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The Effects of L-Arginine on Oxidative and Nitrosative Stress and Inflammation Factors in Patients Infected with *Helicobacter pylori*

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Abstract

Background: *Helicobacter pylori (H. pylori)* plays the primary role in increasing oxidative stress and causing stomach inflammation, peptic ulcers, and gastric malignancy in the infected patients. L-arginine (Arg) has antibacterial and anti-inflammatory effects.
Objectives: The current study aimed at investigating the beneficial effects of L-arginine on inflammation and oxidative stress in patients infected with *H. pylori* with dyspeptic symptoms.
Methods: The current randomized, double-blind controlled, clinical trial was conducted on 34 patients with *H. pylori* infection referred to the center of digestive disorders affiliated to Isfahan University of Medical Sciences, Isfahan, Iran, in order to undergo endoscopy from December 2016 to September 2017. Patients were classified into two groups (control and treatment); the control group only received triple-drug therapy (including Amoxicillin, Clarithromycin, and Omeprazole), and the treatment group received standard triple-drug therapy and L-Arg capsules for three weeks. Gastric biopsies and serum samples were taken from all patients before and after the study. *H. pylori* infection was examined by a rapid urease test and antioxidant indices including superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (TAC) were evaluated in gastric biopsies. In addition, serum samples were used to measure the inflammation factors including interleukin (IL)-8 and tumor necrosis factor (TNF)-α.
Results: Level of SOD activity increased significantly in the treatment group compared with that of the control group (4.91 ± 95.21 vs. 4.0 ± 44.11 IU/mg) ($P = 0.001$). In the treatment group, compared with the control group, the level of TAC increased significantly (0.35 ± 0.60 vs. 0.30 ± 0.9 mM/L) ($P = 0.006$) and the level of GPX activity increased significantly in the treatment group compared with the control group (10.68 ± 2.39 vs. 5.16 ± 2.12 IU/mg) ($P = 0.000$). Regarding the inflammation factor, IL-8 decreased significantly in the treatment group compared with the control group (8.00 ± 1.94 vs. 10.28 ± 2.10 pg/mL) ($P = 0.002$); also TNF-α decreased significantly in the treatment group compared with the control group (9.71 ± 2.69 vs. 12.24 ± 3.29 pg/mL) ($P = 0.036$), while there was no significant difference in high sensitivity C-reactive protein (hs-CRP) decrease between the treatment and the control groups (2.34 ± 1.28 vs. 3.04 ± 1.58 mg/L) ($P = 0.16$).
Conclusions: Consumption of L-arginine increased antioxidant indices and decreased inflammation in patients infected with *H. pylori*.

Keywords: *Helicobacter pylori*, Factor, Inflammation, L-Arginine, Nitrosative Stress, Oxidative Stress

1. Background

Helicobacter pylori (H. pylori) is the main etiological factor and the most frequent cause of gastric lesions such as peptic ulcer and gastric cancer (1). Approximately, 50% of the world's population are infected with *H. pylori* (2). Infection with these bacteria leads to increased production of inflammation factors such as interleukins (IL) and C-reactive proteins (CRP) in the gastric epithelial cells (3). It is shown that *H. pylori* infection increases the IL-6 and IL-8 expression in stomach cells (4). IL-8 is a potent mediator, which activates and recruits basophils, T-cells, and neutrophil to the infected location (5). In addition, an increase of oxidative and nitrosative stress in gastric cells is associated with *H. pylori* infection (5), and this is one of the main mechanisms leading to epithelial injury (6). Alteration in damaged tissue proliferation and apoptosis is the consequence of *H. pylori* infection (7). Reactive oxygen species (ROS) are produced from activated phagocytic leukocytes and *H. pylori* itself in infected gastric cells (8). The produced superoxide radicals from these sources interact with higher amounts of Nitric Oxide (NO) radicals in the stomach and in turn, leads to the production of nitrite peroxide radical, which intensifies oxidative stress

