The Effect of Oral Administration PUFAs on Oxidative Stress in Patients Infected by *Helicobacter pylori* with Dyspeptic Symptom

Rasoul Sharifi¹, Mohammad Nouri², Homayun Dolatkhah³, Rasoul Estakhri⁴, Masoud Shirzomhammadi⁵ and Mehran Miroilaei⁶

¹Basic Biochemistry, Department of Biology, Ahar Branch, Islamic Azad University, Ahar, Iran
²Clinical Biochemistry, Department of Clinical Biochemistry and Laboratories Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, East Azerbaijan, Iran
³Clinical Biochemistry, Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, East Azerbaijan, Iran
⁴Pathology, Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
⁵Liver and Gastrointestinal Disease, Department of Internal Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, East Azerbaijan, Iran
⁶Biochemistry, Cell and Molecular Biology, Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

**Abstract**

**Objective:** *Helicobacter pylori* is a main etiological parameter in chronic active gastritis, gastric duodenal ulcers and gastric malignancy. Particular polyunsaturated fatty acids (PUFA) play roles in inhibitory effect on bacterial propagation. Therefore, this study investigated the protective effects of PUFAs against oxidative stress in patient infected with *H. pylori* with dyspeptic symptom.

**Methods:** This study is a double blind clinical trial whose target population was 34 patients infected with *H. pylori* with dyspeptic symptom. Patients were divided into two groups. The first group was treated with standard therapy without supplement (control), and the second group was treated with Standard Therapy and PUFAs supplement, ω-3, ω-6 and ω-9 (case group) for 2 weeks. Two biopsies from Antrum and body of stomach of all patients were collected before and after the treatment. The biopsy samples were used for quick urease test and measurement of Superoxide Dismutase, Glutathione Peroxidase, and total antioxidant capacity.

**Results:** In gastric mucosa mean levels of total capacity antioxidant were significantly increased in case group comparing with control group. Also, the mean of superoxide dismutase enzyme activity and glutathione peroxidase activity increased significantly in case group compared with control group (p value <0.001).

**Conclusion:** The findings revealed that administration of PUFAs supplement can increase total antioxidant capacity and activity of antioxidant enzymes in patients infected with *H. pylori.*

**Keywords:** *H. pylori,* Oxidative stress; Dyspepsia; PUFAs

**Introduction**

*Helicobacter pylori* is a gram-negative bacterium, single cell, multi-flagella which affects approximately 75% of the people in the world, and it is considered as a main etiological parameter in chronic active gastritis, gastric duodenal ulcers and gastric malignancy. *H. pylori* infection is the most important causes in peptic ulcers and other gastrointestinal disorders [1,2]. Previous investigations have revealed that *H. pylori* infection could often lead to increased pre-inflammation cytokines secretor such as interleukins and C-reactive proteins in the gastric epithelial cells [3-9]. In addition, this pathogen could be elevating Reactive Oxygen species (ROS) levels in gastric mucosa, and treatment infection leads to decrease of oxidative stress [10]. According to the previous investigations which were performed in mouse gastric tissue, increasing of superoxide radicals secreted from of *H. pylori* increases oxidative stress levels. This increasing may be due to production of oxygen species such as superoxide produced by neutrophil cells recruited to the gastric epithelial cells. This radical can react with the presence of NO in gastric juice and lead to the produce nitrite peroxide which is highly toxic and harmful for gastric tissues [11,12]. Although proper treatment regimen with clarithromycin is accepted as the standard triplet therapy, drug resistance is reported the most common issue in eradication of *H. pylori* infections [13-15]. Currently, findings have suggested that particular polyunsaturated fatty acids (PUFAs) play roles in inhibitory effect on bacterial propagation. The mechanisms have been described for PUFAs bacteria inhibitory effects in gastric including disrupt cell membrane and modulation the synthesis of mucosal anti-inflammatory prostaglandins, for example Prostaglandin E2 (PGE2) [16]. In fact, the improvement in duodenal ulcer treatment has led to the increasing in nutritional consumption of PUFAs [17]. Previous studies have revealed that the superoxide dismutase enzyme plays crucial roles in elimination of ROS including superoxide radicals to protect against the oxidative lesions and maintain homeostasis effects [18,19]. Therefore, evaluation of superoxide dismutase enzyme activity (SOD) is considered as an important parameter for analysis of oxidative lesions created by *H. pylori* infection [20]. In addition, according to the findings, determination of glutathione peroxides enzyme (GPX) activity is considered useful for assessment of *H. pylori* infection due to depletion glutathione storages which may lead to producing free radicals [21,22]. Due to high prevalence of *H. pylori* infection and increasing of oxidative stress markers in patients infected with this bacteria and probable beneficiary effect of PUFAs, this study aimed at assessing total antioxidant capacity (TAC), SOD and GPX enzymes activity in stomach tissue in the *H. pylori* infected patients.

