Interplay between *Helicobacter pylori* and host gene polymorphisms in inducing oxidative DNA damage in the gastric mucosa

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Infection by *Helicobacter pylori* is the most important risk factor for gastric cancer. However, only a small fraction of colonized individuals, representing at least half of the world’s population, develop this malignancy. In order to shed light on host-microbial interactions, gastric mucosa biopsies were collected from 119 patients suffering from dyspeptic symptoms. 8-Hydroxy-2'-deoxyguanosine (8-oxo-dG) levels in the gastric mucosa were increased in carriers of *H.pylori*, detected either by cultural method or by polymerase chain reaction, and were further increased in subjects infected with strains positive for the *cagA* gene, encoding the cytotoxin-associated protein, cagA. Oxidative DNA damage was more pronounced in males, in older subjects, and in *H.pylori*-positive subjects suffering from gastric dysplasia. Moreover, 8-oxo-dG levels were significantly higher in a small subset of subjects having a homozygous variant allele of the 8-oxoguanosine-glycosylase 1 (*OGG1*) gene, encoding the enzyme removing 8-oxo-dG from DNA. Conversely, they were not significantly elevated in glutathione S-transferase M1 (*GSTM1*)-null subjects. Thus, both bacterial and host gene polymorphisms affect oxidative stress and DNA damage, which is believed to represent a key mechanism in the pathogenesis of gastric cancer. The interplay between bacterial and host gene polymorphisms may explain why gastric cancer only occurs in a small fraction of *H.pylori*-infected individuals.

Introduction

Mortality rates for stomach cancer have steadily declined during the last decades in most countries (1), due to the so-called ‘unplanned triumph’ of medicine (2). Nevertheless, this type of cancer, and chiefly gastric adenocarcinoma, still remains the second commonest cancer in the world (3). After the first report suggesting an association between infection with the Gram-negative bacterium *Helicobacter pylori* (*H.pylori*) and both chronic gastritis and peptic ulcer (4), *H.pylori* has become the first bacterium linked to a human cancer, and has been categorized by IARC as a Group 1 human carcinogen, with an attributable risk for gastric cancer and mucosa-associated lymphoma tumor (MALT) of 50–60% (5). This infection is considered to be the most important risk factor for gastric cancer, also taking into account that *H.pylori* colonizes the stomach of at least half of the world’s population. However, the odds ratios characterizing the association between *H.pylori* infection and gastric cancer are somewhat weak (6,7).

A hallmark of infection by *H.pylori*, which remains in the gastric lumen without invading the gastric mucosa, is infiltration of neutrophiles and monocytes producing reactive oxygen species (ROS) and nitrogen species (8). However, the mechanisms underlying the involvement of *H.pylori* in the pathogenesis of chronic type B gastritis and peptic ulcer disease, followed by atrophic gastritis, intestinal metaplasia, and carcinoma in a small proportion of infected individuals, are poorly defined (9). Therefore, there is a need to shed light on the host-microbial interaction mechanisms. In particular, the pathogenicity of *H.pylori* infection is modulated by a variety of polymorphic genes belonging both to this bacterium and to the human host.

The circular *H.pylori* genome contains 1667867 base pairs and 1590 predicted coding sequences (10). The most important *H.pylori* polymorphic genes associated with gastric cancer are *cagA* and *vacA* (11). The *cagA* gene, encoding the cytotoxin-associated protein, cagA, is commonly used as a marker for the entire *cag* pathogenicity island (PAI), containing several genes (*cag* E, G, H, I, L, M) required for the release of proinflammatory cytokines such as interleukin (IL)-8 from gastric epithelial cells (11). *CagA* increases cell proliferation in the absence of a corresponding increase in apoptosis, a finding that explains the enhanced risk for gastric carcinoma associated with *H.pylori* *cagA*+ infection (12). Furthermore, *H.pylori* *cagA*+ infected subjects have more frequently *p53* mutations than *H.pylori* *cagA*– infected subjects (13). The *vacA* gene, encoding for the active vacuolating cytotoxin vacA, induces gastric epithelial cell damage (14).

