The Effect of Oral Administration of Polyunsaturated Fatty Acids on the Nitrosative Stress of Patients Infected With Helicobacter pylori With Dyspeptic Symptoms

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Abstract
Objectives: Helicobacter pylori is the cause of many stomach diseases such as chronic gastritis, peptic lesion, gastric cancer, and dyspepsia. It is indicated that the process of inflammation is associated with nitrosative stress. Besides, polyunsaturated fatty acids (PUFAs) have strong protective effects against inflammatory diseases, as well as bactericidal effects. Therefore, this study evaluated the beneficiary effects of PUFAs against nitrosative stress in patients infected with this bacterium with dyspeptic symptoms.

Materials and Methods: This study was a double-blinded clinical trial and the participants were 34 patients infected with H. pylori with dyspeptic symptoms. The patients were divided into 2 groups, and written consent was obtained from all participants. The control group was treated with a current antibiotic regimen and the case group was treated with an antibiotics regimen and PUFAs supplement for 2 weeks. Then, biopsy and juice samples from patients’ stomachs were obtained before and after the treatment. The stomach biopsies were used for a quick urease test and juice samples were used via the Griess method for investigating nitric oxide (NO) levels.

Results: In gastric mucosa, the mean levels of NO significantly reduced (P<0.0001) after treatment in the case group (5.36 ± 1.16 mmol/L) compared with the control group (2.72 ± 1.13 mmol/L).

Conclusions: The results showed that using omega supplement can improve the nitrosative stress occurred in patients with H. pylori infections and is useful for decreasing NO. Thus, it is suggested that the consumption of omega fatty acids in combination with standard therapy is effective in H. pylori eradication.

Keywords: H. pylori, Nitrosative stress, Dyspepsia, PUFAs

Introduction
The available evidence indicates that nitrosative stress is involved in the pathogenesis of more than one hundred diseases (1). In the gastric juice of healthy people, the physiological level of nitric oxide (NO) is relatively high which destroys pathogens from food and mouth cavity (2-5). NO is a multifunctional radical which has a high affinity for attachment to proteins with iron and copper (6). This radical is produced from different cells such as vein endothelial cells, nerve cells, neutrophils, and macrophages (7, 8). The isoenzymes of NO synthesis exist in stomach mucosa (7). In addition, NO is produced via the non-enzymatic reduction of nitrate from saliva and food in the gastric juice (9). Further, this radical prevents bacterial activity because of NO interaction with the terminal oxidase of most aerobic bacteria. It has been reported that Helicobacter pylori produces superoxide radical (O2•-) in high amounts which interacts with the NO of gastric juice. This process may anticipate a mechanism which allows H. pylori to resist the NO of the gastric juice. Therefore, the growth and colonization of these bacteria are provided in the antrum of the stomach (2-5). The measurement of NO in biological systems requires great attention because NO undergoes a series of reactions in the presence of several biological solutions and then changes into nitrite or nitrate. Furthermore, it has a relatively short half-life of about less than a second in biological systems (8). The reactions of changing NO into nitrate and nitrite are as follows:

NO + O2→ ONO2- + H+→ NO3- + H+
2NO + O2→ N2O4 + H2O → NO2- + NO3-
NO + NO2→N2O3 + H2O → 2NO2-

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Nitrite (NO2-) and nitrate (NO3-) are the final products of NO in organisms. The relative amount of nitrite and nitrate is variable and thus their exact amount is unpredictable. Therefore, the best index for determination of total NO in biologic liquids is the calculation of the total amount of nitrite and nitrate.

It should be mentioned that superoxide radical is produced through the transformation of this bacteria from bacillus to the coccoid form. Thus, the reactions between NO and \(O_2^-\) leads to the production of peroxynitrite (OONO\(^-\)) which is highly toxic and detrimental for stomach tissues (10, 11). Since 1997, standard therapy has been introduced for the eradication of \(H. pylori\), and then it became universal. This therapy involves the proton pump inhibitor-clarithromycin and amoxicillin or metronidazole administered for one week (11-14) although the eradication of bacteria is not really successful by this method. In addition, the administration of antibiotics in a long time did not show satisfactory results in patients (15-17). Regarding \(H. pylori\) infection, the reduction of duodenal ulcer incidence depends on the increasing consumption of dietary polyunsaturated fatty acids (PUFAs) (18). It has led to high interest in the antibacterial effects of PUFAs. Different studies investigated the effect of the nutrition diet on the prevention of some diseases. These studies showed that the consumption of ω-3 fatty acid supplement causes the inhibition of oxidative stress and increases antioxidant activity (19,20) while decreasing lipid peroxidation in some diseases such as stomach cancer (21).

However, no study is available regarding the relation between \(H. pylori\) infection and the positive effect of these fatty acids in reducing nitrosative stress in stomach tissues. Therefore, for better and further evaluations of most lesions, the determination of the amount of NO in stomach mucosa can be helpful as complementary parameters.