**Materials and Methods**

**Sample collection**

In this study, thirty-four patients were divided into two groups, the case and control, who were referred to the clinical endoscopy of medical...
The mean of GPX activity in case group who were treated with combination of PUFAs and standard triplet-antibiotics (11.03 ± 2.50 IU/mg protein) was increased significantly (p value<0.001) in comparison with GPX activity of control group who were treated solely with standard therapy (10.14 ± 3.51 IU/mg protein) (Figure 1).

As it can be seen in Figure 2 the mean activity of glutathione peroxidase was compared in two groups. The results showed that the mean of GPX activity in case group who were treated with combination of PUFAs and standard triplet-antibiotics (11.03 ± 2.50 IU/mg protein) was increased significantly (p value<0.001) in comparison with GPX activity of control group who were treated solely with standard therapy (4.58 ± 2.30 IU/mg protein).

Finally, we determined a TAC in the gastric mucosal in both patients of two groups. The results revealed that PUFAs therapeutic regime can increase (p value<0.0001) TAC in the case group subjects (1.08 ± 0.17 mmol/L) compared with the individuals who were treated only with standard triplet-antibiotics 10.14 ± 3.51 IU/mg protein (Figure 1).

Statistical analysis
The mean of TAS, MDA, amounts and mean of GPX and SOD activity of gastric biopsy of samples was calculated, and due to the independently of studied groups, the mean of results was calculated in each group using SPSS statistical software (21 version), and normal distribution of results were examined by Shapiro Wilkes test. The results which had normal distribution were compared in two groups with Independent sample t-Test. When these tests were considered significant that p value was less than 0/05 (p<0/05).

Results
Several clinical parameters such as fast blood sugar (FBS), cholesterol and triglyceride were assessed in case and control subjects (Table 1). According to the findings, there were no significant differences between the factors assessed in case and control groups (p value>0.005). In the present study, when the sexuality types and age parameters were compared in patients and normal individuals, there were not found any significant differences in both groups (p value>0.05).

The glutathione peroxidase and superoxide dismutase enzyme activity levels were assessed in case and control groups using ultraviolet colorimetric assay. The findings revealed that the SOD activity was significantly different in case group (p value<0.001), and it was increased in the subjects that obtained combinations of PUFAs therapeutic supplementary agent and standard triplet-antibiotics regime (19.77 ± 3.02 IU/mg protein) compared with the individuals who were treated only with standard triplet-antibiotics 10.14 ± 3.51 IU/mg protein (Figure 1).

