pylori-mediated stomach cancer formation.

Keywords Helicobacter pylori, oxidative stress, comet method, DNA chain fractures.



This is an open-access article under the [CC-BY 4.0](https://creativecommons.org/licenses/by/4.0/) license

Introduction

The pathophysiology of gastritis and peptic ulcer is significantly influenced by Helicobacter pylori, which is classified as a class I carcinogen by the World Health Organization. Helicobacter pylori is a meticulous, gram (-), flagellated bacterium that only colonizes humans. (1). Atrophic gastritis, intestinal metaplasia, and gastric cancer may develop in a small percentage of Helicobacter pylori infections (2), despite the fact that the majority of patients are asymptomatic. People infected with Helicobacter pylori have an approximately fourfold increased risk of developing stomach cancer than those who are not affected (3). It is believed that this is the result of elevated oxidative stress brought on by inflammation. During the respiratory burst, neutrophils and macrophages produce huge amounts of oxygen and nitrogen radicals, which can interact with DNA and lead to genetic alterations such chain breaks, point mutations, and chromosome abnormalities that can ultimately result in cancer. When radicals react with DNA, they produce 8-hydroxydeoxyguanosine (8-OHdG), the most mutagenic form the DNA damage. When 8th carbon of guanine in DNA bases is oxidized by the hydroxyl radical, 8OHdG is produced. During DNA replication, 8OHdG pairs with adenine rather than cytosine, leading to the G:C T: A mutation (4). In order to remove 8-OHdG molecules from DNA, 8 oxoguanine glycosylases perform a cleavage reaction. If the damage isn't fixed properly, it will return. Oncogenic and tumor suppressor gene mutations typically cause this base substitution (5). The frequency of DNA strand breaks can be

quickly and easily visualized using the comet assay (single cell gel electrophoresis), which is a quick, simple, and sensitive procedure. Initially documented by Singh et al. in 1989. Later, with some tweaks, (6)'s technique became standard in studies of genotoxicity and apoptosis. The frequency of 8-OHdG residues on the DNA can be determined by comet assay and is considered an indicator of oxidative DNA damage (7). When the bacterial endonuclease form amidopyrimidine DNA glycosylase (fpg) is used to generate additional breaks in the regions where this damage is present. The purpose of this research was to ascertain whether or not treating *Helicobacter pylori* infections with clarithromycin and amoxicillin for 2 weeks reduces the number of DNA chain breaks and fpg-sensitive areas.

Methods

The study comprised 19 patients whose biopsy samples tested positive for *Helicobacter pylori* at the Endoscopy Unit of the Department of General Surgery at Al Zahraa hospital in Wasit Province. There were 11 female and 8 male cases, with a mean age of 39 years and 13 months. None of the study participants smoked, used vitamins or drugs that could interact with the oxidant/antioxidant system, and they all followed similar dietary and lifestyle habits. They had gone an entire month without taking any antibiotics. None of them suffered from conditions like diabetes, high blood pressure, or cancer. The kidneys, thyroid, and liver all performed as expected. Clarithromycin (1 g) and amoxicillin (1 g) were administered to patients for two weeks after a 3 ml heparinized blood sample was obtained. At the completion of the treatment, blood samples were obtained from the cases again. Number of DNA strand breaks and fpg-sensitive areas quantified by single-cell gel electrophoresis (6) Low melting point agar gel was put on a microscope slide, and then heparinized blood was added. All of the cell contents, excluding the nucleus, were lysed by submerging the slide in a salty lysis solution. The cells were washed with a pH 8 buffer to get rid of any leftover material. A high alkaline environment (pH >11) was maintained for the remaining double-stranded DNA on the slide, which allowed the double-helical structure to open in regions where chain breaks were present. pH was adjusted following alkaline electrophoresis, and ethidium bromide-stained samples were studied using a fluorescence microscope.

DNA that is electrophoretically separated and moving towards the anode resembles a comet. The star's head is represented by the unbroken DNA, and the tail by the fragments. According to how they look under a fluorescence microscope, damaged DNAs are classified into one of five groups. For the study, two copies of each slide were made. 100 bits of DNA were analyzed on each slide, the average of the counts obtained from the two slides was collected, and the number of DNA in each category was determined and the frequency of chain breaks was expressed as arbitrary units (au), showing the % DNA in the tail region. After lysis, the DNA was treated with fpg, a bacterial enzyme, at 37°C for 1 hour to determine the prevalence of fpg-sensitive areas, an indicator of oxidative DNA damage. The total quantity of oxidative base damage was calculated by deducting the number of chain breaks observed in the fpg-incubated slide from the number of chain breaks observed in the control slide. The data was summarized as a mean and standard deviation. The results of the pre- and post-treatment phases were statistically compared using the non-parametric Wilcoxon test. Significant results were seen at the p0.05 level. Spearman correlation coefficient was utilized in correlation analysis.