Human polymorphic genes that have been associated so far with *H.pylori*-related gastric cancer include IL-1β, IL-1 receptor β, tumor necrosis factor (TNF)-α, and IL-10 (11). These genes encode inflammatory cytokines and activities that inhibit acid secretion and activate intracellular pathways related to inflammation and apoptosis. Subjects bearing adverse polymorphisms for these genes undergo more severe consequences following *H.pylori* infection as compared with subjects bearing wild-type polymorphisms (11).

Oxidative stress plays an important role in the pathogenesis of *H.pylori*-induced mucosal damage (8,15–20). Inflammation accompanying *H.pylori* infection represents a

Abbreviations: *H.pylori*, *Helicobacter pylori*; 8-oxo-dG, 8-hydroxy-2'-deoxyguanosine; GSTM1, glutathione S-transferase M1; OGG1, 8-oxoguanosine-glycosylase 1; ROS, reactive oxygen species.

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main source of oxidative stress. In addition, the chronic gastritis induced by H. pylori infection is characterized by overexpression in the gastric epithelium of nuclear genes encoding for mitochondrial proteins (21). Mitochondrial damage is important for H. pylori establishment and growth because secretion of urease by H. pylori appears to be insufficient to adequately neutralize the local environment (22). All factors related to H. pylori, human host and oxidative stress interact with dietary factors. Therefore, the global risk for the development of gastric cancer results from the interaction between environmental exposure (e.g., ingested nitrates) and endogenous risk factors including polymorphic genes of both bacteria and host.

These pathogenetic considerations bear relevance for the primary and secondary prevention of gastric cancer. As an example, subjects infected by cagA+ H. pylori strains are expected to receive more benefit from H. pylori eradication than those infected by cagA− strains. Taking into account that the clinical outcome is related to H. pylori genotypes (23), anti-H. pylori therapy should be targeted to selected patient groups considering the multifaced role of H. pylori (24).

Insofar, the genetic variables characterizing H. pylori infection, including human and bacterial polymorphic genes and DNA damage, have been mainly analyzed separately. The aim of the herein reported study, whose outline is summarized in Figure 1, was to analyze in vivo the interplay among H. pylori and host susceptibility factors in inducing DNA damage in the human gastric mucosa colonized by H. pylori strains. To this purpose, we evaluated in parallel oxidative DNA damage, H. pylori gene polymorphisms (cagA and vacA), and human host polymorphisms involved in the response to oxidative stress. These included the glutathione S-transferase M1 (GSTM1) gene, which is involved in detoxification of electrophiles and ROS, and 8-oxoguanine DNA glycosylase 1 (OGG1)/AP lyase, which catalyzes removal from DNA of 8-oxodeoxyguanosine, the most abundant lesion generated by oxidative stress. DNA damage of gastric cells was evaluated in terms of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG), a sensitive marker of DNA oxidation. The results obtained indicate that the interaction among H. pylori and human adverse polymorphisms is crucial in inducing remarkable levels of oxidative DNA damage.

Materials and methods

Subjects

A total of 119 patients suffering from dyspeptic symptoms were enrolled in the study. Dyspeptic symptoms included epigastric pain, upper abdominal discomfort, nausea and vomiting, early satiety, heartburn, and regurgitation lasting for at least 3 months. Patients receiving bismuth compounds, antisecretory drugs, or antibiotics during the 4 weeks before endoscopy were excluded. Other exclusion criteria included gastrointestinal surgery, pregnancy or lactation, regular use of acetylsalicylic acid and other non-steroidal anti-inflammatory drugs, malignancy, known allergy to penicillin, severe liver, heart or kidney disease, or prior treatment for H. pylori infection. Of the 119 subjects, 50 were males and 69 females. The age was in the 7–84 years range, with an overall mean (±SD) of 49.7 ± 17.2 years. BMI was available for all subjects. A questionnaire was administered regarding smoking habits and diet, with particular reference to the intake of alcohol, fish, and vegetables, salt, cuts, and grilled food. A written informed consent was obtained from all patients and the protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Sassari, Italy.

Collection of gastric mucosa biopsies

For each patient, eight biopsy specimens, consisting of superficial mucosa, were collected, four of which from the antrum, two from the rugal fold, and two from the corpus of the stomach. Biopsy specimens for histology were immediately fixed in 10% buffered formalin. Two biopsies from the antrum, two from the rugal fold and two from the corpus were stained with hematoxylin and eosin and with Giemsa to grade the density of H. pylori. The stage of gastritis was evaluated by using the Sydney system (25). During histological examination, the presence of dysplasia, intestinal metaplasia, and inflammation were assessed and graded.

