Is there an oxidative stress in children with *Helicobacter pylori* infection?

Duran Arslan, MD, Kader Kose, PhD, Tahir E. Patiroglu, MD.

It is well known that there is a causal relationship between acute or chronic gastritis, duodenal ulcer, gastric cancer, lymphoma of stomach, and *Helicobacter pylori* (HP) colonization in the gastric mucosa of adults. Most features of this infection in children differ from those in adults. Epidemiological data show that childhood is the important period for acquisition of HP infection. Although the disease acquired at an early age and lasting for years is claimed to be a risk factor for cancer development in adulthood, this subject is controversial. However, such claims would indicate the importance of early diagnosis and treatment of HP infection. It is reported that reactive oxygen species (ROS) appearing during HP infection in adults have an important role in gastroduodenal damage, and that eradication rates improve with the addition of antioxidant drugs to conventional anti-HP regimens. Although oxidative stress is reported to play a role in tissue damage, which is produced by HP infection, it is not completely established. This study aimed to investigate the status of malondialdehyde (MDA), which is a carbonyl compound and a product of oxidative stress, and superoxide dismutase (SOD) activity in gastric tissue and erythrocytes, and presence of HP infection by means of histology.

**Methods.** At the Pediatric Gastroenterology Department of Erciyes University, Kayseri, Turkey, between January 2004 to August 2005, 39 children undergoing upper gastrointestinal endoscopy were investigated for malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity in gastric tissue and erythrocytes, and presence of HP infection by means of histology.

**Results:** There is an increase of the oxidative stress parameter, MDA, in gastric tissue, but not in erythrocytes in HP (+) patients. The antioxidant enzyme, SOD, levels both in gastric tissue and erythrocytes were not different between HP (+) and HP (-) patients. In 8 HP infected children after treatment with an anti-HP regimen, no change was observed except for tissue SOD activity, which is increased after therapy. No correlation was observed between histological findings and tissue, and erythrocyte MDA levels and SOD activities.

**Conclusion:** Oxidative stress has some role in tissue damage in HP infection in children.

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staged individually using scores from zero to 3 by a pathologist, unaware of the patients’ diagnoses (Table 1). Sum of acute and chronic inflammatory scores was defined as total inflammation score. Histological evaluation is considered as the gold standard, so the patients were divided into 2 groups; that is, HP (+) and HP (-) on histological sections. Eight of the patients diagnosed as HP gastritis and treated with omeprazole, amoxicillin, and clarithromycin were evaluated again 4 weeks after the end of treatment. Antral tissue samples were also put into plastic tubes, weighed and kept at -20°C until biochemical analyses. In addition, blood samples obtained just before endoscopy were taken into heparinized plastic tubes and centrifuged at 1500 g for 15 minutes at 4°C. Plasma was discarded, and erythrocyte pellet was washed 3 times with cold isotonic saline. The packed cells were then subdivided and kept at -20°C until MDA and SOD measurements. Antral tissue samples stored at -20°C were thawed. After washing 3 times with isotonic saline, we suspended and homogenized samples in ice-cold 0.1 M phosphate buffer, pH 7.0 (10% W/V), and used in biochemical measurements. The MDA levels in erythrocyte and tissue homogenates were assessed by spectrophotometrical methods based on measuring concentrations of pink chromogen compound of MDA and thiobarbituric acid (TBA). The evaluation was performed using a standard curve obtained from MDA-TBA reaction. The SOD activity in erythrocytes and tissue homogenates was assayed by its ability to inhibit the reduction of nitroblue tetrazolium (NBT), with xanthine–xanthine oxidase used as superoxide anion generator. One unit of SOD is defined by the amount of the enzyme to inhibit the rate of NBT reduction by 50%. Protein content of tissue samples and hemoglobin (Hb) levels of erythrocytes with Dubkin’s reagent, were also measured. Both MDA and SOD were expressed as per gram of Hb in erythrocytes (nmol MDA/g Hb and U SOD/g Hb), and as per milligram of protein in tissue homogenates (nmol MDA/mg protein and U SOD/mg protein).

Statistical analysis was carried out using SPSS for Windows. Age, SOD-tissue, and SOD-erythrocyte levels, MDA-tissue, and MDA-erythrocyte levels were expressed as mean ± standard deviation and comparison of these parameters between the groups were made using t-test. Histological scores were expressed as median (minimum – maximum) and the parameters of the groups were compared using Mann-Whitney U-test. Correlation between tissue and erythrocyte MDA levels, SOD activities, and histological scores was investigated. Parameters obtained both before and after treatment were compared using Wilcoxon’s paired 2 tests. The p-values less than 0.05 were considered significant.

