0021-7557/06/82-05/325
Jornal de Pediatria

doi:10.2223/JPED.1529

REVIEW ARTICLE

Gastroduodenal peptic ulcer and *Helicobacter pylori* infection in children and adolescents

Paulo F. S. Bittencourt,[1] Gifone A. Rocha,[2] Francisco J. Penna,[3] Dulciene M. M. Queiroz[4]

Abstract

Objective: To show important aspects of gastroduodenal peptic ulcer and of *Helicobacter pylori* infection in children and adolescents.

Sources: Technical textbooks and MEDLINE and LILACS databases including publications between 1966 and 2006.

Summary of the findings: The etiology of peptic ulcer in children and adolescents may be primary, associated with *H. pylori* infection, or secondary, in which etiopathogenic mechanisms rely upon the underlying disease. The infection is acquired predominantly in childhood, with prevalence rates between 56.8 and 83.1% in children who live in the poorest Brazilian regions, amounting to nearly 10% in children aged less than 10 years in industrialized countries. The infection can be diagnosed by invasive methods, which investigate the presence of the bacterium, or of DNA, RNA or bacterial products in biopsy fragments of the gastric mucosa obtained at endoscopic examination; it can also be diagnosed through noninvasive methods, which include the detection of anti-*H. pylori* antibodies in serum, urine or saliva samples, detection of bacterial antigens in stool samples, and the carbon 13-labeled urea breath test. However, upper gastrointestinal endoscopy is the method of choice for the diagnosis of peptic ulcer, as it allows collecting fragments from the gastric mucosa during the procedure for the diagnosis of infection and for histopathological analysis.

Conclusions: *H. pylori* infection is the major cause of peptic ulcer among children. Eradication of the bacterium with antimicrobial therapy results in the cure of the disease, and is therefore indicated for all children with *H. pylori* infection with an active, recurrent, healed, or complicated peptic ulcer.

J Pediatr (Rio J). 2006;82(5):325-34: Peptic ulcer, etiology, pathophysiology, diagnosis, *Helicobacter pylori,* children, adolescents.

Introduction

Hippocrates, in 460 B.C., reported a case whose diagnosis was later confirmed as peptic ulcer (PU). In the 18th century, clinicians, who were famous for their accuracy regarding the identification of symptoms, hardly ever missed the diagnosis of perforated PU, which was almost invariably fatal at that time. Legal Medicine compendia, such as that written by Albertus in 1725, mentioned that perforated ulcer was a rare cause of death, and that it should be distinguished from poisonings.

In the second half of the 19th century, ulcers were predominantly gastric. Only after some decades did duodenal ulcer become predominant.[1] In 1962, experts suggested that an environmental factor found in the late 19th century and early 20th century in the western world caused a cohort effect on individuals born at such time. Environmental factors associated with early urbanization, social stress and with the economic crisis after World War I were incorrectly regarded as predisposing factors to the disease.[2]

Contrast radiology was the first complementary method used for the diagnosis of PU. Later on, with the development and improvement of gastrointestinal endoscopy, it was found that radiological methods had high rates of false-positive (21%) and false-negative (32%) results.[3] Schindler was the first author to describe the characteristics of PU on endoscopy, which is currently the method of choice for the diagnosis of the disease.[4]

Up until about 2 decades ago, the pathogenesis of PU was ascribed to an unbalance between acid secretion and mucosal defense mechanisms, whose etiology was unknown. Nevertheless, in 1982, in Australia, Warren &

1. Doutorando, Programa de Pós-Graduação da Saúde da Criança e Adolescente, Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brasil.
2. Doutor, professor adjunto, Departamento de Propedêutica Complementar, Faculdade de Medicina, UFMG, Belo Horizonte, MG, Brasil.
3. Doutor, professor titular, Departamento de Pediatria, UFMG, Belo Horizonte, MG, Brasil.
4. Doutor, professor titular, Departamento de Propedêutica Complementar, Faculdade de Medicina, UFMG, Belo Horizonte, MG, Brasil.

Manuscript received Jul 19 2006, accepted for publication Aug 08 2006.

Suggested citation: Bittencourt PF, Rocha GA, Penna FJ, Queiroz DM. Gastroduodenal peptic ulcer and *Helicobacter pylori* infection in children and adolescents. J Pediatr (Rio J). 2006;82:325-34.

Marshall isolated a bacterium, later called *Helicobacter pylori*, from gastric mucosal fragments obtained from patients with gastritis and duodenal ulcer.[5] Subsequent studies worldwide confirmed the initial hypothesis that this bacterium was associated with the pathogenesis of peptic ulcer disease in adults[6,7] and children.[8,9] In 1994, the World Health Organization (WHO)[10-12] admitted the role of infection caused by this bacterium in the pathogenesis of gastric carcinoma, based on epidemiological evidence and on biological feasibility; more recently, the disease has been experimentally reproduced in animals.[13]

The description of this bacterium and its association with peptic ulcer disease was so important to Medicine that Australian researchers were awarded the Nobel Prize in Physiology or Medicine in 2005.

Epidemiology

The prevalence of PU is difficult to estimate, due to the subjective nature of its symptoms and also because it is frequently mistaken for other dyspeptic disorders. Besides current evidence of a decrease in the incidence of PU, the proportion between males and females has also changed in the last decades. Available data are based on statistics about perforated ulcer cases which, although regarded with skepticism for not representing the whole spectrum of peptic ulcer disease, have been the most uniform material for such assessments.[14]

The actual incidence of PU among children is still unknown. It is common knowledge, though, that it is rare in children aged less than 10 years. Large pediatric centers in North America diagnose on average four to six new cases every year.[15,16] At the Division of Pediatric Gastroenterology of Hospital das Clínicas of Universidade Federal de Minas Gerais, Brazil, the annual average amounts to 7.6 new cases,[17] which is quite similar to the average observed at Universidade Federal de São Paulo/ Escola Paulista de Medicina, Brazil (7.2 patients/year).[18] Some studies have shown a male predominance for this disease.[16,18]

As previously mentioned, *H. pylori* is a crucial factor in the pathogenesis of PU. Most children with duodenal PU test positive for the bacterium. As occurs among adults, *H. pylori*-negative duodenal ulcer is uncommon among children. It might, however, occur in other disorders (e.g.: Crohn's disease), or be secondary to the use of medications, such as nonsteroidal anti-inflammatory drugs.[19]

After the role of *H. pylori* in the pathogenesis of PU was acknowledged, several studies have shown the association between peptic ulcer disease and infection by more virulent strains of *H. pylori*. Quick and rampant urbanization, combined with poor living conditions in booming cities at the beginning of the Industrial Revolution, in the early 20th century, probably caused a decrease in hygiene standards in most of the population, which may have contributed towards a greater exposure to more virulent strains of *H. pylori*. Subsequently, improved knowledge about the importance of hygiene measures to prevent the dissemination of diseases prompted government actions whose aims were to improve health conditions and the water supply to urban households. Such measures probably managed to reduce the rates of *H. pylori* infection. Thus, the increase and decrease in the prevalence of PU are historical hallmarks of the increase and decrease in *H. pylori* infection rates. Low socioeconomic background and its natural consequences, such as poor hygiene, overcrowding, and absent or insufficient sanitation, predispose to the acquisition of the bacterium, being currently regarded as the major markers for the presence of *H. pylori* infection.[20]

Man is the main reservoir for *H. pylori*. It is transmitted from person to person via the fecal-oral[21] and oral-oral[22] routes. Infection is predominantly acquired in childhood.[23-25] Studies in our setting[23,14] and in industrialized countries[23] have demonstrated the importance of mothers[23,24] and siblings[24-26] in its transmission. Infection rates significantly increase with age, which is due to the cohort effect, i.e., the higher prevalence among older individuals shows greater risk for the acquisition of the infection than these individuals had as a child.