Microbiological analyses

One antral biopsy was immediately transported in Portagater pylori (bioMerieux, S.p.A., Rome, Italy) for culture, streaked on Columbia agar plates (bioMerieux) and incubated for 3 ± 5 days at 37°C, in an atmosphere of 12% CO2 and 100% relative humidity. Bacterial growth was identified as H. pylori on the basis of colony morphology and positive biochemical reactions for catalase, urease, oxidase, γ-glutamyltransferase, alkaline phosphatase and negative hippurate hydrolysis and nitrate reduction tests.

The minimum inhibitory concentration for amoxicillin, clarithromycin, tetracycline and metronidazole was determined by the E-test (AB Biodisk, Uppsala, Sweden). The breakpoints used to define resistant H. pylori were as follows: amoxicillin resistance, MIC >8 μg/ml; clarithromycin resistance, MIC >2 μg/ml; trimethoprim resistance, MIC >8 μg/ml; metronidazole resistance, MIC >0.5 μg/ml. (26).

PCR analyses of H. pylori

Antral biopsy DNA was extracted using the QIAamp Tissue Kit (QIAGEN, Chatsworth, CA). Specific primers for H. pylori 16S rRNA (27) were used to amplify DNA fragments of 521 bp. Two sets of oligonucleotide primers were designed to amplify a conserved region in the vacA gene. The first set, VAGF and VAGR (27), was used to amplify a 570 bp product for m1 and m2 from the middle region of the vacA gene. The second set, VA1F and VA1R (28), was used to amplify a 259 bp product for s1 and s2 from the signal sequence region of the vacA gene. One set of primers, F1 and B1, was also used for the amplification of the conserved region of the cagA gene (29). Following initial denaturation at 94°C for 1 min, each reaction consisted of 35 cycles at 94°C for 1 min, annealing (53°C for VA1F/R, VAGF/R and 55°C for F1/B1) and extension for 2-3 min and then at 72°C for 10 min.

Each PCR mixture was subjected to gel electrophoresis on 1% agarose gels. A 100 bp DNA ladder (Sigma-Aldrich, St Louis, MO) was used as a reference marker. Two previously described PCR assays for vacA signal and mid-region genotyping were combined in a multiplex format (28). VacA alleles were amplified in a 50 μl reaction containing: 5 μl DNA extract from gastric biopsy; 200 μM (each) dNTP (GibcoBRL Life Technologies, Paisley, UK); 0.3 μM each of the primers VA1-F and VA1-R and 0.48 μM each of the primers VAG-F and VAG-R (MWG Biotech, Ebersberg, Germany): 2.0 μM MgCl2; 20 μM Tris–HCl, pH 8.4; 50 μM KCl; 0.2% w/v glycerol; and 1 U Taq polymerase (GibcoBRL). Thermal cycling conditions were...
94°C for 5 min followed by 35 cycles at 94°C (30 s), 53°C (1 min) and 72°C (1.5 min) and a final elongation at 72°C for 5 min. The PCR products were separated by electrophoresis on 2% agarose gel in TBE buffer (90 mM Tris–HCl, 90 mM boric acid and 2 μM EDTA), and stained in ethidium bromide. American Type Culture Collection (ATCC) H. pylori strains were subjected to amplification and used as positive controls.