and stomach injury (9). It is noteworthy that approximately, half of the world's population are infected with *H. pylori*, which highlights the need for the eradication of the bacteria. Standard triple-drug therapy, based on Clarithromycin, is popular and the main regimen for *H. pylori* eradication (10); but considering the fact that *H. pylori* is resistant to antibiotics, there is a considerable interest to find natural agents with low toxicity to eradicate the bacteria. However, nutritional supplementation contains essential nutrients, which modulate immune, inflammatory, and metabolic pathways, and offers a therapeutic model to reduce the risk of cancer in patients infected with these bacteria. In this respect, special attention is paid to the amino acid L-arginine (Arg), which has direct effects on the immune system (11). *In vitro* and *in vivo* studies on patients with cancer showed that Arg dietary supplement stimulated many immune system activities such phagocytic activity of macrophage and natural killer cells (NKC). Another study showed that this cationic amino acid interacts with negative groups on the bacterial surface and inhibits its growth and colonization (12). It is shown that Arg decreases the inflammation factors such as IL and MDA (malondialdehyde) levels, and increases the activity of antioxidant enzymes and total antioxidant capacity (13-17). In addition, Arg is a precursor of NO, which is a potent antiseptic factor in the stomach (14).

The effects of Arg on the antioxidant status of the gastric tissue as well as the serum inflammatory factors in patients with *H. pylori* infection are not studied.

Therefore, the current study aimed at clarifying the Arg role in the improvement of oxidative stress and its effect on the production of inflammation factor.

2. Methods

2.1. Patients

The current randomized, double-blind, controlled clinical trial was conducted on 34 patients with *H. pylori* infection. Patients with upper gastrointestinal discomfort referring to the center of digestive disorders at Al-Zahra Hospital affiliated to Isfahan University of Medical Sciences to undergo endoscopy from December 2016 to September 2017 were selected and equally divided into two groups. Allocation of patients into each group was based on the inconvenience sampling method with a coin-flip. Considering previous researches and the mean of expression for caspase-3, and due to 95% confidence interval (CI), and 80% power, the sample volume was calculated as 17 cases in each group. The written informed consent was obtained from all patients. The control group received standard triple-drug therapy (including Omeprazole, Amoxicillin, and Clarithromycin). The duration of antibiotic consumption was different, depending on location and resistance to antibiotics; generally the consumption period was two weeks. The control group received Clarithromycin 500 mg twice daily, Amoxicillin 1 g twice daily, and Omeprazole 20 mg twice daily for 14 days. The treatment group received standard triple-drug therapy and L-Arg capsule (1500 mg/day) for three weeks. Arg capsules were purchased from ALLMAX Nutrition company (Canada), and the treatment group received three capsules daily. Patients with gastric cancer, renal failure, and the ones using nitroglycerine, protein supplements, and Arg previously were excluded from the study. Gastric biopsies and serum samples were taken from all patients before and after the study in fasting status by a gastroenterologist. The biopsies were stored in a nitrogen tank at -70°C and sera were kept at -20°C. *H. pylori* infection was examined by a rapid urease test; and antioxidant indices including superoxide dismutase (SOD), glutathione peroxidase (GPX), and TAC were evaluated in gastric biopsies. In addition, sera samples were used to measure the inflammation markers including IL-8, hs-CRP, and TNF-α after making sure that the instrument was calibrated.

2.2. Determination of TAC in Gastric Biopsies

Evaluation of TAC was performed using the FRA (ferric reducing ability) method. Based on this method, Fe^{+3} to Fe^{+2} reduction in the presence of TPTZ (Tripyridyl-S-Triazine) leads to violet Fe^{+2}-TPTZ complex and this is an index of antioxidant capacity of stomach juice. This complex is blue and the increase of Fe^{+2}-TPTZ concentration can be measured at 593 nm using a spectrophotometer JENWAY 6505 (18).

2.3. Determination of Antioxidant Enzyme Activities

Determination of SOD activity in gastric biopsy was performed by the Ransod method and Randox Kit (England). Based on this method, O_2^- radicals produced using xanthine and xanthine oxidase reacts with 2-(4-iodophenol)-3-(4-nitrophenol)-5-pheniltetrazolium chloride (INT) and lead to form a red formazan dye. This reaction is inhibited by SOD and its activity is determined as an inhibition percent (19). A unit of SOD activity is defined as 50% inhibition of the rate of INT reduction under the assay conditions.

GPX activity of biopsies was measured by Randox Kit (England) based on the method of Paglia and Valentine. based on this method, reduced glutathione is oxidized by GPX in the presence of cumene hydroperoxide, then glutathione reductase converts the oxidized glutathione to reduce glutathione and following that nicotinamide adenine dinucleotide phosphate (NADPH) is converted to $NADP^+$. The absorbance reduction of NADPH was measured at 340 nm (20). Consumption 1 μM of NADPH per minute is equal to 1 GPX unit.