In Brazil, there has been a paucity of studies assessing the prevalence of this infection among healthy children. Appropriate diagnostic methods for epidemiological surveys in children should include the carbon 13-labeled urea breath test and detection of bacterial antigens in stool samples, due to the low accuracy of serological tests in children aged less than 10 years.[27] Given these premises, there have been studies only in the poorest Brazilian regions. On the outskirts of Fortaleza, state of Ceará, and in the rural area of Melquíades, state of Minas Gerais, the prevalence rates for this infection have been greater than 56.8%,[24,25] amounting to 75.4% among children aged 12 to 14 years.[25] The prevalence is even higher among riparian and indigenous populations of the state of Rondônia, in northern Brazil, reaching 83.1%.[28] Incidence data have shown that infection is acquired quite early in underprivileged Brazilian regions, ranging from 5.7% per year among children aged up to 5 years in Melquíades to 25.0% per year among children aged up to 2 years among the riparian populations of Rondônia.[24,28]

On the other hand, in industrialized countries, the infection affects only 10% of children aged less than 10 years, and a sharp decrease in its prevalence has been observed.[29]

Etiopathogenesis

PU can be primary, associated with *H. pylori* infection, with a chronic clinical course, affecting mainly the

duodenum, without associated systemic disease and being more prevalent among children older than 10 years; or secondary, with an acute clinical course, commonly affecting the stomach in children aged less than 6 years, especially newborns and infants, in whom etiopathogenic mechanisms rely on the underlying disease.[17,30] Although injury to the mucous membrane is highly predominant in primary PU, and changes in the mucosal defense mechanisms are prevalent in secondary PU,[30] usually, the disease originates from an unbalance between defense mechanisms and aggressive factors that act upon the gastroduodenal mucosa. In the stomach, the major protective barrier consists of the confluence between epithelial cells and continuous secretion of mucus bicarbonate (HCO_3^-). This layer, which is approximately 200-300 µm thick, provides efficient protection.[31] It has been demonstrated that the mucus lining the superficial epithelial cells contains bipolar phospholipids which, due to their high polarity, prevent the backdiffusion of mineral acids, such as hydrochloric acid (HCl), from the gastric lumen into the mucosa. However, nonionized organic compounds, such as bile salts or acetylsalicylic acid, which have a relatively low pKa, can quickly reach the superficial mucosal cells by nonionic diffusion and accumulate within these cells, where they dissociate and cause cellular injury.[32] The concept of barrier was developed by Code & Scholer[33] and Davenport,[34] who proposed the rupture of the barrier representing the initial step in the injury to the mucosa with subsequent release (cascade) of histamine-like substances, mucosal bleeding, and development of acute gastritis. These findings can be observed in human beings after the intake of aspirin or concentrated ethanol, as well as in animals experimentally exposed to different mucosal irritants.[35,36]

On the other hand, differently from what occurs in the gastric mucosa, where protection is provided by a mucus barrier, hydrogen ions (H^+) easily penetrate the mucosal cells of the duodenum, leading to a transient pH reduction. This change activates the basolateral sodium-bicarbonate cotransporters, with consequent excessive migration of HCO_3^- from the extracellular space into the intracellular one, activating the HCO_3^-/chloride (Cl^-) exchange in the apical portion of the cell membrane. These events result in the increase of HCO_3^- secretion which, combined with the mucus layer, neutralizes the H^+ ions that reach the duodenal lumen, warranting the neutrality and integrity of the mucosa.[37,38]

Even though the pathogenesis of peptic ulcer disease has not been fully elucidated, *H. pylori* infection in adults causes remarkable changes in gastric physiology, especially regarding the acid secretion mechanisms that are closely related to pathogenesis. In infection, mainly by cytotoxin-associated gene A (*cag*A)-positive strains, there is increased expression of proinflammatory cytokines, including interleukin-8 (IL-8), IL-1ß and tumor necrosis factor alpha (TNF-α),[39,40] which have a large effect on the mucus and on the HCO_3^- concentration on the cell surface, as well as on acid secretion. This is because they act on D cells by inhibiting somatostatin production with consequent hypergastrinemia and increase in acid secretion.[41,42] Among other consequences of the infection, it should be noted that the bacterium induces the release of several compounds (e.g.: cyclooxygenase 2 (COX-2)), which may compromise mucosal protection through the formation of other proinflammatory substances, such as reactive oxygen species (ROS).[31]

When infection is restricted to the antral mucosa and is followed by a remarkable increase in plasma gastrin, acid secretion is excessively high. Since infection also reduces the duodenal secretion of HCO_3^- and of mucus, the duodenal mucosa becomes permeable and is "attacked" by H^+ ions and other irritants, being replaced with metaplastic gastric mucosa. The bacterium in the gastric mucosa migrates and colonizes the metaplastic gastric areas in the duodenum, where it stimulates local inflammatory response, predisposing to the formation of the ulcer niche.

Reduction in the number of D cells and the inhibition of somatostatin production followed by hypergastrinemia are observed in *H. pylori*-positive children.[43] Since children with duodenal ulcer are more frequently colonized by more virulent strains of *H. pylori*, at least in part, the mechanisms of ulcer formation in children are similar to those found in adults.

H. pylori *virulence factors*

Several virulence factors have been associated with the development of severe diseases linked to *H. pylori* infection.

The *cagA* gene is a pathogenicity island (cag-*PAI*) marker.[40] The island genes encode proteins with several functions, including *cagA* protein translocation, of 120 kDa, into the cytoplasm of gastric epithelial cells, where, after being phosphorylated by *c-src* and *Lyn* kinases, the gene binds to and activates SHP-2 cell phosphatase, causing rearrangement of the cytoskeleton and formation of pedestals that allow greater bacterial adherence.[44] Several island genes are involved in stimulating IL-8 production by gastric epithelial cells. IL-8 is a powerful chemotactic factor and an activator of polymorphonuclear leukocytes and macrophages, contributing to a stronger inflammatory response in patients colonized by cag-PAI-positive strains. Therefore, patients infected by *cag*A-positive strains have larger bacterial density in the gastric mucosa, more severe epithelial injury, more intense polymorphonuclear leukocyte infiltration, and higher levels of proinflammatory cytokines, which endorses the commonly reported association between infection by *cag*A-

positive strains and peptic ulcer disease in children[45,46] and adults,[6,7,47] or gastric carcinoma in adults.[48-50] In Brazil, 95.0 and 62.3% of strains isolated from children with and without duodenal ulcer, respectively, are cagA-positive.[45]

The vacuolating cytotoxin A (*vac*A) gene, found in all *H. pylori* strains, encodes the VacA protein, an exotoxin that directly induces the formation of intracytoplasmic vacuoles and apoptosis of epithelial cells.[51] The toxin also increases epithelial permeability, which can make the passage of toxic substances into the epithelium easier and also help the diffusion of nutrients to the mucus layer, favoring the survival of *H. pylori*. The *vac*A has an immunomodulatory function; therefore, it sometimes stimulates the inflammatory response of the gastric mucosa by different mechanisms (e.g.: by increasing the expression of COX-2 in T cells, in neutrophils and in macrophages), and sometimes inhibits the IL-2-mediated response of T cells.[52] In *vac*A, there are two families of signal sequences called s1 and s2, with variations such as s1a, s1b and s1c, and two alleles located in the middle region of the gene, m1 and m2.[53] The vacA s1 *H. pylori* strains are more virulent than s2 strains, and are more commonly observed in patients with PU and gastric carcinoma than in those with gastritis.[53,54] In our population, 85.0 and 58.3% of children with and without duodenal ulcer, respectively, are colonized by s1 strains.[55]