Table 1: Demographic findings of patients in this study.
against infection agent. However, natural selection makes the necessary potential in bacteria to resist local oxidative pressure [30]. These oxidative defensive mechanisms by host cells not only do not damage bacteria seriously, but also, they are deleterious to host cell itself [31]. These two mechanisms causing free radicals in both bacteria and host cells result in intensive oxidative pressure in \( H. \text{pylori} \) infection. NO molecules have tendency to react with superoxide radicals resulted from \( H. \text{pylori} \) or white cells which is led to peroxynitrite in gastric tissues. This, in turn, strengthens the oxidative stress, and the bacteria become resistant to NO bactericidal effect [31]. According to the therapeutic effects of fatty acids in several illnesses, the present study aimed to assess antibacterial effects and beneficiary effects of fatty acids in reducing oxidative stress in gastric patients infected by \( H. \text{pylori} \). Previous studies (Toorang and colleagues (2009), Mahdavi and colleagues (2011), Sarbolouki and colleagues (2010) have mentioned various mechanism for the beneficiary effects of omega fatty acids in increasing serum's total antioxidant capacity. Omega-3 fatty acids may increase catalase levels in both of cytoplasm and peroxisome. Therefore, it may improve the defense against free radicals. It has been showed that supplementary consumptions with PUFAs are replaced with PUFAs which are damaged by free radicals. In addition, silencing of gene expression by PUFAs can inhibit oxidative stress resulted in apoptosis. Furthermore, PUFAs can play a role in oxidative stress reduction by alteration prostaglandins synthesis and gene expression as well as regulating antioxidant enzymes [32-34]. It has been shown recently that omega fatty acids may play crucial roles in regulating gene expression of inflammatory factors in various cell lines. This can be effective in reducing oxidative stress. It has been proved that \( \omega-6 \) linoleic acid can inhibit the growth of \( H. \text{pylori in vitro} \). This inhibitory effect depends on the unsaturated condition of fatty acid, that is, the number of double bonds in fatty acid molecules [35]. Several studies revealed that consumption of \( \omega-3 \) PUFAs anti-inflammatory effects while consumption of \( \omega-6 \) PUFAs produced strong inflammatory factors by locating in cell membrane and metabolization [35]. Correia et al. have proved that inhibitory ability of \( \omega-3 \) PUFAs in bacteria growth and its colonization solely depends on DHA in mice gastric [36,37]. It has been shown that 100 μM concentration of DHA reduces \( H. \text{pylori} \) growth while concentration greater than 250 μM inhibits the survival of \( H. \text{pylori} \) irreversibly. In addition, it has been demonstrated that DHA may change expression and metabolism of outer membrane proteins and the phenotype of lipopolysaccharides [36,37]. It should be mention that DHA is significantly less effective in the eradication of \( H. \text{pylori} \) from mice gastric mucous comparing with the triplet standard therapy. As a result, if DHA is combined to triplet standard therapy based on clarithromycin, there could be better results in eradication of \( H. \text{pylori} \) comparing with triplet standard therapy alone. None of the mice treated with standard therapy / DHA did not show gastric colonization by bacteria after 2 months of treatment [36]. In this study it has been proved that the combination of family fatty acid omega and standard therapeutic regimes compared with standard therapy with clarithromycin significantly improved oxidative stress. The more the fatty acids are unsaturated, the more effective in reducing oxidative stress. Since the enzymes levels of elongase and desaturase is lower in gastric epithelial, it is not supposed the consumed fatty acids including linoleic acid and alpha-linolenic acid are change to long chains fatty acids such as arachidonic acid and docosahexaenoic acid. Therefore, the reductive effects of fatty acids’ oxidative stress are not related to their metabolites. Nevertheless, this effect is related to fatty acids themselves [37]. Not only do these fatty acids influence fluidity and functions of gastric cells by participating in the structure of cell membrane phospholipids, but also, they cause alteration of signaling pathways in these cells [38]. Infection to \( H. \text{pylori} \) in gastric cells results

---

**Figure 1:** Comparison of mean of SOD activity in gastric mucosa of control and case groups after treatment.

**Figure 2:** Comparison of mean of GPX activity in gastric mucosa of control and case groups after treatment.

**Figure 3:** Comparison of TAC level in gastric mucosa of control and case groups after treatment.
in IL-8 and therefore it leads to illness intensification. As it has been proven these fatty acids inhibits IL-8 expression in gastric cells in vitro [39,40].

Conclusion

The findings revealed that fatty acid omega supplementary consumption can reduce the oxidative stress conditions appeared in patients with H. pylori infections. Finally, the analysis of catalase activity and malondialdehyde levels in gastric mucosa as well as comparison of membrane phospholipids profiles in before and after PUFAs treatment regimes is suggested for future investigations.

Acknowledgements

This project consumed huge amount of work, research, and dedication. Still, implementation would not have been possible if we did not have a support of many individuals and organizations. Therefore, we would like to extend our sincere gratitude to all of them.

First of all, we are thankful to Tabriz Liver and Gastrointestinal Disease Research Center and Tehran Islamic Azad University Science and Research Branch for their financial and logistical support and for providing necessary guidance concerning projects implementation.

We are also grateful to Tabriz Endoscopy Department of Imam Reza and Shahid Madani Hospital for provision of expertise, and technical support in the implementation. Without their superior knowledge and experience, the Project would like in quality of outcomes, and thus their support has been essential.