Results and Discussion

Results

In Table 1, we see the comparison between the baseline frequency of DNA chain breaks in *Helicobacter pylori* infections and the frequency of fpg-sensitive areas, which are markers of oxidative DNA damage. There was no discernible reduction in DNA strand breakage after treatment. In contrast, it was shown that the number of fpg-sensitive regions reduced considerably following treatment ($p=0.001$), and that the number of fpg-sensitive regions before to treatment was positively connected with age ($r=0.59$; $p=0.05$).

Table 1: - DNA damage parameters before and after treatment in patients which infected with H. Pylori

	Before Treatment	After Treatment (N= 19)
Frequency of DNA Strand Breaks	99±22	101± 24 (au)
Fpg-Sensitive Zones	159± 28	135± 21*

* $P < 0,001$

Discussion

Recent studies have shown that gastric inflammatory illnesses are associated with an increase in oxidative damage (8-10), and that accumulated oxidative DNA damage in tumor suppressor genes like p53 may play a significant role in the development of gastric tumors (11). DNA damage is higher in *Helicobacter pylori*-infected gastric mucosa than in uninfected normal mucosa, as shown in studies that directly measured 8-OHdG residues on DNA (12-14) and studies that evaluated DNA chain breaks and fpg-sensitive areas (15-18). DNA damage measured by the comet method was shown to be higher in individuals over the age of 50 than in young persons, according to a study conducted by Laderia et al. (16) on patients with *helicobacter pylori* (+) gastritis. The same group of researchers (17) found that *Helicobacter pylori* infection was associated with increased DNA damage in the stomach mucosa and peripheral leukocytes.

If *Helicobacter pylori* infection raises oxidative DNA damage, then the level of DNA damage should drop after the infection is cleared. After *Helicobacter pylori* eradication, both the 8-OHdG level in the antral mucosa and gastric fluid mutagenicity decreased (13). The 8-OHdG level in the antral mucosa of patients with extensive gastritis infected with *Helicobacter pylori* was found to be higher than that of uninfected gastritis patients. Hahm et al. (14) also found that after eradicating *Helicobacter pylori*, the 8-OHdG level in stomach mucosa biopsy samples was lower than it had been before therapy. On the other hand, there are research that find the complete reverse. DNA damage

was found to be greater in the normal mucosa than in the gastritis area in a study of epithelial cells isolated from antral biopsies of *Helicobacter pylori*-infected individuals conducted by Everett et al. (15), and to have increased in the gastritis area after 6 weeks of eradication, but to have remained greater in the normal mucosa. Researchers found that DNA damage in *Helicobacter pylori*-infected gastric epithelial cells was significantly lower than in uninfected cells, and they hypothesized that this difference was related to higher cell turnover in gastritis. Similarly, Farinati et al. (12) discovered that in *Helicobacter pylori*-infected cases, the 8OHdG level increased after eradication in biopsy samples from the antrum, leading them to the conclusion that eradication did not mitigate the harm that had already been done. Urinary 8-OHdG levels in *Helicobacter pylori*-infected children showed no significant change between pre- and post-eradication studies (19).

Two weeks of treatment with clarithromycin and amoxicillin decreased base oxidation but had no effect on DNA chain breaks, as shown by our research. DNA chain breaks can be formed for a variety of reasons, including oxidative stress, UV, radiation, and DNA repair (20). Only oxygen and nitrogen radicals, however, can create 8-OHdG residues. Antibiotic therapy for *Helicobacter pylori*-associated gastritis and peptic ulcers may also be effective in the prevention of cancer due to a decrease in 8-hydroxy-2'-deoxyguanosine residues following treatment with clarithromycin and amoxicillin.

Conclusion

This study demonstrates that a two-week treatment with clarithromycin and amoxicillin effectively reduces oxidative DNA damage, as indicated by a significant decrease in fpg-sensitive regions, although it does not affect DNA chain breaks in *Helicobacter pylori*-infected patients. These findings suggest that mitigating oxidative DNA damage through antibiotic treatment may reduce the risk of *Helicobacter pylori*-associated gastric carcinogenesis. The positive correlation between age and fpg-sensitive regions highlights the need for targeted interventions in older populations. Future research should explore the long-term effects of antibiotic treatment on oxidative DNA damage and investigate additional mechanisms by which *Helicobacter pylori* influences DNA integrity to develop comprehensive strategies for cancer prevention.