The *H. pylori* blood-group antigen-binding adhesin A (BabA) binds to Lewis b and H-1 antigens, expressed in the gastric mucosa. The adherence of the BabA-mediated bacterium seems to play a crucial role in the transfer of bacterial virulence factors, which cause injury to the gastric mucosa, either directly or as a result of the inflammatory response and/or autoimmunity.[56] In addition, firmly adhered bacteria are less exposed to gastric acidity and are not eliminated by peristalsis.[57] The presence of the *bab*A2 gene, which encodes BabA, has been associated with duodenal ulcer in adults.[47,58] In a study of our group, we demonstrated the presence of the gene in all *H. pylori* strains that were evaluated, regardless of whether they were isolated from children with or without duodenal ulcer.[59]

The adherence of the bacterium to the gastric mucosa is also mediated by the sialic acid-binding adhesin A (SabA) protein, which binds to glucoconjugate residues of sialic acid expressed on the surface of epithelial cells due to the inflammatory or neoplastic process. The expression of sialic acid, which seldom occurs in the healthy gastric mucosa, is induced by *H. pylori* infection, contributing to its chronicity. SabA participates in the neutrophil activation by mechanisms that do not involve bacterial opsonization.[60]

Genes that encode proteins in the outer membrane of the pathogen, such as outer inflammatory protein A (OipA) and adhering lipoprotein AB (AlpAB), have been associated with higher virulence of strains, although the functions of their products are unknown.[61]

Recently, a probable *H. pylori* virulence factor has been described, called duodenal ulcer promoting gene A (*dup*A). The *dup*A, also located in the bacterial genome region that encodes surface proteins, corresponds to the fusion of *jhp*0917 and *jhp*0918 genes, with the insertion of a cytosine (C) or thymine (T) base pair. On describing the gene, the authors found an association with duodenal ulcer in adults.[62] These findings, however, have not been confirmed in Brazilian children and adults, thus indicating possible geographic differences.[63]

Genetic factors

Genetic factors are implicated in the pathogenesis of PU. This disease usually affects children with family history of this disorder, and the concomitance between monozygotic twins is three times greater than among dizygotic twins.[64]

Moreover, the disease is much more frequent among individuals with O blood type, which is equivalent to the Lewis b antigen. This higher frequency has been ascribed to the fact that the bacterial load is larger in these individuals, since, as previously mentioned, *H. pylori* has an adhesin called *BabA*, which binds to the Lewis b antigens.[56]

It should be underscored that the host's genetic factors can also determine immunological and inflammatory response to the infection, and can thus contribute to the outcome of a severe disease. Recent studies have shown that polymorphisms in regions that stimulate genes that encode proinflammatory cytokines can change gene transcription, with consequent increase in cytokine expression.[65,66] Thus, polymorphic alleles at positions -31 and -511 of the *IL1B* gene represent phenotypes that release high amounts of IL-1ß. Similarly, the polymorphic allele 2 of the *IL1RN* gene, which encodes the IL-1 receptor antagonist (IL-1ra), also increases IL-1ß production.[65,66,67] These polymorphisms have been associated with a greater risk for gastric carcinoma.[68-70] On the other hand, there are few studies on the role of these polymorphisms in the risk of PU. Recently, our group has demonstrated that the presence of polymorphic allele 2 of the IL1RN gene increases the risk for duodenal ulcer by 2.5 times in children.[71] This increase is likely to cause more exuberant inflammation in the antrum, with a decrease in somatostatin levels and an increase in gastrin levels, followed by an increase in acid secretion by the parietal cells, predisposing to the development of PU. However, this association has not been observed in adults,[72,73] neither in our population.[74] These differences may indicate different etiopathogenic mechanisms for PU in children and adults.

Clinical aspects

The clinical symptoms of primary PU in children can vary considerably according to their ages. The disease has an insidious clinical course, alternating between symptomatic and asymptomatic periods.

Newborns and infants can have hemorrhage, visceral perforation, or both, as first manifestations of the disease, even in the absence of any triggers, such as stress, which is regarded as the major cause of PU in this age group. In children, injuries often occur in the stomach, as a single lesion, but they frequently develop as multiple, small and bleeding erosions.[75] In children up to 6 years old, PU can initially present as nausea and vomiting, but hematemesis is the most common symptom, although it is difficult to establish the symptomatology. In school-aged children, older than 7 years, abdominal pain, albeit atypical and difficult to locate precisely, has been described in virtually all cases. In this age group, the most common symptoms include vomiting and bleeding, the latter of which is characterized either by hematemesis or melena.[75] Older children and adolescents have similar symptoms to those of adults. Abdominal pain is usually epigastric, but can also be present in the right lower quadrant. It can be intermittent or, more rarely, continuous; it can also be cyclic, with remission periods that last weeks or months. There is no correlation with food intake, and it often occurs in early morning. Some authors have pointed out that variability is the most consistent aspect of pain caused by PU in children.[75] In a study carried out in Brazil evaluating 43 children and adolescents aged 4 to 17 years, the authors demonstrated that abdominal pain was present in 90.7% of cases;[18] that it was most frequently epigastric (79.5%); that it consisted of a burning sensation in 56.4% of children; that it improved with food intake in 51.3%; and that decreased appetite (74.4%) and vomiting (69.8%) were the symptoms most commonly associated with pain.[18] Other symptoms, such as pyrosis, sialorrhea, sense of fullness, anorexia, weight loss, abdominal distension, eructation, meteorism and iron deficiency anemia seldom occur.[17,76]

The differential diagnosis of primary PU should be established with clinical entities that cause vomiting, abdominal pain and/or upper gastrointestinal bleeding, such as parasitic diseases, peptic esophagitis, esophageal and/or gastric varices, Zollinger-Ellison syndrome, food intolerance, allergic gastroenteropathy, Crohn's disease, pancreatitis, bile duct diseases, and gastritis (lymphocytic, hypertrophic or autoimmune), among others.[30]

Secondary PU is characterized by more acute symptoms and, in most cases, upper gastrointestinal bleeding is the major complaint, accompanied or not by abdominal pain. The underlying clinical disease and the depth and extension of the ulcers determine the severity and prognosis of the bleeding event.[17]

Endoscopic aspects

Contrast radiological examination of the stomach and duodenum has already been used for the diagnosis of ulcerated lesions before the advent of esophagogastroduodenoscopy. However, even with the double contrast technique, which is more sensitive, results have not been satisfactory. Furthermore, it cannot be easily used in younger children, in addition to exposing them to radiation.[16] Upper gastrointestinal endoscopy (UGIE) is the method of choice for the diagnosis of peptic ulcer disease, even in very young children.[77] Even though it is invasive and costly, especially in children, because in most cases it is performed in a hospital under general anesthesia, this method is safe and reliable. In addition to establishing the diagnosis and locating the ulcer, the UGIE allows obtaining biopsy fragments for histopathological analysis and for *H. pylori* investigation.[78] Moreover, modern videoendoscopes allow storing the images for later reassessments.