**Detection of 8-oxo-dG in gastric mucosa**

Antral biopsy DNA was purified by a phenol–chloroform procedure using an automatic DNA extractor (Genepure 341, Applied Biosystems, Foster City, CA) working under oxygen-free helium atmosphere. Due to the small size of collected biopsies (1–2 mm) the total amount of DNA extracted was 3.05 ± 2.23 μg (mean ± SD) only. Accordingly, 8-oxo-dG was detected by 32P-post-labeling, as previously described (30,31). Briefly, DNA (1–2 μg) was depolymerized to 3’-monophosphate nucleotides by incubation with micrococcal nuclease and spleen phosphodiesterase at 37°C for 3.5 h. Unmodified dGp nucleotides were selectively removed by incubation with 80% v/v trichloroacetic acid for 10 min at room temperature. The samples were dried by vacuum centrifugation and 3’-phosphate-8-oxo-dG molecules were labeled by incubation with T4 polynucleotide kinase in the presence of AT-γ-32P (64 μCi, specific activity 750 Ci/mmol) (ICN, Irvine, CA) at 37°C for 40 min. The mixture was subjected to nuclease P1 digestion (2.7 U at 37°C for 30 min). 32P-labeled 8-oxo-dG was purified by monodirectional thin layer chromatography in unbuffered 1.5 M formic acid, identified by electronic autoradiography (InstantImager, Packard, Meriden, CT) and quantified by calculating the ratio of 32P-labeled 8-oxo-dG to 32P-labeled normal nucleotides.

Positive reference standards were obtained by incubating calf thymus DNA with 0.1 U micrococcal nuclease, 50 mM MgCl2 and 2 U Platinum DNA polymerase (Life Technologies, Rockville, MD) and 1 μl of diluted Sybr green as fluorescent tracer to quantify the PCR amplification products. Polymerase was hot-start activated and amplification was performed for 94°C for 1 min, followed by 45 cycles at 94°C for 1 min and 72°C for 1 min for 40 cycles, using a rotating real-time thermocycler (Corbett Research, Mortlake, Australia). The fluorescent signal was detected before each denaturation cycle. The specificity of the reaction products was tested by analyzing the melting curves of PCR products.

**OGGI1 polymorphism**

The OGG1 gene Ser326Cys polymorphism, resulting from a C→G transversion in exon 7, was evaluated by QPCR using a gene–specific molecular beacons discriminating the occurrence of this genetic variant either on both alleles (homozygous mutant) or on one allele (heterozygous) or on no allele (wild-type). The sequences of PCR primers containing molecular beacons were: h-OGGI1-Cys(326)mut: 5’-HEX-CCGGATCTCGCCAACTGGCCATTGCATCCGAC-3’ and h-OGGI1-Ser(326)wt: 5’-FAM-CCGGATCTGCGAACTGGCCATTGCATCCGAC-CATCCGAC-3’. The PCR primers were sequences: Primer 1 (5’-CCGCTCTTGATCTCTCC-3’) and Primer 2 (5’-CTCTTGAGACACTTCT-3’). The reaction mixture (50 μl) contained: 5 μl 10× PCR buffer, 0.4 μl 100 mM dNTP mix, 2 μl 50 mM MgCl2, 1 μl 10 mM Primer 1, 1 μl 10 mM Primer 2, 1 μl Beacon wild-type, 1 μl Beacon mut, 0.5 μl Platinum Taq polymerase, 1 μl (200 ng) DNA and 37.1 μl sterile water. The hot-start reaction was performed at 94°C for 2 min, followed by 45 cycles at 94°C for 30 s, 65°C for 20 s, 55°C for 30 s and 72°C for 30 s. The HEX signal was acquired at the end of the 65°C step and the FAM signal at the end of the 55°C step.

**Results**

**Prevalence of H. pylori infection as related to gender, age and histopathology**

Of the 119 subjects under study, 69 (58.0%) had H. pylori detectable by culturing antral biopsies, and 77 (64.7%) were positive for H. pylori, as detected by PCR. There was agreement between H. pylori culture and PCR in 69 positive cases and in 42 negative cases. The PCR was positive in the absence of H. pylori growth in culture in eight cases, while no positive H. pylori culture was obtained in PCR-negative samples.

Table I (first two columns) shows the prevalence of H. pylori positivity by PCR as related to gender, age, and histopathological alterations of the gastric biopsies examined. The H. pylori infection prevalence was similar in males (33 out of 50, i.e. 66.0%) and females (44 out of 69, i.e. 63.8%). The age distribution was almost identical in H. pylori-negative and H. pylori-positive subjects. The prevalence of positivity for H. pylori culture was affected by the gastritis stage, being 0% in Stage 1, 55% in Stage 2, 65% in Stage 3, and 100% in Stage 4 (P < 0.001). Likewise, the prevalence of PCR positivity was 0% in Stage 1, 82% in Stage 2, 95% in Stage 3, and 100% in Stage 4 (P < 0.001).