References

1. Dolatkhah H, Babaei S, Rahbani-Nobar M (2011) Diabetes Mellitus and Helicobacter pylori. Lambert Acad Publ 139: 103-112.
2. Dolatkhah H, Rahbani-Nobar M, Fattahi E, Ansari M, Mirza-Aghazadeh A, et al. (2011) Evaluation of glycemic control, gastric juice nitric oxide and oxidative stress in diabetic patients infected by Helicobacter pylori. Journal of Medical Genetics and Genomics 3: 1-6.
3. Houghton J, Macera-Bloc LS, Harrison L, Kim KH, Korah RM (2000) Tumor Necrosis Factor Alpha and Interleukin 1β Up-Regulate Gastric Mucosal Fas Antigen Expression in Helicobacter pylori infection. Infection and Immunity 68: 1189-1195.
4. Basso D, Scrigner M, Toma A, Navaglia F, Di Mario F, et al. (1996) Helicobacter pylori infection enhances mucosal interleukin-1β, interleukin-6, and the soluble receptor of interleukin-2. International Journal of Clinical and Laboratory Research 26: 207-210.
5. Noach L, Bosma N, Jansen J, Hoek F, Van Deventer S, et al. (1994) Mucosal Tumor Necrosis Factor-α, Interleukin-1/3, and Interleukin-8 Production in Patients with Helicobacter Pylori Infection. Scandinavian Journal of Gastroenterology 29: 425-429.
6. Karttunen R, Karttunen T, Yousfi M, El-Zimey H, Graham D, et al. (1997) Expression of mRNA for interferon-γ, interleukin-10, and interleukin-12 (p40) in normal gastric mucosa and in mucosa infected with Helicobacter pylori. Scandinavian Journal of Gastroenterology 32: 22-27.
7. Jung HC, Kim JM, Song IS, Kim CV (1997) Helicobacter pylori induces an array of pro-inflammatory cytokines in human gastric epithelial cells: Quantification of mRNA for interleukin-8, -1α/b, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1 and tumour necrosis factor-α. Journal of Gastroenterology and Hepatology 12: 473-480.
8. Yun CH, Lundgren A, Azem J, Sjöling Å, Holmgren J, et al. (2005) Natural killer cells and Helicobacter pylori infection: bacterial antigens and interleukin-12 act synergistically to induce gamma interferon production. Infection and Immunity 73: 1482-1490.
9. Jafarzadeh A, Hassanshahi G, Nemat M (2009) Serum levels of high-sensitivity C-reactive protein (hs-CRP) in Helicobacter pylori-infected peptic ulcer patients and its association with bacterial CagA virulence factor. Digestive Diseases and Sciences 54: 2612-2616.
10. Suzuki H, Mori M, Seto K, Kai A, Kawaguchi C, et al. (1999) Helicobacter pylori-associated gastric pro-and antioxidant formation in Mongolian gerbils. Free Radical Biology and Medicine 26: 679-684.
11. Bergaust L, Van Spanning RJ, Frostegård Å, Bakken LR (2012) Expression of nitrous oxide redox in Paracoccus denitrificans is regulated by oxygen and nitric oxide through FnrP and NFR. Microbiology 158: 826-834.
12. Inoue M, Nishikawa M, Sato EF, Ah-Mee P, Kashiba M, et al. (1999) Cross-talk of NO, superoxide and molecular oxygen, a majesty of aerobic life. Free Radical Research 31: 251-260.
13. Malfertheiner P, Megraud F, O'Morain C, Bell D, Porr B, et al. (1997) Current European concepts in the management of Helicobacter pylori infection—The Maastricht Consensus Report. European Journal of Gastroenterology and Hepatology 9: 1-2.
14. Chey WD, Wong BC (2007) American College of Gastroenterology guideline on the management of Helicobacter pylori infection. The American Journal of Gastroenterology 102: 1808-1825.
15. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, et al. (2007) Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut 56: 772-781.
16. Das U, Ramadevi G, Rao K, Rao M (1989) Prostaglandins can modify gamma-radiation and chemical induced cytotoxicity and genetic damage in vitro and in vivo. Prostaglandins 38: 689-718.
17. Tarnawski A, Hollander D, Gergely H (1987) Protection of the gastric mucosa by linoleic acid—a nutrient essential fatty acid. Clinical and Investigative Medicine, Medicine Clinique et Experimentale: 10: 132-135.
18. Nakamura A, Park AM, Nagata K, Sato EF, Kashiba M, et al. (2000) Oxidative cellular damage associated with transformation of Helicobacter pylori from a bacillary to a coccoid form. Free Radical Biology and Medicine 28: 1611-1618.
19. Beckerman K, Rogers H, Corbett J, Schreiber R, McDaniel M, et al. (1993) Release of nitric oxide during the T cell-independent pathway of macrophage activation, its role in resistance to Listeria monocytogenes. The Journal of Immunology 150: 889-895.
20. Noguchi K, Kato M, Moriya T, Suzuki T, Saito M, et al. (2002) Analysis of cell damage in Helicobacter pylori-associated gastritis. Pathology International 52: 110-118.
21. Jung HK, Lee KE, Chu SH, Yi SY (2001) Reactive oxygen species activity, mucosal lipoperoxidation and glutathione in Helicobacter pylori-infected gastric mucosa. Journal of Gastroenterology and Hepatology 16: 1336-1340.
22. Yoshikawa T, Minamiyama Y, Ichikawa H, Takahashi S, Naito Y, et al. (1997) Role of lipid peroxidation and antioxidants in gastric mucosal injury induced by the hypoxanthine-xanthine oxidase system in rats. Free Radical Biology and Medicine 23: 243-250.
23. Benzle IFF, Strain JJ (1999) Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299: 15-27.
24. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine 70: 158-169.
25. L'Abbé MR, Fischer PW (1990) Automated assay of superoxide dismutase in blood. Methods in Enzymology 186: 232-237.
26. Zhu Y, Zhou X, Wu J, Su J, Zhang G (2014) Risk factors and prevalence of Helicobacter pylori infection in persistent high incidence area of gastric carcinoma in Yangzhou city. Gastroenterology Research and Practice 2014: 1-10.
27. Miyazawa M, Suzuki H, Masaoka T, Kai A, Suematsu M, et al. (2003) Suppressed apoptosis in the inflamed gastric mucosa of Helicobacter pylori-colonized INOS-knockout mice. Free Radical Biology and Medicine 34: 1621-1630.
28. Dietrich M, Block G, Hudes M, Morrow JD, Norkus EP, et al. (2002) Antioxidant supplementation decreases lipid peroxidation biomarker F2-isoprostanes in plasma of smokers. Cancer Epidemiology Biomarkers and Prevention 11: 7-13.
29. Farinati F, Della Libera G, Cardin R, Molaro A, Plebani M, et al. (1996) Gastric antioxidant, nitrates, and mucosal lipoperoxidation in chronic gastritis and Helicobacter pylori infection. Journal of Clinical Gastroenterology 22: 275-281.
30. Debowski AW, Carnoy C, Verbrugghe P, Nilsson HO, Gaaultt JC, et al. 2012. Xer reconstituzione e genome integrity in Helicobacter pylori, a pathogen without topoisomerases IV. PloS ONE 7: e33310.
31. Park AM, Nagata K, Sato EF, Tamura T, Shimono K, et al. (2003) Mechanism of strong resistance of Helicobacter pylori respiration to nitric oxide. Archives of Biochemistry and Biophysics 411: 129-135.
32. Toorang F, Djazayeri A, Jalali M, Eshraghian M, Farvid M, et al. (2008) The Effect of Oral Administration PUFAs on Oxidative Stress in Patients Infected by Helicobacter pylori with Dyspeptic Symptom. Med Chem 7: 324-328. doi: 10.4172/2161-0444.1000476.
effect of supplementation with omega-3 fatty acids on HbA1c, total antioxidant capacity and superoxide dismutase and catalase activity in type 2 diabetic patients. Iran J Food Sci Tech 3: 1-8.