References

- [1] International Agency for Research of Cancer. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Monogr. Eval. Carcinog. Risks Hum. 61:177-241, 1994.
- [2] NIH Consensus Development Panel on *Helicobacter pylori* in peptic ulcer disease. *Helicobacter pylori* in peptic ulcer disease. JAMA 1994; 272: 65–69.
- [3] Hahm KB, Lee KJ, Kim JH, et al. *Helicobacter pylori* infection, oxidative DNA damage, gastric carcinogenesis, and reversibility by rebamipide. Dig Dis Sci 43(suppl 9): 72S-77S, 1998.
- [4] Cheng KC, Cahill DS, Kasai H, et al. 8Hydroxyguanine an abundant form of oxidative DNA damage, causes G → T and A → C substitutions. J Biol Chem 267;166-172, 1992.
- [5] Hussain SP, Harris CC. Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumor suppressor genes. Cancer Res 58: 4023-4037, 1998.

- [6] Singh NP, McCoy MT, Tice RR, et al. A simple technique for quantification of low levels of DNA damage in individual cells. *Exp Cell Res* 175:184-191, 1988.
- [7] Collins AR, Raslova K, Somorovska M, et al. DNA damage in diabetes: correlation with a clinical marker. *Free Radic Biol Med* 25: 373-377, 1998.
- [8] Farinati F, Della Libera G, Cardin R, et al. Gastric antioxidant, nitrites, and mucosal lipoperoxidation in chronic gastritis and *Helicobacter pylori* infection. *J Clin Gastroenterol* 122: 275281, 1996.
- [9] Farinati F, Cardin R, Degan P, et al. Oxidative DNA damage accumulation in gastric carcinogenesis. *Gut* 42: 351-356, 1998.
- [10] Danese S, Cremonini F, Armuzzi A, et al. , CagA-positive strains affect oxygen free radicals generation by gastric mucosa. *Scand J Gastroenterol* 36: 247-250, 2001
- [11] Choi J, Yoon SH, Kim JE, et al. Gene-specific oxidative DNA damage in *Helicobacter pylori* infected human gastric mucosa. *Int J Cancer* 99: 485-490, 2002.
- [12] Farinati F, Cardin R, Russo VM, et al. Differential effects of *Helicobacter pylori* eradication on oxidative DNA damage at the gastroesophageal junction and at the gastric antrum. *Cancer Epidemiol Biomarkers Prev* 13 (11Pt 1): 17221728, 2004.
- [13] Nishibayashi H, Kanayama S, Kiyohara T, et al. *Helicobacter pylori*-induced enlarged-fold gastritis is associated with increased mutagenicity of gastric juice, increased oxidative DNA damage, and an increased risk of gastric carcinoma. *J Gastroenterol Hepatol* 18: 1384-1391, 2003.
- [14] Hahm KB, Lee KJ, Choi SY, et al. Possibility of chemoprevention by the eradication of *Helicobacter pylori*: oxidative DNA damage and apoptosis in *H. pylori* infection. *Am J Gastroenterol* 92: 1853-1857, 1997.
- [15] Everett SM, White KL, Drake IM, et al. The effect of *Helicobacter pylori* infection on levels of DNA damage in gastric epithelial cells. *Helicobacter* 7:271-280,2002.
- [16] Laderia MS, Rodrigues MA, Salvadori DM, et al. DNA damage in patients infected by *Helicobacter pylori*. *Cancer Epidemiol Biomarkers Prev* 13: 631-637, 2004.
- [17] Sidransky D, Tokino T, Hamilton SR et al. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992; 256: 102–105.
- [18] Dale A, Thomas JE, Darboe MK, Coward WA, Harding M, Weaver LT. *Helicobacter pylori* infection, gastric acid secretion, and infant growth. *J Pediatr Gastroenterol Nutr* 1998; 26: 393–397.
- [19] Fox JG, Blanco MC, Yan L et al. Role of gastric pH in isolation of *Helicobacter mustelae* from the feces of ferrets. *Gastroenterology* 1993; 104: 86–92.
- [20] Owen RJ. *Helicobacter* – species classification and identification. *Br Med Bull* 1998; 54: 17–30.