As shown in Table I, the mean stage of gastritis and the prevalence of severe gastritis (Stages 3–4) were significantly more elevated in H. pylori-positive subjects than in H. pylori-negative subjects. In contrast, occurrence of either dysplasia or enteroend metaplasia of the gastric mucosa was not significantly affected by H. pylori infection. The stage of gastritis (mean ± SD) was slightly but significantly more advanced in males than in females (2.6 ± 0.7 versus 2.3 ± 0.7, P < 0.05). The subjects affected by dysplasia were older than those unaffected (63.6 ± 6.3 versus 48.9 ± 16.6 years among all subjects, P < 0.05), a difference that was not significant in H. pylori-positive subjects (59.7 ± 3.8 versus 48.9 ± 2.9 years) but borderline to statistical significance in H. pylori-negative subjects (66.5 ± 11.1 versus 48.8 ± 2.9 years, P = 0.07).

**Table I. Characteristics of the examined subjects as related to detection of H. pylori by PCR in gastric biopsies and positivity for cagA**

<table>
<thead>
<tr>
<th>Characteristics of subjects</th>
<th>H. pylori-negative</th>
<th>H. pylori-positive (all)</th>
<th>H. pylori + (cagA+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number (males/females)</td>
<td>42 (17/25)</td>
<td>77 (33/44)</td>
<td>60 (25/35)</td>
</tr>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>50.5 ± 18.7</td>
<td>49.3 ± 16.6</td>
<td>52.2 ± 16.0</td>
</tr>
<tr>
<td>Stage of gastritis (mean ± SD)</td>
<td>2.1 ± 0.7</td>
<td>2.5 ± 0.6a</td>
<td>2.6 ± 0.6a</td>
</tr>
<tr>
<td>Subjects affected by severe gastritis (Stages 3–4)</td>
<td>12 (28.6)</td>
<td>34 (44.2)b</td>
<td>28 (46.7)b</td>
</tr>
<tr>
<td>Subjects affected by dysplasia</td>
<td>4 (9.5%)</td>
<td>3 (3.9%)</td>
<td>3 (5.0%)</td>
</tr>
<tr>
<td>Subjects affected by enteroend metaplasia</td>
<td>8 (19.1%)</td>
<td>17 (22.1%)</td>
<td>13 (21.7%)</td>
</tr>
</tbody>
</table>

Values are number of subjects (n), except for age that is mean (± standard deviation) for each group.

aP < 0.01, compared with H. pylori-negative.

bP < 0.05, compared with H. pylori-negative.
Sensitivity of *H. pylori* isolates to antibiotics and prevalence of *VacA* and *CagA* genotypes

Of the 59 *H. pylori* isolates tested for sensitivity to 4 antibiotics, none was resistant to either tetracyclin or amoxicillin, while 2 of them (3.4%) were resistant to either clarythromycin or metronidazol. No significant relationship was detected between antibiotic resistance and other tested variables, including *H. pylori* genotypes and host age, gender, gene polymorphisms, stage of gastritis and oxidative DNA damage in the gastric mucosa.

All the *H. pylori* strains detected displayed a s2-m2 polymorphism for the *vacA* gene, while the s1-m1 adverse polymorphism was not detected in any strain. The *cagA* genotype was present in 60 of the 76 tested *H. pylori* strains positive at PCR (79.0%). As shown in Table I (third column), positivity of *H. pylori* for *cagA* was independent of age and did not affect the stage of gastritis and occurrence of either dysplasia or enteroid metaplasia of the gastric mucosa.

Oxidative DNA damage in gastric biopsies

The mean (±SE) levels of 8-oxo-dG in the gastric biopsies of all 119 examined subjects were 4.0 ± 0.4 8-oxo-dG/10^5 nucleotides. A variety of factors, including gender, age, histopathological alterations, *H. pylori* infection and host, and *H. pylori* gene polymorphisms, affected the intensity of oxidative DNA damage. Table II summarizes the host and *H. pylori* factors that significantly affected this end-point either in all subjects or in *H. pylori*-infected subjects.