The most common endoscopic finding in children with *H. pylori* infection is a nodular lesion with a cobblestone pattern predominantly located in the antral mucosa, which is better visualized when covered by blood from the biopsy site.[77]

PUs are continuity solutions of the gastrointestinal mucosa that extend through the *muscularis mucosae*, reaching into the submucosa and into the *muscularis propria*. Most of the time, it is a single lesion; however, there may be several lesions sometimes. In adults, gastric ulcers should be distinguished from neoplastic lesions. In children, malignant lesions are very rare, but the characteristics of the borders, base and mucosa around the lesion should be recognized by all endoscopists. In peptic lesions, usually round or oval-shaped, the base consists of granulation tissues, it is flat, smooth and regular, and often covered by a white or grayish white fibrinoid exudate. In the initial ulcer stages, fibrin is thick and contains necrotic debris or hematin deposition, and the borders are smooth, regular, clearly defined, raised, and a bit higher than the base. In the mucous membrane around the lesion, the terminal ends of the folds extend towards the borders in a regular fashion. Most gastric peptic ulcers are located in the lesser curvature, in the *incisura angularis*, in the lower third of the gastric body, proximally to the antrum and anteriorly to the pyloric region. In 50% of the cases, duodenal ulcers are located in the anterior wall of the bulb.[79] The endoscopic aspect of the ulcer depends on the time at which it is observed, i.e., on its stage, according to the life cycle described by Sakita.[80] This cycle is divided into three stages: an initial stage, called active (A-active), followed by an intermediate stage, in which the ulcer is in the process of healing (H-healing) and, finally, the last stage, in which the ulcer has scarred (S-scar). All of these stages, according to their

characteristics, are subdivided into other two stages. After healing of the duodenal ulcer, the bulb might show a deformed architecture and, in extreme cases, this can cause segmental stenosis.

Diagnosis of H. pylori *infection*

There are several methods for the diagnosis of *H. pylori* infection. The microbiological and histopathological methods, and molecular biology techniques for direct detection of the pathogen in the gastric mucosa, are considered invasive, since mucosal fragments are obtained through esophagogastroduodenoscopy. The noninvasive or indirect methods consist of detection of anti-*H. pylori* antibodies in serum, urine and saliva samples, detection of *H. pylori* antigens in stool samples, and of the carbon 13-labeled urea breath test, non-radioactive isotope.

It has been recommended that at least two tests be used for a more accurate diagnosis of the infection. It is also necessary that noninvasive methods be validated for the population to be assessed.

Preformed urease test and H. pylori *investigation in histological sections and stained smears*

Since bacterial load is lower in children, test sensitivity increases when at least two mucosal fragments are analyzed, one from the antrum and another one from the gastric corpus. There may be false-negative results due to the irregular distribution of the bacterium in the gastric mucosa or due to the use of antimicrobials or proton pump inhibitors (PPIs) in case of the urease test.

The accuracy of investigation in histological sections relies mainly on the pathologist's experience.[17,81] Carbol fuchsin staining has proved sensitive, simple and inexpensive for the detection of *H. pylori* in smears or histological sections.[82,83]

Culturing

The isolation of *H. pylori* from gastric mucosal fragments is the most specific method for the diagnosis of this infection. It also allows detecting virulence factors and susceptibility to antimicrobials, enabling an improved therapy.

The use of at least two fragments from the gastric mucosa, one from the antrum and another one from the gastric body, adequate specimen transportation, use of a selective and indicator medium, and the team's experience are essential for the successful isolation of *H. pylori*.

Despite the high specificity of this method, sensitivity rates range from 77.0 to 100%.[84-86] Queiroz et al., in Brazil, found a sensitivity of 94.6%.[87]

Molecular biology techniques

These techniques are used for the diagnosis of *H. pylori* infection, genotyping of *H. pylori* virulence markers and determination of susceptibility to antimicrobials, in isolated strains or in biopsy fragments. The sensitivity of polymerase chain reaction (PCR) is higher than 95.0%.[75,88,89] Tissue culture of bacterial DNA can be performed using fluorescence *in situ* hybridization (FISH), with similar sensitivity to that of PCR.[90]

H. pylori virulence factors, such as cagA and *vac*A, can be identified by several molecular biology methods, such as PCR,[45,55,91,92] PCR followed by line probe assay (LiPA),[93] real-time PCR and reverse transcription PCR (rtPCR).[94] Since there are regional variations in the sequence of genes that encode these factors, primers described in the literature must be tested for different populations. Amplification followed by sequencing is necessary to identify other *H. pylori* virulence genes, such as *bab*A,[59] *dup*A,[62,63] *sab*A and *oip*A.[95]

Carbon 13-labeled urea breath test

Studies with adults[96] and with children older than 6 years[97,98] have shown that the breath test has sensitivity and specificity higher than 95.0%.

Although some reports have described lower specificity for the carbon 13-labeled urea breath test in children aged less than 6 years,[99,100] other studies have found a similar specificity to that observed in adults, regardless of the child's age.[101] Excellent results have been observed in our population, for children aged less than 6 years (sensitivity of 88.0% and specificity of 95.0%) and for older children as well (sensitivity of 100% and specificity of 98.0%).[102]

The carbon 13-labeled urea breath test is currently regarded as the method of choice to assess therapeutic response in adults and in children.

False-negative results are obtained when the patient is on PPIs and antimicrobials that may decrease bacterial density. PPIs must be discontinued 15 days and antimicrobials 1 month prior to the test. On the other hand, the use of histamine-2 (H_2) receptor antagonists and of antacids has a negligible interference with the test results; therefore, their use should be discontinued 48 hours before the test.

Detection of H. pylori *antigens in stool samples*

The detection of *H. pylori* in stool samples is achieved by an immunoenzyme assay using monoclonal or polyclonal antibodies; several kits are commercially available. Since it is a noninvasive test, it can also be used in epidemiological studies. There have been some reports of lower sensitivity of this method for the diagnosis of infection in children aged less than 6 years.[103] Nevertheless, the test has high sensitivity and specificity rates (approximately 95.0%) in our population, even among children aged less than 6 years.[102] It should be highlighted that appropriate transportation and maintenance of samples are crucial for successful results.

Gastrointestinal bleeding and the use of antimicrobials and of PPIs reduce the test sensitivity, which is not changed, however, by H_2 receptor antagonists and antacids.

Immunochromatographic tests for the detection of antigens in the feces, recently released into the market, are easily applied and allow having the results within few minutes. Their sensitivity and specificity are similar to those obtained from immunoenzyme assays.[104,105]

Detection of anti-H. pylori *antibodies*

H. pylori infection produces a cellular and humoral immune response in the host, which triggers the production of anti-*H. pylori* antibodies (IgM, IgA and IgG). IgM antibodies can be detected very early on. IgA and IgG antibodies only have detectable levels approximately 3 weeks to 3 months after the onset of acute infection and can be detected for up to 2 years after bacterial eradication.

Among several available methods, the enzyme-linked immunosorbent assay (ELISA) is the most widely used, since it is quick, easy-to-use, reproducible, inexpensive, and highly accurate; several kits have been commercially available. However, it should not be used for the diagnosis of infection in children aged less than 12 years, because its sensitivity is too low in this age group (44.4% for children aged 2 to 6 years and 76.7% for those aged 7 to 11 years).[27] As immunoenzyme assays for the detection of antibodies in the urine[106] and saliva[107] are even less sensitive and specific, they are not recommended for the diagnosis of infection in children. On the other hand, the sensitivity and specificity of the detection of anti-*H. pylori* antibodies by immunoblotting are acceptable for the diagnosis in children. In our population, a sensitivity of 95.0% and a specificity of 86.0% have been reported for children aged 2 to 16 years.[108]

Treatment

Peptic ulcer associated with H. pylori *infection*

H. pylori is the major cause of PU, and its eradication results in the cure of patients, being indicated for all children with active, recurrent, healed or complicated disease.