2.4. Evaluation of Inflammation Factors

The levels of TNF-α and IL-8 were measured by the commercially available enzyme-linked immunosorbent assay (ELISA) kits of AviBion Human TNF-α and AviBion Human IL-8 according to the manufacturer's instruction (both kits were purchased from Orgenium Company, Helsinki, Finland). The sensitivity of both ELISA kits is less than 4 pg/mL and their assay range is 3.9 - 250 pg/mL; their concentration was measured using the ELISA microplate reader (Awareness, Model stat fax 2100, USA) and the immunoturbidimetry assay was used to evaluate serum level of hs-CRP (21). The serum CRP-anti-CRP polyclonal antibody (latex-fixed) complex creates turbidity. This turbidity is equal to serum CRP level and is measurable at $\lambda = 500$ nm by auto-analyzer (Abbot-Alcyon 300-USA).

2.5. Measurement of NO in Stomach Juices

Griess colorimetric assay is a direct and easy method to measure two metabolites of NO (nitrite and nitrate) performed in two stages. First, nitrate is reduced to nitrite and then Griess reagent is added to the test sample, which changes nitrite into the azo compound, in dark purple, and can be measured in a wavelength of 540 nm by a spectrometer JENWAY 6505 (England) (22).

2.6. Statistical Analysis

The mean of each variable was calculated in each group and the data were analyzed with IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA) with independent samples t-test. All data were expressed as the mean $\pm$ standard error (SE) and $P < 0.05$ was considered as the level of significance.

3. Results

3.1. Demographic Data

The levels of some clinical parameters were measured in the treatment and control groups. Differences in the parameters between the groups were not significant (Table 1). In addition, there were no significant differences between the groups in terms of gender, weight, and age ($P > 0.05$).

3.2. Inflammation Factors

In Table 2, the levels of serum inflammation markers in the two groups were summarized. The levels of serum IL-8 and TNF-α decreased significantly in the treatment group, compared with the control group after consumption of arginine, while serum hs-CRP decrease was not significant between the two groups.

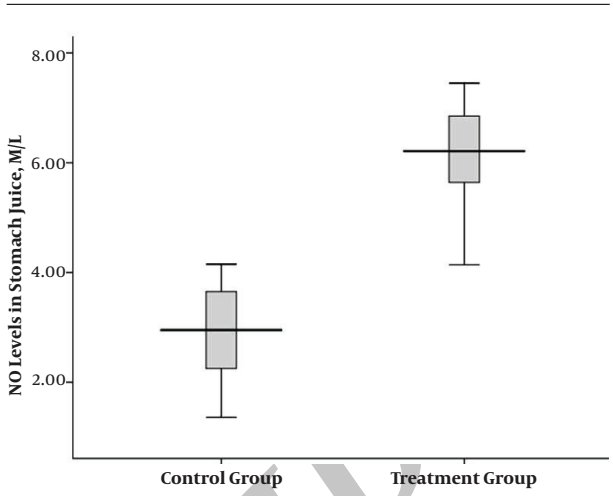

Figure 1. TAC level of gastric biopsies in the treatment and control groups

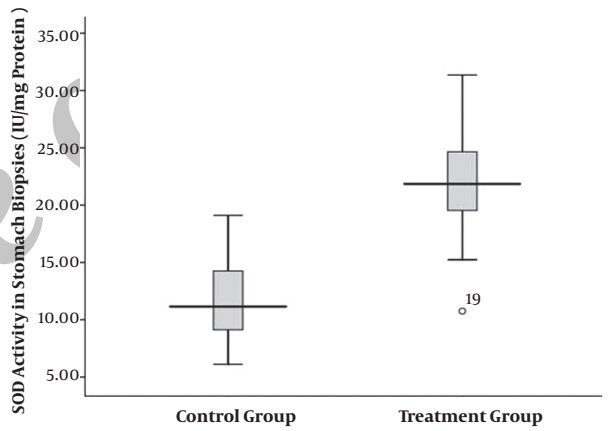

Figure 2. SOD activity of gastric biopsies in the treatment and control groups

3.3. Antioxidant Indices

TAC level of gastric biopsies significantly increased after consumption of arginine supplement in the treatment group, compared with the control group (Figure 1). TAC level in the treatment and control groups were 0.35 ± 0.60 and 0.30 ± 0.9 mM/L, respectively ($P = 0.006$).