8-Oxo-dG levels were significantly higher in the 69 females than in the 50 males recruited for the study. By assuming a cut-off value of 50 years, which was approximately the overall mean age of all subjects, 8-oxo-dG levels were higher in subjects aged ≥50 years than in subjects aged <50 years. This difference did not reach the statistical significance threshold among all subjects (4.3 ± 0.6 versus 3.1 ± 0.4, means ± SE) but was significant in *H. pylori*-infected subjects (Table II). As shown in Figure 2, there was no correlation between age and 8-oxo-dG in *H. pylori*-negative subjects (r = −0.211, not significant), but a significant correlation was recorded in *H. pylori*-positive subjects (r = 0.285, P < 0.05). As to histopathological alterations, oxidative DNA damage was significantly increased in *H. pylori*-positive subjects bearing gastric dysplasia (Table II), while the difference was not significant in *H. pylori*-negative subjects (2.5 ± 0.6 versus 1.5 ± 0.5). Smoking habits and dietary factors were not significantly associated with either 8-oxo-dG levels in the gastric mucosa or histopathological alterations. Alcohol consumption was moderate in all subjects. There was an inverse correlation between 8-oxo-dG levels and BMI (BMI, r = −0.378, P < 0.05). BMI was higher in males (26.0 ± 0.7, mean ± SE) than in females (23.2 ± 1.0, P < 0.05), and in ex-smokers (27.0 ± 0.7) than in either current smokers (23.9 ± 1.4, P < 0.01) or never smokers (22.8 ± 1.0, P < 0.01). Moreover, BMI significantly correlated with age (r = 0.466, P < 0.01).

*H. pylori* infection and the *H. pylori* genotype had a profound effect on oxidative DNA damage in gastric mucosa. In fact, as shown in Table II, 8-oxo-dG levels were almost twice as high in *H. pylori*-infected subjects, irrespective of the *H. pylori* detection method (culture or PCR), as compared with *H. pylori*-negative subjects. Within *H. pylori*-positive

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**Table II.** Host and *H. pylori* (HP)-related factors affecting 8-oxo-dG levels in the gastric mucosa

<table>
<thead>
<tr>
<th>Variability factor</th>
<th>Number of subjects</th>
<th>8-oxo-dG/10^5 nucleotides (mean ± SE)</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>119</td>
<td>3.0 ± 0.4 versus 4.6 ± 0.6^a</td>
<td>1.5</td>
</tr>
<tr>
<td>Males versus females</td>
<td>50 versus 69</td>
<td>2.6 ± 0.4 versus 4.7 ± 0.5^b</td>
<td>1.8</td>
</tr>
<tr>
<td>HP-negative versus HP-positive (culture)</td>
<td>50 versus 68</td>
<td>2.4 ± 0.4 versus 4.6 ± 0.5^c</td>
<td>1.9</td>
</tr>
<tr>
<td>HP-negative versus HP-positive (PCR)</td>
<td>42 versus 76</td>
<td>3.7 ± 0.5 versus 9.9 ± 2.9^d</td>
<td>2.7</td>
</tr>
<tr>
<td>OGG1 polymorphism: wild-type versus mutant</td>
<td>82 versus 6</td>
<td>3.5 ± 0.6 versus 9.9 ± 2.9^e</td>
<td>2.8</td>
</tr>
<tr>
<td>OGG1 polymorphism: heterozygous versus mutant</td>
<td>31 versus 6</td>
<td>2.3 ± 0.4 versus 3.5 ± 0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>GSTM1 polymorphism: positive versus null</td>
<td>63 versus 56</td>
<td>2.7 ± 0.4 versus 4.0 ± 0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>HP-infected subjects</td>
<td>76</td>
<td>3.2 ± 0.4 versus 5.3 ± 0.7^f</td>
<td>1.7</td>
</tr>
<tr>
<td>Age (&lt;50 versus ≥50 years)</td>
<td>35 versus 41</td>
<td>4.4 ± 0.4 versus 9.3 ± 3.4^g</td>
<td>2.1</td>
</tr>
<tr>
<td>Gastric dysplasia: unaffected versus affected</td>
<td>71 versus 5</td>
<td>1.7 ± 0.3 versus 5.1 ± 0.5^h</td>
<td>3.0</td>
</tr>
<tr>
<td>HP cagA-negative versus cagA-positive</td>
<td>16 versus 60</td>
<td>4.2 ± 0.5 versus 12.6 ± 1.8^i</td>
<td>3.0</td>
</tr>
<tr>
<td>OGG1 polymorphism: wild-type versus mutant</td>
<td>52 versus 5</td>
<td>3.7 ± 0.6 versus 12.6 ± 1.8^j</td>
<td>3.4</td>
</tr>
<tr>
<td>OGG1 polymorphism: heterozygous versus mutant</td>
<td>19 versus 5</td>
<td>2.7 ± 0.4 versus 4.0 ± 0.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

^aP < 0.05. ^bP < 0.01. ^cP < 0.001.