Even though there is no ideal therapeutic regimen, several treatments have been successfully used in adults, with eradication rates between 80 and 90%. The most efficient treatments include one PPI and two antimicrobials. At least one of the antimicrobials should be systemic, i.e., it should be excreted in active form into the gastric mucosa after being absorbed. There are few compounds with such quality, which include macrolides and imidazole derivatives (tinidazole or metronidazole). In Brazil, high eradication rates have been obtained with the use of PPIs, clarithromycin and furazolidone.[109,110] Other antimicrobials include amoxicillin, metronidazole, tetracycline, bismuth and quinolones, which are often used as second-line treatments. However, there is a paucity of studies on the treatment of infection in children. In addition to assessing a small number of patients, these studies substantially differ in terms of design, drug dosage and length of treatment, making it difficult to choose the best treatment regimen for the eradication of the pathogen in children.[111]

In Sweden, Tindberg et al.[112] found eradication rates lower than 67.0% with therapeutic regimens containing azithromycin, tinidazole and lansoprazole. Better results were obtained by Kato et al.[113] in Japan with treatments that included PPIs, amoxicillin and clarithromycin or metronidazole, which eradicated the pathogen in 77.4 and 87.5% of children, respectively. According to Oderda et al.,[111] triple regimens including bismuth, metronidazole and clarithromycin, or PPIs, amoxicillin and tinidazole, or PPIs, metronidazole and clarithromycin, for 1 to 2 weeks, yielded eradication rates greater than 85.0% in the treatment of children in Italy. Recently, Francavilla et al.,[114] in Italy, have obtained eradication rates of 97.5% by using a sequential regimen consisting of omeprazole and amoxicillin for 5 days, followed by omeprazole, clarithromycin and tinidazole for another 5 days. In Brazil, an eradication rate greater than 85% was observed in children treated with furazolidone, amoxicillin and metronidazole, given in three daily doses for 7 days.[43,115] Differently from the results obtained for adults in industrialized countries, treatment regimens including PPIs, clarithromycin and amoxicillin were not efficient for the treatment of children in Brazil.[116] Alternative regimens, containing other antimicrobials, should be used with caution. Quinolones are contraindicated for pediatric use, and tetracyclines cannot be used in children aged less than 8 years.

Resistance, especially to clarithromycin and to metronidazole, has been described, with rates that vary according to region. In our setting, clarithromycin resistance has amounted to 29.0%, whereas metronidazole resistance has reached 45.5%.[117] The microbiological methods recommended to assess resistance include the E-test and agar dilution. Mutations that provide clarithromycin resistance (adenine-to-guanine transition at position 2142 or 2143, and adenine-to-cytosine transition at position 2142) in the gene that encodes RNA 23S can be directly investigated in the DNA of isolated bacteria or in the DNA obtained from the freshly collected tissue fragment, or kept frozen by PCR followed by restriction fragment length polymorphism analysis (PCR-RFLP),[118] real-time PCR,[119] LiPA[120] or FISH.[121-123]

H. pylori-*negative peptic ulcer*

H_2 receptor antagonists are safe and efficient drugs used for the healing of PU. They inhibit acid secretion, competing with the H_2 receptors of parietal cells, and reduce

pepsinogen secretion. Oral cimetidine, in two doses of 20 to 30 mg/kg/day, and oral ranitidine, in two doses of 5 to 10 mg/kg/day, heal the ulcer in 80 to 90 and 80 to 100% of cases, respectively, after 8 weeks of treatment.[17,124]

PPIs are the most potent acid-secretion blockers available on the market, and omeprazole is the drug pediatricians have more experience with. PPIs whose action is dose-dependent specifically block the H^+-K^+-ATPase of the apical membrane of the parietal cell, with consequent suppression of acid secretion. In case of younger children, unable to swallow an intact capsule, the contents should be removed from the capsule and mixed in an acidic medium, preventing the protective films from dissolving while traveling down through the esophagus. Omeprazole soluble in water and in fruit juice is already commercially available, making its administration to children a lot easier.[30] The drug should be given in the morning, 30 minutes before breakfast.[125] The dose ranges from 0.7 to 3.3 mg/kg/day, but it has not yet been clearly defined for pediatric patients.[126] The total healing of ulcers is obtained in up to 100% of cases after 6 weeks. Individual response to intravenous omeprazole varies considerably, and persistent increase of pH levels above 4.0, is only observed in few patients.[125]

Antacids can bring some benefits to the treatment of PU, but high doses are necessary to neutralize gastric acidity, making its administration more difficult, especially to younger children.[16] Sucralfate has no effect on gastric acid secretion; however, it can contribute to the healing of ulcers by increasing the blood flow in the mucosa, as well as bicarbonate secretion and gastric mucus.[16]

H. pylori-*negative bleeding peptic ulcer*

Deep ulcers in the lesser curvature of the stomach or in the postero-inferior wall of the duodenal bulb are more likely to have severe bleeding, due to their vicinity to large vessels. Endoscopic stigmata of bleeding, described by Forrest et al., serve as a guide to endoscopic treatment and are useful indicators for the prognosis of bleeding. The endoscopic treatment of patients with active PU is indicated in special situations, such as in PU with active spurting bleeding (Forrest Ia) or oozing bleeding (Forrest Ib) and in those ulcers with visible vessel (Forrest IIa). The safest and most commonly used method of endoscopic hemostasis in the pediatric population in our setting consists of a local injection of an epinephrine 1:10,000 solution.[127] Other methods include thermal coagulation with argon or Nd:Yag laser, and the mechanical method with placement of metal clips.[127] The surgical treatment of patients with hemorrhagic PU is indicated in cases of uncontrollable bleeding, with severe hemodynamic consequences and unresponsiveness to clinical and endoscopic treatment.[128]