Materials and Methods
Study Design
The present study was a double-blinded, before and after the clinical trial (intervention) in which neither the specialist nor the patients were aware of the treatment and many of the potential errors were prevented accordingly. In general, 34 patients (including 18 males and 16 females) were divided into two groups of 17 after referring to the Endoscopy Department of the Clinic of Tabriz University of Medical Sciences.

Treatment and Sampling
After the definite diagnosis of dyspeptic symptoms, biopsy and juice were obtained from the patients’ gastric. The biopsy was used for \(H. pylori\) detection via a quick urease test and juices were used for NO measurement. The patients were divided into the case and control groups. The control group was treated with a common antibiotic regimen including omeprazole, amoxicillin, and clarithromycin, and the case group was treated with a common antibiotic regimen and PUFA capsule (three capsules per day) for 2 weeks. The patients received capsules including Natural Factors Ultimate–Omega Factors 90 Softgels (SKU 2260), as well as soft gels containing wild fish oil blend (molecularly distilled, ultra-purified, anchovy, sardine and/or mackerel) 400 mg, eicosapentaenoic acid 120 mg, docosahexaenoic acid 80 mg, organic flaxseed oil blend (Linum usitatissimum, seed) 400 mg, alpha-linolenic acid 200 mg, oleic acid (OA) 60 mg, borage seeds (Borago officinalis) 400 mg, gamma-linolenic acid 75 mg, and OA 55 mg. On the other hand, patients with digestive system cancer and kidney disease in addition to diabetic patients, and those who have currently consumed omega supplements were excluded from this study. The patients’ stomach biopsies and juices were obtained in a fasting condition by a gastrointestinal specialist. All patients completed written informed consent, and the study was carried out under the supervision of a gastrointestinal specialist. In the end of capsule consumption, endoscopy was accomplished again for all patients in a fasting condition, and the eradication of \(H. pylori\) and deletion of active chronic gastritis were evaluated by enrolling stomach biopsy. The enrolled juice samples were transferred to the related department for NO evaluation.

Identifying Fatty Acid Pattern in Omega-3, 6, and 9 Capsules Prescribed to the Patients
To ensure the exact amount of fatty acids in the capsules administered to patients with “Natural Factors Ultimate-Omega Factors” brand name (Natural Factors North America, SKU 2260), fatty acid patterns in the capsules were determined by following the protocol of Bligh and Dyer (22). Then, after the primary purification of the lipids, the direct transesterification of fatty acids was done based on the protocol of Lepage et al (23). Finally, the area under curve of each fatty acid was measured by Peak simple 3.59 software, and data were shown in percentage scale (24).

Measurement of Gastric Juice Nitric Oxide
The Griess colorimetric method was used for the measurement of two metabolites of NO (nitrite and nitrate) which was performed in 2 simple stages. In the first stage, nitrate in a reduction reaction was converted to nitrite and, in the second stage, the Griess reagent was added to the test sample altering nitrite into a dark purple compound called “Azo”, which can be measured in 540 nm using a spectrometer (25).

Demographic Parameters Measurement
Several clinical parameters were assessed, including fasting blood sugar, cholesterol, and triglyceride using Pars-Azmoon kits, as well as some demographic parameters in
cases and control groups (Table 1).

**Statistical Analysis**
The mean of NO amount was calculated and analyzed using SPSS statistical software (21 version) and the independent sample t test method. The significant level was less than 0.05 ($P<0.05$).

**Results**

**Demographic Data of the Studied Groups**
There was no significant difference between the factors obtained in cases and control groups (Table 1). In addition, no significant difference was found in both groups when the sexuality types and age parameters were compared between patients and normal individuals ($P>0.05$).

**Determination of the Pattern of Fatty Acids in Omega-3, 6, and 9 Capsules Prescribed to Patients**

Table 2 presents the percent of the fatty acid pattern in “Natural Factors Ultimate-Omega Factors” capsules. The percent of Omega-3, -6, and -9 fatty acids was equal to 22.76%, 28.71%, and 16.04%, respectively.

The NO levels were evaluated in both groups using the colorimetric method. The findings showed that NO levels were significantly different ($P<0.001$) and decreased in subjects who consumed a PUFA supplement and standard triplet-antibiotics regime ($2.72\pm1.13\text{M/L}$) compared with those who were treated only with standard triplet-antibiotics ($5.36\pm1.16\text{M/L}$), related data of which are depicted in Figure 1.