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**Fig. 2.** Correlation between age and oxidative DNA damage in the gastric mucosa of 119 gastritis patients, either positive or negative for *H. pylori* (HP). The equations of the regression lines are y = 3.76 − 0.026x; r = −0.211; not significant (HP negative) and y = 1.18 + 0.063x; r = 0.285; P < 0.05 (HP positive).
subjects, 8-oxo-dG levels were as much as 3-fold higher in subjects infected by cagA-positive strains.

Prevalence of GSTM1 and OGG1 polymorphisms and their influence on oxidative DNA damage

Almost half of the examined subjects (46.8%) had a GSTM1-null genotype. The OGG1 polymorphism was detected as wildtype in the 68.8% of the examined subjects, as heterozygous in the 26.0%, and as homozygous mutant in the 5.2%.

When comparing GSTM1-null subjects with GSTM1-positive subjects, a trend for an increased oxidative DNA damage in GSTM1-null subjects was observed in all and HP positive subjects, without reaching the statistical significance threshold (Table II). Conversely, homozygous mutation for the OGG1 Ser326Cys polymorphism exerted a significant and strong effect on oxidative DNA damage as compared with either wild-type or heterozygous subjects. This effect was observed both in all subjects and in H. pylori-positive subjects (Table II), whereas no significant difference was noted in H. pylori–free subjects (wild-type 1.7 ± 0.6, heterozygous 3.0 ± 1.5, and homozygous 1.7 ± 0.1 8-oxo-dG/10^5 nucleotides). Heterozygosity for OGG1 did not affect oxidative DNA damage, no significant difference between wild-type and heterozygous subjects being detected in either H. pylori-negative, H. pylori-positive, or all subjects.

Discussion

Oxidative damage is a crucial step of H. pylori pathogenicity, being mechanistically related to the link between H. pylori infection and gastric carcinoma (8). Occurrence of H. pylori-related oxidative DNA damage has been so far demonstrated under different experimental conditions and by using various methodological approaches (8,15–21). In addition, the role of oxidative stress in the pathogenesis of stomach cancer is supported by epidemiological data showing that this cancer is prevented by dietary antioxidants (32). The results of the present study, evaluating the levels of 8-oxo-dG in gastric biopsies from 119 subjects, confirm that this indicator of oxidative DNA damage is increased in gastric samples colonized with H. pylori, as detected both by cultural method and, with a higher sensitivity, by PCR. In addition, our results shed light on the interplay between H. pylori and host susceptibility factors in the induction of oxidative DNA damage.

8-Oxo-dG is a modified nucleoside resulting from oxidative DNA damage, which may affect the initiation step of H. pylori-related gastric carcinogenesis. However, it is most likely that this molecular marker of exposure may also reflect oxidative stress and inflammatory mechanisms involved in the H. pylori-host interaction. These mechanisms play a major role by inducing epigenetic effects and signal transduction alterations that contribute to the promotion of the multistage and multimechanistic carcinogenesis process (33,34).

Of the investigated H. pylori gene polymorphisms, we could not evaluate the role of vacA because all detected H. pylori strains were vacA+. The finding that H. pylori strains circulating in Sardinia are devoid of vacA-related adverse activity contributes to explain the low incidence of gastric cancer in this region (35), which contrasts with the high prevalence of H. pylori infection in the population (36,37). In fact, vac2/m2 H. pylori strains are the least virulent (38,39). A similar discrepancy between H. pylori infection prevalence and gastric cancer prevalence has been reported in other geographic areas, for instance in certain African, Indian and Latin American populations. This phenomenon has been referred to as the ‘African enigma’ (3).