After consumption of arginine supplement, SOD activity of gastric biopsies significantly increased in the treatment group compared with the control group (Figure 2). SOD activity in the treatment and control groups were 4.91 ± 95.21 and 4.0 ± 44.11 IU/mg, respectively ($P = 0.001$).

GPX activity of gastric biopsies increased significantly in the treatment group compared with the control group after consumption of arginine supplement (Figure 3). GPX activities in the treatment and control groups were 10.68

Table 1. Demographic Results of Subjects (N = 17)

Variables	Control	Treatment	P Value
Age, y	53 ± 11.51	58 ± 10.69	0.191, Based on t-test
Weight, kg	86.2 ± 17.85	79.47 ± 19.95	0.322, Based on t-test
Gender			0.734, Based on Chi-Square test
Male (n = 16)	8	8	
Female (n = 18)	9	9	
FBS, mg/dL	87.09 ± 10.61	93.23 ± 12.11	0.126, Based on t-test
Systolic BP, mmHg	12.08 ± 1.09	12.67 ± 1.24	0.154, Based on t-test
Diastolic BP, mmHg	7.08 ± 0.75	7.42 ± 0.81	0.223, Based on t-test
HDL, mg/dL	41.35 ± 12.77	44.76 ± 11.16	0.412, Based on t-test
Cholesterol, mg/dL	128.28 ± 28.46	115.03 ± 19.121	0.134, Based on t-test
LDL, mg/dL	88.76 ± 28.77	94.64 ± 34.47	0.592, Based on t-test
Triglyceride, mg/dL	89.35 ± 18.53	80.62 ± 14.00	0.131, Based on t-test

Table 2. Levels of Inflammation Markers in the Study Groups

Information Factor	Control Group	Treatment Group	P Value Based on t-Test
IL-8, pg/mL	1028 ± 2.10	8.00 ± 1.94	0.002
hS-CRP, mg/L	3.04 ± 1.58	2.34 ± 1.28	0.16
TNF-α, pg/mL	12.24 ± 3.92	9.71 ± 2.69	0.036

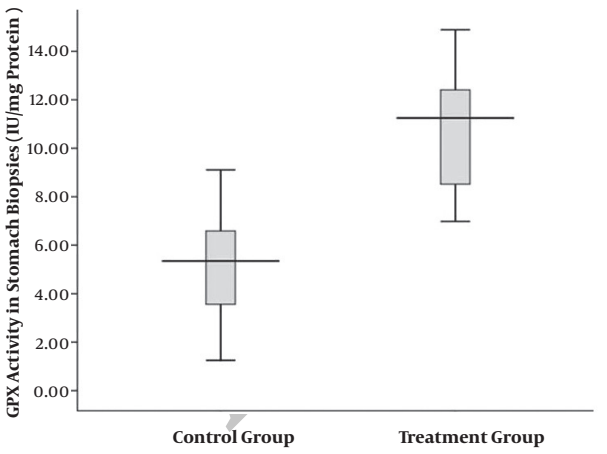

Figure 3. GPX activity of gastric biopsies in the treatment and control groups

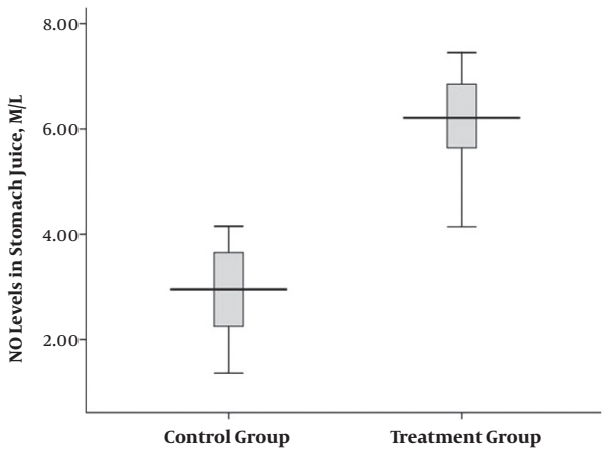

Figure 4. NO level of gastric biopsies in the treatment and control groups

± 2.39 and 5.16 ± 2.12 IU/mg, respectively (P < 0.0001).