References

1. Baron JH, Sonnenberg A. Publications on peptic ulcer in Britain, France, Germany and the US. Eur J Gastroenterol Hepatol. 2002;14:711-5.
2. Susser M, Stein Z. Civilization and peptic ulcer. Lancet. 1962;1:115-9.
3. Dooley CP, Larson AW, Stace NH. Double-contrast barium meal and upper gastrointestinal endoscopy. A comparative study. Ann Intern Med. 1984;101:538-45.
4. Schindler R. Lehrbuch und Atlas der Gastroskopie. Munchen: Lehmann; 1923.
5. Marshall BJ, Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. 1983;1:1273-5.
6. Blaser MJ. Intrastrain differences in Helicobacter pylori: a key question in mucosal damage? Ann Med. 1995;27:559-63.
7. Graham DY. Campylobacter pylori and peptic ulcer disease. Gastroenterology. 1989;96(2 Pt 2 Suppl):615-25.
8. Blecker U. Helicobacter pylori-associated gastroduodenal disease in childhood. J La State Med Soc. 1998;150:419-29.
9. Queiroz DM, Rocha GA, Mendes EN, Carvalho AS, Barbosa AJA, Oliveira CA, et al. Differences in distribution and severity of Helicobacter pylori gastritis in children and adults with duodenal ulcer disease. J Pediatr Gastroenterol Nutr. 1991;12:178-81.
10. International Agency for Research Cancer, Shistosomes, liver flukes and Helicobacter pylori. Lyon: IARC; 1994.
11. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med. 1991;325:1127-31.
12. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med. 1991;325: 1132-6.
13. Fujioka T, Honda S, Tokieda M. Helicobacter pylori infection and gastric carcinoma in animal models. J Gastroenterol Hepatol. 2000;15 Suppl:D55-9.
14. Castro LP, Coelho LGV. Gastroenterologia. 2ª ed. São Paulo: Medsi; 2004.
15. Drumm B, Rhoads JM, Stringer DA, Sherman PM, Ellis LE, Durie PR. Peptic ulcer disease in children: etiology, clinical findings and clinical course. Pediatrics. 1988;82:410-4.
16. Walker WA, Durie PR, Hamilton JR, Walker-Smith JA, Watkins JB. Pediatric gastrointestinal disease. 2nd ed. St Louis: Mosby; 1996.
17. Carvalho AS. Úlcera péptica. J Pediatr (Rio J). 2000;76 Suppl 2:S127-34.
18. Kawakami E, Machado RS, Fonseca JA, Patrício FR. Aspectos clínicos e histológicos da úlcera duodenal em crianças e adolescentes. J Pediatr (Rio J). 2004;80:321-5.
19. Anand BS, Graham DY. Ulcer and gastritis. Endoscopy. 1999;31: 215-25.
20. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev. 2000;22:283-97.
21. Goodman KJ, Correa P, Tengana Aux HJ, Ramírez H, DeLany JP, Guerrero Pepinosa OG, et al. Helicobacter pylori infection in Colombian Andes: a population-based transmission pathways. Am J Epidemiol. 1996;144:290-9.
22. Lee A, Fox JG, Otto G, Dick EH, Krakowka S. Transmission of Helicobacter spp. A challenge to the dogma of faecal-oral spread. Epidemiol Infect. 1991;107:99-109.
23. Malaty HM, Kumagai T, Tanaka E, Ota H, Kiyosawa K, Graham DY, et al. Evidence from a nine-year birth cohort study in Japan of transmission pathways of Helicobacter pylori infection. J Clin Microbiol. 2000;38:1971-3.
24. Rocha GA, Rocha AMC, Silva LD, Santos A, Bocewicz C, Queiroz RM, et al. Transmission of Helicobacter pylori infection in families of preschool-aged children from Minas Gerais, Brazil. Trop Med Int Health. 2003;8:987-91.
25. Rodrigues MN, Queiroz DM, Bezerra Filho JG, Pontes LK, Rodrigues RT, Braga LL. Prevalence of Helicobacter pylori infection in children from an urban community in north-east Brazil and risk factors for infection. Eur J Gastroenterol Hepatol. 2004;16:201-5.
26. Goodman KJ, Correa P. Transmission of Helicobacter pylori among siblings. Lancet. 2000;355:358-62.
27. Oliveira AM, Rocha GA, Queiroz DM, Mendes EN, Carvalho AS, Ferrari TCA, et al. Evaluation of enzyme-linked immunosorbent assay for the diagnosis of Helicobacter pylori infection in children from different age groups with and without duodenal ulcer. J Ped Gastroenterol Nutr. 1999;28:157-61.
28. Cunha RP, Alves FP, Rocha AM, Rocha GA, Camargo LM, Nogueira POP, et al. Prevalence and risk factors associated with Helicobacter pylori infection in native population from Brazilian Western Amazon. Trans Royal Soc Trop Med Hyg. 2003;97:382-6.
29. Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am. 2000;29: 559-78.
30. Carvalho SD, Penna SJ. Úlcera Péptica Gastroduodenal. In: Silva RL, editor. Urgências Clínicas e Cirúrgicas em gastroenterologia e hepatologia pediátricas. Rio de Janeiro: Medsi; 2004. p. 219-32.
31. Konturek SJ, Konturek PC, Brzozowiski T, Konturek JW, Pawlik WW. From nerves and hormones to bacteria in the stomach; Nobel prize for achievements in gastrology during last century. J Physiol Pharmacol. 2005;56:507-30.
32. Teorell T. On the permeability of the stomach mucosa for acid and some other substances. J Gen Physiol. 1940;94:308-14.
33. Code CF, Scholer JF. Barrier offered by gastric mucosa to absorption of sodium. Am J Physiol. 1955;183:604-8.
34. Davenport HW. A history of gastric secretion and digestion. Oxford: Oxford University Press; 1992.