Our finding that 8-oxo-dG levels were higher in the antral mucosa specimens colonized with cagA+ H. pylori strains than in those colonized with cagA– H. pylori strains confirms the crucial role of this gene as a pathogenetic determinant triggering biological effects amenable to the carcinogenesis process. CagA has been demonstrated to be associated with severe gastritis (40) and peptic ulcer (41) due to the fact that this gene encodes proteins that increase gastric mucosa inflammation and stimulate the secretion of higher levels of proinflammatory cytokines (42). CagA+ H. pylori induced the production of various cytokines by the gastric mucosa, such as IL-1beta and IL-8, while cagA– strains were weak inducers of these molecules (43). A correlation exists between infection with cagA+ strains and increase of protein alterations, expression of IL-8 and inducible nitric oxide synthase (iNOS) and gastric pathology (44). Occurrence of higher levels of oxidized and nitrated proteins in H. pylori cagA+ than in H. pylori cagA– infected subjects is associated with a significant oxidative and nitrative stress in the stomach mucosa (44).

These mechanisms explain why, in the population of dyspeptic subjects investigated in the present study, H. pylori infection was strictly related to the appearance of gastritis, as demonstrated by the increasing frequency of H. pylori positivity from Stage 1 to Stage 4 gastritis. This is consistent with the conclusion that induction of inflammation is a primary pathogenetic mechanism of H. pylori, as further supported by the finding that oxidative damage was significantly increased in gastric mucosa, but only in H. pylori-infected subjects. While severe gastritis was significantly more frequent in H. pylori carriers, no correlation of H. pylori infection with either dysplasia or enteroid metaplasia was found. This finding may be related to the different mechanisms involved in these long-term histopathological alterations. In fact, gastric dysplasia was related to age in H. pylori-negative subjects, while the influence of bacterial infection was prevailing on age-related factors in H. pylori-positive subjects.

A host-related factor affecting oxidative DNA damage was represented by age. In fact, 8-oxo-dG levels in gastric mucosa were higher in subjects aged >50 years than in younger subjects, but the difference was statistically significant only in H. pylori-infected subjects. A similar relationship between age and DNA damage, detected by comet assay, has been recently reported in a group of Brazilian patients (45). These findings suggest that H. pylori infection represents a risk factor triggering the chronic accumulation of DNA damage, leading to degenerative processes of the gastric mucosa and cancer.

As to the investigated host gene polymorphisms, irrespective of H. pylori infection, 8-oxo-dG levels were not significantly higher in GSTM1-null subjects, compared with GSTM1 carriers. Previous studies indicated that the GSTM1 homozygous deletion, either alone or in association with the GSTP1-null polymorphism, did not confer an increased risk for gastric cancer in Japanese (46) or Chinese (47) subjects.
increased gastric cancer risk but only in combination with GSTT1 for the double null genotype (48).

Conversely, in spite of the low frequency of the OGG1 homozygous Ser326Cys polymorphism, our study provided evidence that this variant allele is associated with a remarkable and significant increase in the occurrence of oxidative DNA damage in case of H. pylori infection. Note that the necessity of collecting gastric biopsies limited the size of the study population but allowed us to evaluate in parallel 8-oxo-dG in the target tissue and the gene encoding the enzyme removing this modified nucleoside from DNA. Previous studies reported that this polymorphism is associated with an increased risk of various cancers (49,50).

In a Chinese population, a borderline influence of OGG1 polymorphism on stomach cancer was related to dietary risk factors (51). The possible role of OGG1 in gastric carcinogenesis is supported by the selective upregulation of its expression in malgun cells, the morphologically modified epithelial cells characterizing H. pylori gastritis. Malgun cells represent a typical morphological change involved in an early stage of carcinogenesis (52). In this view, our results indicate a role of OGG1 homozygous mutant polymorphism in increasing DNA damage but only as related to H. pylori infection.

In conclusion, our study provides evidence that induction of oxidative DNA damage in gastric mucosa is a pathogenic mechanism that mainly depends on the highly pathogenic H. pylori cagA+ strains. However, genetic polymorphisms of the host related to the repair of oxidative DNA damage do also play an important role. The interplay between H. pylori and host genetic polymorphisms contributes to explain why gastric cancer only occurs in a small fraction of H. pylori-infected subjects.

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References


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