3.4. NO

After consumption of arginine supplement, NO level of gastric juice increased significantly in the treatment group compared with the control group (Figure 4). NO levels in the treatment and the control groups were 2.92 ± 0.88 and 6.17 ± 0.90 M/L, respectively (P = 0.001).

4. Discussion

Oxidative stress is one of the main problems related to *H. pylori* infection (23); these bacteria promote oxidative stress and inflammation in damaged epithelial gastric cells. *Helicobacter pylori* infection causes recalling and recruiting neutrophils to injured gastric epithelial cells and this phenomenon results in superoxide radical pro-

duction, and therefore, intensifies injury to the host cells (24). Many studies showed that Arg consumption inhibits oxidative stress, increases antioxidant enzymes activities, and decreases lipid peroxidation in some of the inflammation related diseases (25). It is observed that some amino acids disrupt bacterial cell wall integrity in high concentration. Wessolowski et al., showed that Arg hexamer has antimicrobial effects and another study reported that peptides with antibacterial effects have lysine and arginine in their structures, which interact with negative groups in the bacterial surface, and then, form special hairpin structure (26). In addition, arginine rich-peptides are permeable to bacterial cell walls and this amino acid can increase the interface between bacteria and arginine in hexamers (27, 28). *H. pylori* cannot synthesize cholesterol; therefore, it obtains non-esterified cholesterol from gastric epithelial cell membrane and converts it to a-glycosylated form, which incorporates into bacteria membrane (24). Recently, it is observed that a-glycosylated cholesterol is a mechanism for drug resistance, since it helps *H. pylori* to escape from host immune system and phagocytosis and remain immune to bile salts (29). On the other hand, cholesterol acts as a bridge to attach to the host cell membrane; therefore, cholesterol depletion can be a mechanism against colonization of these bacteria (30). It is hypothesized that NO is produced from L-arginine, and inhibits the cholesterol production in gastric epithelial cells. Therefore, L-arginine has indirect effects on *H. pylori* survival through regulation of cholesterol availability to these bacteria in gastric epithelial cells (31). In the current study, Arg improved tissue antioxidant indices and decreased serum inflammatory markers and consequently, increased tissue NO level. Hnia et al. showed that L-arginine supplementation decreased inflammation markers such as IL-6, IL-1β, and TNF-α in mice with Duchenne muscular dystrophy (32). In another study, Boger et al. demonstrated that Arg consumption can improve serum NO level and decrease superoxide anion in hypercholesterolemia rabbits; therefore, restored endothelial function increased NO production and protected NO from early breakdown by superoxide anion in these animals (33). In addition, it is observed that dietary arginine supplementation (3 g/day for eight weeks) increased serum TAC levels without changes in GPX and SOD activities in prediabetic patients (34). There are several proposed mechanisms that might explain the antioxidant properties of Arg. Huang et al. showed that L-arginine inhibited xanthine oxidase activity, a kind of metallo-flavoprotein, which produces H_2O_2 and O_2o and is a mechanism of free radicals production (35). Another possible mechanism is that Arg can improve oxidative stress by decreasing cellular attachment and homocysteine production (36). Furthermore, Lin et al. reported that *H. pylori* induced IL-8 production in host epithelial gastric cell, and therefore, intensified illness (37). The potential mediators in IL-8 expression in stomach cells are PKCδ and EGFR (epidermal growth factor receptor). PKC belongs to a family of protein-serine/threonine kinases that acts as integrators of mitogenic signals in many cellular responses (37). In the current study, both oxidative stress and inflammation markers level decreased by arginine consumption; therefore, L-arginine can inhibit IL-8 production and its related signals. According to previous studies, high dosage of Arg causes diarrhea in patients; therefore, the 1500 mg/day dosage was selected in the current study, which was one of the limitations of the study.

4.1. Conclusion

The results of the current study clearly suggested that alternative therapies such as Arg combined with the standard therapy, which changes inflammation markers level and oxidative stress status, might be identified as a new therapeutic strategy to manage *H. pylori* infection.

Footnotes

Conflict of Interest: The authors declared no conflict of interest.

Ethical Approval: The current study protocol was approved by the ethical committee of the local university and the procedures were conducted based on the 1964 Helsinki declaration and its latest modifications.

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