**Results.** Thirty-nine patients (20 females and 19 males), aged between 7 and 17 years (mean 12.6 ± 2.8 years), underwent upper gastrointestinal (GI) endoscopy. The ages and sexes of HP (+) and HP (-) groups were similar (Table 2). Regarding the endoscopy results, 13 (33%) patients were normal, 11 (28%) had findings of antral gastritis, 9 (23%) nodular gastritis, 2 (5%) duodenitis, and one (2.5%) gastric ulcer (induced by steroid), duodenal ulcer, erosive gastritis, and congestive gastropathy. Histological scores of HP (+) patients were higher than those of HP (-) patients. Acute inflammation, chronic inflammation, lymphoid follicle, and total inflammation scores of HP (+) patients were significantly higher than those of HP (-) patients (Table 2). The mean gastric tissue MDA levels of HP (+) patients were higher than those of HP (-) patients. There was no difference in erythrocyte MDA levels and tissue and erythrocyte SOD activities between HP (+) and HP (-) patients (Table 2). Evaluation of 8 treated patients showed that tissue and erythrocyte MDA levels and erythrocyte SOD activities were comparable both before and after treatment (Table 3). Mean tissue SOD activities were significantly increased in patients following treatment (Table 3). Erythrocyte SOD activities in HP (-) patients and the post treatment group were higher than HP (+) and the pre treatment group, but the difference was not significant (Tables 2 & 3). No correlation was found between histological findings and tissue, and erythrocyte MDA levels and SOD activities.

**Discussion.** In this prospective study of 39 patients undergoing upper GI endoscopy, the rate of HP infection was found to be 64% (25/39). This figure suggests that HP infection is acquired at an early age in our country. The HP infection is usually associated with inflammation in gastric mucosal tissue, which is typically in the form of type B chronic gastritis. The mechanisms underlying the different clinical pictures (gastritis, stomach ulcer, duodenal ulcer, lymphoma, stomach cancer) produced by the microorganism in the stomach, and duodenum cannot be fully explained. It is known that virulence of the microorganism and factors related to host, play an important part. The basic component of the inflammation caused by HP is neutrophil leukocytes in adults and lymphocytes in children. In the chain of events initiated by the invasion of stomach epithelium by HP, chemotactic factors, and cytokines are released from epithelial cells, and interleukins tumor necrosis factor-alpha, and interferon-gamma from mononuclear cells, stimulated by epithelial cells, and HP. They all cause the aggregation of the neutrophils in the region as well as other inflammatory cells and induce antibody production by stimulating B lymphocytes. Mucosal
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### Table 1 - Histopathologic grading for *Helicobacter pylori.*

<table>
<thead>
<tr>
<th>Score</th>
<th>Acute inflammation</th>
<th>Chronic inflammation</th>
<th>Lymphoid follicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absent</td>
<td>There is no increase</td>
<td>Absent</td>
</tr>
<tr>
<td>1</td>
<td>Mild colonization or scattered organisms</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>1/3 of pits and surface epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Moderate colonization</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>1/3-2/3 of pits and surface epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Large clusters or severe colonization</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>2/3&lt; of pits and surface epithelium</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Neutrophilic infiltration of the lamina propria, pits or surface epithelium
†Increase in lymphocytes and plasma cell in lamina propria

### Table 2 - Comparison of bacillus (+) and bacillus (-) groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HP(+) (n=25)</th>
<th>HP(-) (n=14)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>12.60 ± 2.5</td>
<td>12.5 ± 3.8</td>
<td>0.915</td>
</tr>
<tr>
<td>Gender (Female/Male)</td>
<td>13/12</td>
<td>7/7</td>
<td>0.904†</td>
</tr>
<tr>
<td>Acute inflammation score</td>
<td>1 (1-2)</td>
<td>0 (0-1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chronic inflammation score</td>
<td>2 (1-3)</td>
<td>0 (0-1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymphoid follicle score</td>
<td>2 (0-3)</td>
<td>0 (0-3)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Total inflammation score</td>
<td>5 (3-7)</td>
<td>1 (0-5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SOD-tissue (unit / mg-protein)</td>
<td>0.93 ± 1.74</td>
<td>6.06 ± 2.61</td>
<td>0.853</td>
</tr>
<tr>
<td>SOD- erythrocyte (unit / g-Hb)</td>
<td>2437.9 ± 268.4</td>
<td>2633.4 ± 346.3</td>
<td>0.057</td>
</tr>
<tr>
<td>MDA-tissue (nmol / mg-protein)</td>
<td>2.15 ± 0.98</td>
<td>1.11 ± 0.54</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>MDA-erythrocyte (nmol / g-Hb)</td>
<td>411.3 ± 78</td>
<td>413.0 ± 84.9</td>
<td>0.948</td>
</tr>
</tbody>
</table>