35. Brzozowski T. Experimental production of peptic ulcer, gastric damage and cancer models and their use in pathophysiological studies and pharmacological treatment - Polish achievements. J Physiol Pharmacol. 2003;54 Suppl 3:99-126.
36. Bukhave K, Rask-Madsen J, Hogan DL, Koss MA, Isenberg JI. Proximal duodenal prostaglandin E2 release and mucosal bicarbonate secretion are altered in patients with duodenal ulcer. Gastroenterology. 1990;99:951-5.
37. Konturek SJ, Mrzozowski T, Drozdowicz D, Pawlik W, Sendur R. Gastroprotective and ulcer healing effects of solon, a synthetic flavonoid derivative of sophoradin. Hepatogastroenterology. 1987;34:164-70.
38. Isenberg JI, Hogan DL, Koss MA, Selling JA. Human duodenal mucosal bicarbonate secretion. Evidence for basal secretion and stimulation by hydrochloric acid and a synthetic prostaglandin E1 analogue. Gastroenterology. 1986;91:370-8.
39. Crabtree JE, Covacci A, Farmery SM, Xiang Z, Tompkins DS, Perry S, et al. Helicobacter pylori induced interleukin-8 expression in gastric epithelial cells is associated with cagA-positive phenotype. J Clin Pathol. 1995;48:41-5.
40. Censini S, Lnage C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci USA. 1996;93:14648-53.
41. McColl KE, Fullarton GM, Chittajalu R, el Nujumi AM, MacDonald AM, Dahill SW, et al. Plasma gastrin, daytime intragastric pH, and nocturnal acid output before and at 1 and 7 months after eradication of Helicobacter pylori in duodenal ulcer subjects. Scand J Gastroenterol. 1991;26:339-46.
42. Queiroz DM, Mendes EN, Rocha GA, Moura SB, Resende LM, Barbosa AJ, et al. Effect of Helicobacter pylori eradication on antral gastrin- and somatostatin-immunoreactive cell density and gastrin-somatostatin concentrations. Scand J Gastroenterol. 1993;28: 658-64.
43. Queiroz DMM, Moura SB, Mendes EN, Rocha GA, Barbosa AJA, Carvalho AS. Effect of Helicobacter pylori eradication on G-cell and D-cell density in children. Lancet. 1994;343:1191-93.
44. Selbach M, Moese S, Hurwitz R, Hauck CR, Meyer TF, Backert S. The Helicobacter pylori CagA protein induces cortactin dephosphorylation and actin rearrangement by c-Src inactivation. EMBO J. 2003;22:515-528.
45. Queiroz DM, Mendes EN, Carvalho AS, Rocha GA, Oliveira AMR, Soares AST, et al. Factors associated with Helicobacter pylori by a cagA-positive strain in children. J infect Dis. 2000;181:626-30.
46. Oderda G, Figura N, Bayeli PF. Serologic IgG recognition of Helicobacter pylori cytotoxin associated protein, peptic ulcer and gastroduodenal pathology in childhood. Eur J Gastroenterol Hepatol. 1995;5:605-9.
47. Oliveira AG, Santos A, Guerra JB, Rocha GA, Rocha AM, Oliveira CA, et al. babA2 and cagA-positive Helicobacter pylori strains are associated with duodenal ulcer and gastric carcinoma in Brazil. J Clin Microbiol. 2003;41:3964-6.
48. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. 1995;55:2111-5.
49. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40:297-301.
50. Queiroz DMM, Mendes EN, Rocha GA, Oliveira AMC, Oliveira CA, Magalhães PP, et al. cagA-positive Helicobacter pylori and risk for developing gastric carcinoma in Brazil. Int J Cancer. 1998;78:135-9.
51. Cover TL, Blanke SR. Helicobacter pylori VacA, a paradigm for toxin multifunctionality. Nat Rev Microbiol. 2005;3:320-2.
52. Monteccuco C, de Bernard M. Immunosuppressive and proinflammatory activities of the VacA toxin of Helicobacter pylori. J Exp Med. 2003;198:1767-71.
53. Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem. 1995;270:17771-7.
54. Ashour AA, Magalhães PP, Mendes EN, Collares GB, Gusmão VR, Queiroz DM, et al. Distribution of vacA genotypes in Helicobacter pylori strains isolated from Brazilian adult patients with gastritis, duodenal ulcer or gastric carcinoma. FEMS Immunol Med Microbiol. 2002;33:173-8.
55. Gusmão VR, Mendes EN, Queiroz DM, Rocha GA, Rocha AM, Ashour AA, et al. vacA genotypes in Helicobacter pylori strains isolated from children with and without duodenal ulcer in Brazil. J Clin Microbiol. 2000;38:2853-7.
56. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. Helicobacter pylori adhesin binding fucosylated histo-blood antigens revealed by retagging. Science. 1998;279:373-7.
57. Rad R, Gerhard M, Lang R, Schoniger M, Rosch T, Schepp W, et al. The Helicobacter pylori blood group antigen-binding adhesin facilitates bacterial colonization and augments a nonspecific immune response. J Immunol. 2002;168:3033-41.
58. Olfat FO, Zheng Q, Oleastro M, Voland P, Boren T, Karttunen R, et al. Correlation of the Helicobacter pylori adherence factor BabA with duodenal ulcer disease in four European countries. FEMS Immunol Med Microbiol. 2005;44:151-6.
59. Silva JP, Guerra JB, Rocha GA, Rocha AMC, Bittencourt P, MMDA Cabral, et al. babA2 as a risk factor for duodenal ulcer. Helicobacter. 2004;9:549.
60. Unemo M, Aspholm-Hurtig M, Ilvert D, Bergström J, Borén T, Danielsson D, Borén T, et al. The sialic binding SabA adhesin of Helicobacter pylori is essential for nonopsonic activation of human neutrophils. J Biol Chem. 2005; 280:15390-7.
61. Figueiredo C, Machado JC, Yamaoka Y. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2005;10 Suppl1: 14-20.
62. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of Helicobacter pylori. Gastroenterology 2005;128:833-48.
63. Queiroz DM, Gomes LI, Rocha GA, Soares TF, Rocha AM, Godoi LM. dupA-positive H. pylori strains are highly prevalent in Brazil and are not associated with duodenal ulcer in Brazilian adults and children. Gastroenterology. 2006;130:A144.
64. Rotter JI. Genetic aspects of ulcer disease. Compr Ther. 1981;7: 716-25.
65. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol. 1998; 47:195-8.
66. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, et al. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in Helicobacter pylori infection. Gastroenterology. 2002;123:1793-803.
67. Vilaichone RK, Mahachai V, Tumwasorn S, Wu JY, Graham DY, Yamaoka Y. Gastric mucosal cytokine levels in relation to host interleukin-1 polymorphisms and Helicobacter pylori cagA genotype. Scand J Gastroenterol. 2005;40:530-9.
68. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404:398-402.
69. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, et al. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology. 2001;121:823-9.
70. Rocha GA, Guerra JB, Rocha AM, Saraiva IE, da Silva DA, de Oliveira CA, et al. IL1RN polymorphic gene and cagA-positive status independently increase the risk of noncardia gastric carcinoma. Int J Cancer. 2005;115:678-83.
71. Queiroz DMM, Bittencourt P, Guerra JB, Rocha AMC, Rocha GA, Carvalho AST. IL1RN polymorphism and cagA-positive Helicobacter pylori strains increase the risk of duodenal ulcer in children. Pediatr Res. 2005;58:892-6.
72. Furuta T, El-Omar EM, Xiao F, Shirai N, Takashima M, Sugimura H. V. Interleukin 1beta polymorphisms increase risk of hypochlorhydria and atrophic gastritis and reduce risk of duodenal ulcer recurrence in Japan. Gastroenterology. 2002;123:92-105.
73. Zambon CF, Basso D, Navaglia F, Germano G, Gallo N, Milazzo M, et al. Helicobacter pylori virulence genes and host IL1RN and IL-1beta genes interplay in favouring the development of peptic ulcer and intestinal metaplasia. Cytokine. 2002;18:242-51.
74. Guerra JB, Rocha GA, Rocha AM, Castro Mendes CM, Saraiva IE, Oliveira CA, et al. IL-1 gene cluster and TNFA-307 polymorphisms in the risk of perforated duodenal ulcer. Gut. 2006;55:132-3.
75. Kenneth SN. Doença da úlcera péptica na população pediátrica. Tradução e adaptação Pediatr Clin North Am. 1988;1:121-45.
76. Vandenplas Y, Blecker U. Helicobacter pylori infection in children. Acta Paediatr. 1998;87:1105-12.
77. Hargrove CB, Ulshen MH, Shub MD. Upper gastrointestinal endoscopy in infants: diagnostic usefulness and safety. Pediatrics. 1984;74: 828-31.
78. Magalhães AF, Cordeiro FT, Quilici FA, Machado G, Amarante HM, Prolla JC, et al. SOBED- Endoscopia Digestiva Diagnóstica e Terapêutica. 4ª ed. São Paulo: Revinter; 2005.
79. Bahu MG, da Silveira TR, Maguilnick I, Ulbrich-Kulczynski J. Endoscopic nodular gastritis an endoscopic indicator of high-grade bacterial colonization and severe gastritis in children with Helicobacter pylori. J Pediatr Gastroenterol Nutr. 2003;36: 217-22.
80. Sakita T. Endoscopy in the diagnosis of early cancer. Clin Gastroenterol. 1973;2:345-60.
81. Rowland M, Drumm B. Clinical significance of Helicobacter infection in children. Br Med Bull. 1998;54:95-103.
82. Rocha GA, Queiroz DMM, Mendes EN, Lage AP, Barbosa AJA. Simple carbolfuchsin staining for showing C. pylori and other spiral bacteria in gastric mucosa. J Clin Pathol. 1989;42:1004-5.
83. Resende LM, Queiroz DM, Mendes EN, Rocha GA, Coelho LG, Passos MC, et al. Comparison of the urease test and of direct smear examination in the control of treatment of Helicobacter pylori-induced infection. Braz J Med Biol Res. 1993;26:699-702.
84. Westblom TU, Madan E, Gudipati S, Midkiff BR, Czinn SJ. Diagnosis of Helicobacter pylori infection in adult and pediatric patients by using Pyloriset, a rapid latex agglutination test. J Clin Microbiol. 1992;30:96-8.