**Discussion**
Nitrosative stress refers to an increase in NO and its chemical derivatives (26). Recent evidence shows that nitrosative stress is involved in the pathogenesis of many diseases (1). The concentration of NO in healthy persons is relatively high and NO is produced by three isozymes which exist in the stomach (5). *H. pylori* is located in the gastric epithelial cell, exactly in the appearance point of NO synthase in the lamina layer and causes the inhibition of NO production in this site and, easily interacts with cells containing NO synthase (26). Moreover, NO has a tendency to interact with superoxide radical produced from *H. pylori* or the white blood cell thus produces the peroxynitrite radical that is toxic for stomach tissues. This product will intense nitrosative stress (10,11). This process causes *H. pylori* be resistant to NO antibacterial effects (11). Regarding the therapeutic effects of fatty acids in various diseases, the antibacterial and their benefits in decreasing nitrosative stress was proven in this study. Recently, it has been indicated that PUFAs with the regulation of genes involving in inflammation in various cell lines show antibacterial effects (27). Additionally, it has been demonstrated that the linoleic acid, which is a ω-6 fatty acid, can inhibit the propagation and growth of *H. pylori* in vitro. It is sought that this effect depends on the double-bond number in the structure of fatty acids (28). Previous evidence represented that ω-3 PUFAs have anti-inflammatory effects. In contrast, ω-3 and ω-6 PUFAs with a location in the cell membrane produce strong inflammatory factors (29). Based on the findings of a study, 100 μm concentration of docosahexaenoic acid (DHA) decreases *H. pylori* growth while concentration greater than 250 μm prevents this bacterium survival irreversibly (29). PUFAs can disrupt the cell membrane and the synthesis modulation of mucosal anti-inflammatory prostaglandin E2 (30). Correia et al demonstrated that...

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**Table 1.** Demographic Data of Patients in Case and Control Groups

<table>
<thead>
<tr>
<th>Clinical and Pathological Factors</th>
<th>Control (n=17)</th>
<th>Case (n=17)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>51.52±10.47</td>
<td>57.29±10.69</td>
<td>0.688</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.550</td>
</tr>
<tr>
<td>Male (n=18)</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Female (n=16)</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>88.03±9.36</td>
<td>92.76±10.48</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>136.26±28.46</td>
<td>110.18±20.75</td>
<td>0.153</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>93.23±16.85</td>
<td>80.24±10.00</td>
<td>0.187</td>
</tr>
</tbody>
</table>

**Note.** SD: Standard deviation; FBS: Fasting blood sugar.

**Table 2.** The Results of Gas Chromatography Capsules

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Myristate</th>
<th>Palmitate</th>
<th>Palmitoleate</th>
<th>Oleate</th>
<th>Linoleate</th>
<th>Arachidate</th>
<th>Linolenate</th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>3.24</td>
<td>17.20</td>
<td>1.65</td>
<td>28.71</td>
<td>22.76</td>
<td>4.83</td>
<td>16.04</td>
<td>2.67</td>
<td>2.29</td>
</tr>
</tbody>
</table>

**Note.** EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.
the ω-3 PUFA family shows the inhibitory effects on *H. pylori* growth and this inhibition is related to DHA (29). DHA changes bacterial lipopolysaccharide phenotype via altering the expression of outer membrane proteins (31) although DHA alone fails to inhibit *H. pylori* growth significantly compared with standard therapy (29). Therefore, the best results can be achieved if DHA is added to standard therapy. Many unsaturated fatty acids can reduce nitrosative stress (6). Considering that the levels of desaturase and elongase enzymes in stomach epithelial cells are low, it is not assumed that the consumed fatty acids such as linoleic acid and alpha-linolenic acid are converted to fatty acids with long chains such as arachidonic acid and DHA. Therefore, the reduction of the effects of fatty acids by nitrosative stress does not belong to their metabolites but these effects are related to the nature of fatty acid molecules (31). Fatty acids in corporation with the phospholipid structure of stomach epithelial cells not only cause a change in fluidity and their protein functions but also lead to an alteration in the signaling pathway in these cells (32). It has been proven that ω-3 fatty acids prevent the interleukin (IL)-8 production already activated by *H. pylori* (33). To IL-8 gene expression in the infected gastric cell, NF-κB and activator protein 1 played a crucial role as transcriptional factors (34). According to the findings, the estimated glomerular filtration rate test and protein kinase C–delta (PKCδ) may act as potential mediators in the IL-8 gene expression induced by *H. pylori* (35). In general, PKCδ belongs to the serine/threonine protein kinase member which contributed to the synchronization of mitogenic signals during several procedures of response cells (35). However, our results demonstrated that it is conceivable that unsaturated fatty acid, particularly omega 3 families may inhibit the activated PKCδ pathway, leading to a decrease in the produced NO during inflammatory conditions.

**Conclusions**

In general, the findings revealed that the consumption of omega fatty acid supplement can improve the nitrosative stress that occurred in patients with *H. pylori* infections and thus is useful for decreasing NO. It is suggested that the consumption of omega fatty acids in combination with standard therapy is effective in *H. pylori* eradication.

**Conflict of Interests**

Authors have no conflict of interests.

**Ethical Issues**

The ethical code number of 1394.641 was issued for the study by the Ethics Committee of Tabriz University of Medical Sciences. Additionally, the study was registered at Iranian Registry of Clinical Trials (identifier: IRCT20161210031338N3).

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