Chi-square test, SOD - superoxide dismutase, MDA - Malondialdehyde

### Table 3 - Comparison of patients before and after treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment (n=8)</th>
<th>After treatment (n=8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD-tissue (unit / mg-protein)</td>
<td>5.20 ± 1.21</td>
<td>9.08 ± 2.74</td>
<td>0.001</td>
</tr>
<tr>
<td>SOD- erythrocyte (unit / g-Hb)</td>
<td>2382.1 ± 364.6</td>
<td>2615.1 ± 170.7</td>
<td>0.072</td>
</tr>
<tr>
<td>MDA-tissue (nmol / mg-protein)</td>
<td>1.77 ± 0.87</td>
<td>1.80 ± 0.89</td>
<td>0.140</td>
</tr>
<tr>
<td>MDA-erythrocyte (nmol / g-Hb)</td>
<td>408.1 ± 71.9</td>
<td>435.0 ± 56.4</td>
<td>0.413</td>
</tr>
</tbody>
</table>

SOD - superoxide dismutase, MDA - Malondialdehyde
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damage, and consequently disruption in epithelium is mediated by reactive oxygen metabolites, and proteases liberated by activated neutrophils, cytotoxic T cells, and antibodies. The role played by oxidative stress in mucosal damage caused by HP has been demonstrated in studies on adults. Moreover, it is stated that the severity of tissue damage decreases, and the rate of HP eradication increases when antioxidant agents are added to conventional HP treatment protocols. Information on oxidative stress, and its relation with the histological picture in children with HP infection is limited. There are few and controversial data on the role of oxidative damage in HP gastritis in children. Results of investigations on oxidative damage in HP infection in adults and children are different. Oxidative stress and DNA damage were increased in HP gastritis in adults, and their relation to gastric cancer was speculated in several studies. In children; in an early study, Baik et al found that 8-hydroxydeoxyguanosine – a marker of oxidative DNA damage-levels was higher in HP infected children. In a recent study by Shimizu et al, 8-hydroxydeoxyguanosine levels were not different before and after therapy in HP infected children. Brodie et al found that SOD activity was higher in the antral mucosa of HP positive children. But in a study by the same authors, SOD activity was markedly decreased in ulcer edge in HP (+) patients. Akcam et al found that myeloperoxidase, xanthine oxidase, and SOD were not different in the gastric mucosa of children who were infected and noninfected with HP. Cytokine expression profile and intensity in children with HP gastritis was found different from adults.

In the present study, MDA, a product of oxidative stress and a reactive carbonyl compound, levels were higher in HP (+) patients. The HP infection may stimulate some oxidative stress in children. We did not find any difference in SOD activities, an enzyme preventing oxidative damage, in either gastric (antral region) tissue or peripheral blood erythrocytes and MDA levels, in erythrocytes between patients infected with HP and those not infected. As compared with the post treatment group, a significant rise was observed only in tissue SOD levels in pre-treatment levels. Thus, although there is a significant change in oxidative stress products, and no change in protective enzyme activities with HP infection, a rise was observed in SOD levels following treatment. It may be accepted as a restoration effort of the body. The different gastric histology in children may be the cause for this controversial finding. As also seen in our study, the main component of HP infection in children is mononuclear cells (lymphocytes) and although oxidative stress and related events are associated with all inflammatory cells (macrophages, monocytes, lymphocytes, neutrophils), its real source is active neutrophils. In a setting where neutrophil infiltration is not intense, free oxygen radicals, and damage caused by them may not be marked.

In conclusion, it may be claimed that free oxygen radicals may play some role in tissue damage caused by HP gastritis in children. Controversial results on oxidative stress and antioxidant enzymes may result different gastric response to HP between adults and children. For clear interpretations, future studies should be conducted on homogenous and larger groups of children.

References


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