33. Sarbolouki S, Djalali M, Dorosty A, Dizayery S, Eshraghian M, et al. (2010) Effects of EPA and vitamin E on serum enzymatic antioxidants and peroxidation indices in patients with type II Diabetes Mellitus. Iranian Journal of Public Health 39: 82-91.

34. Mahdavi R, Nemati A, Feizi E, Amani M, Alimohammadi Asl H, et al. (2011) Effect of ω-3 Fatty Acid Supplementation on Oxidative Stress in Gastric Cancer Patients Undergoing Chemotherapy. Journal of Ardabil University of Medical Sciences 11: 166-175.

35. Ley C, Mohar A, Guamer J, Herrera-Goepfert R, Figueroa LS, et al. (2004) Helicobacter pylori eradication and gastric preneoplastic conditions a randomized, double-blind, placebo-controlled trial. Cancer Epidemiology Biomarkers and Prevention 13: 4-10.

36. Correia M, Michel V, Matos AA, Carvalho P, Oliveira MJ, et al. (2012) Docosahexaenoic acid inhibits Helicobacter pylori growth in vitro and mice gastric mucosa colonization. PLoS One 7: e35072.

37. Duggan A, Atherton J, Cockayne A, Balsitis M, Evison S, et al. (1997) Clarification of the link between polyunsaturated fatty acids and Helicobacter pylori-associated duodenal ulcer disease: a dietary intervention study. British Journal of Nutrition 78: 515-522.

38. De Caterina R, Bernini W, Cartuccio MA, Liao JK, Libby P (1998) Structural requirements for inhibition of cytokine-induced endothelial activation by unsaturated fatty acids. Journal of Lipid Research 39: 1062-1070.

39. Wetzker R, Böhrer FD (2003) Transactivation joins multiple tracks to the ERK/MAPK cascade. Nature Reviews Molecular Cell Biology 4: 651-657.

40. Chu SH, Kim H, Seo JY, Lim JW, Mukaida N, et al. (2003) Role of NF-κB and AP-1 on Helicobacter pylori-induced IL-8 expression in AGS cells. Digestive Diseases and Sciences 48: 257-265.