85. Yanez P, la Garza AM, Perez-Perez G, Cabrera L, Munoz O, Torres J. Comparison of invasive and noninvasive methods for the diagnosis and evaluation of eradication of Helicobacter pylori infection in children. Arch Med Res. 2000;31:415-21.
86. Ogata SK, Kawakami E, Patricio FR, Pedroso MZ, Santos AM. Evaluation of invasive and non-invasive methods for the diagnosis of Helicobacter pylori infection in symptomatic children and adolescents. Sao Paulo Med J. 2001;119:67-71.
87. Melo FF, Rocha AMC, Rocha GA, Bittencourt P, Soares TF, Almeida LR, et al. Avaliação da cultura para o diagnóstico da infecção por Helicobacter pylori em crianças. In: Anais do XXIII Congresso de Microbiologia; 2005 23-25 nov; Santos (SP), Brasil. p. 1470-1.
88. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of Helicobacter pylori: comparison of staining methods. J Clin Pathol. 2000;53:756-9.
89. Vinette KM, Gibney KM, Proujansky R, Fawcett PT. Comparison of PCR and clinical laboratory tests for diagnosing H. pylori infection in pediatric patients. BMC Microbiol. 2002;40:3720-8.
90. Russmann H, Kempf VA, Koletzko S, Heesemann J, Autenrieth IB. Comparison of fluorescent in situ hybridization and conventional culturing for detection of Helicobacter pylori in gastric biopsy specimens. J Clin Microbiol. 2001;39:304-8.
91. Loeb M, Jayaratne P, Jones N, Sihoe A, Sherman P. Lack of correlation between vacuolating cytotoxin activity, cagA gene in Helicobacter pylori, and peptic ulcer disease in children. Eur J Clin Microbiol Infec Dis. 1998;17:653-6.
92. Gzyl A, Berg DE, Dzierzanowska D. Epidemiology of cagA/vacA genes in H. pylori isolated from children and adults in Poland. J Physiol Pharmacol. 1997;48:333-43.
93. van Doorn LJ, Figueiredo C, Rossau R, Jannes G, van Asbroek M, Sousa JC, et al. Typing of Helicobacter pylori vacA gene and detection of cagA gene by PCR and reverse hybridization. J Clin Microbiol. 1998;36:1271-6.
94. Boonjakuakul JK, Syvanen M, Suryaprasad A, Bowlus CL, Solnick JV. Transcription profile of Helicobacter pylori in the human stomach reflects its physiology in vivo. J Infect Dis. 2004;190; 946-56.
95. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of Helicobacter pylori. Proc Natl Acad Sci U S A. 2000;97:7533-8.
96. Klein PD, Graham DY. Minimum analysis requirements for detection of Helicobacter pylori infection by the ^{13}C-urea breath test. Am J Gastroenterol. 1993;88:1865-9.
97. Kalach N, Briet F, Raymond J, Benhamou PH, Barbet P, Bergeret M, et al. The ^{13}carbon urea breath test for the noninvasive detection of Helicobacter pylori in children: comparison with culture and determination of minimum analysis requirements. J Ped Gastroenterol Nut. 1998;26:291-6.
98. Bazzoli F, Cecchini L, Corvaglia L, Dall'Antonia M, De Giacomo C, Fossi S, et al. Validation of the ^{13}C-urea breath test for the diagnosis of Helicobacter pylori infection in children: a multicenter study. Am J Gastroenterol. 2000;95:646-50.
99. Kindermann A, Demmelmair H, Koletzko B, Krauss-Etschmann S, Wiebecke B, Koletzko S. Influence of age on 13C-urea breath test results in children. J Pediatr Gastroenterol Nutr. 2000;30:85-91. Erratum in: J Pediatr Gastroenterol Nutr. 2000;30:354.
100. Imrie C, Rowland M, Bourke B, Drumm B. Limitations to carbon 13-labeled urea breath testing for Helicobacter pylori in infants. J Pediatr. 2001;139:622-3.
101. Machado RS, Patricio FR, Kawakami E. 13C-urea breath test to diagnose Helicobacter pylori infection in children aged up to 6 years. Helicobacter. 2004;9:39-45.
102. Cardinali LC, Rocha GA, Rocha AM, Moura SB, Soares TF, Esteves AM, et al. Evaluation of [^{13}C]urea breath test and Helicobacter pylori stool antigen test for diagnosis of H. pylori infection in children from developing country. J Clin Microbiol. 2003;41: 3334-5.
103. Oderda G, Rapa A, Ronchi B, Lerro P, Pastore M, Staiano A, et al. Detection of Helicobacter pylori in stool specimens by non-invasive antigen enzyme immunoassay in children: multicentre Italian study. BMJ. 2000;320:347-8.
104. Kato S, Ozawa K, Okuda M, Nakayama Y, Yoshimura N, Konno M, et al; Japan Pediatric Helicobacter Study Group. Multicenter comparison of rapid lateral flow stool antigen immunoassay and stool antigen enzyme immunoassay for the diagnosis of Helicobacter pylori infection in children. Helicobacter. 2004;9: 669-73.
105. Antos D, Crone J, Konstantopoulos N, Koletzko S. Evaluation of a novel rapid one-step immunochromatographic assay for detection of monoclonal Helicobacter pylori antigen in stool samples from children. J Clin Microbiol. 2005;43:2598-601.
106. Megraud F; European Paediatric Task Force on Helicobacter pylori. Comparison of non-invasive tests to detect Helicobacter pylori infection in children and adolescents: results of a multicenter European study. J Pediatr 2005;146:164-7.
107. Gilger MA, Tolia V, Johnson A, Rabinowitz S, Jibaly R, Elitsur Y, et al. The use of an oral fluid immunoglobulin G ELISA for the detection of Helicobacter pylori infection in children. Helicobacter. 2002;7:105-10.
108. Rocha GA, Oliveira AM, Queiroz DM, Carvalho AS, Nogueira AM. Immunoblot analysis of humoral immune response to Helicobacter pylori in children with and without duodenal ulcer. J Clin Microbiol. 2000;38:1777-81.
109. Dani R, Queiroz DM, DIAS MG, Franco JM, Magalhães LC, Moreira LS, et al. Omeprazole, clarithromycin and furazolidone for the eradication of Helicobacter pylori in patients with duodenal ulcer. Aliment Pharmacol Ther. 1999;13:1647-52.
110. Queiroz DM, Dani R, Silva LD, Santos A, Moreira LS, Rocha GA, et al. Factors associated with treatment failure of Helicobacter pylori infection in a developing country. J Clin Gastroenterol. 2002; 35:315-20.
111. Oderda G, Rapa A, Bona G. A systematic review of Helicobacter pylori eradication treatment schedules in children. Aliment Pharmacol Ther 2000;14 Suppl 3:59-66.
112. Tindberg Y, Casswall TH, Blennow M, Bengtsson C, Granström M, Sörberg M. Helicobacter pylori eradication in children and adolescents by a once daily 6-day treatment with or without a proton pump inhibitor in a double-blind randomized trial. Aliment Pharmacol Ther. 2004;20:295-302.
113. Kato S, Konno M, Maisawa S, Tajiri H, Yoshimura N, Shimizu T, et al. Results of triple eradication therapy in Japanese children: a retrospective multicenter study. J Gastroenterol. 2004;39: 838-43.
114. Francavilla R, Lionetti E, Castellaneta SP, Magista AM, Boscarelli G, Piscitelli D, et al. Improved efficacy of 10-Day sequential treatment for Helicobacter pylori eradication in children: a randomized trial. Gastroenterology. 2005;129:1414-9.
115. Carvalho AS, Queiroz DM, Mendes EN, Rocha GA, Nogueira AM, Cabral MM, et al. Triple antimicrobial therapy plus H_2 receptor antagonist or omeprazole in the eradication of Helicobacter pylori in children with duodenal ulcer. Gut. 1998:43 Suppl 1:A74.
116. Kawakami E, Ogata SK, Portorreal ACM, Magni AM, Pardo MLE, Patrício FRS. Triple therapy with clarithromycin, amoxicillin and omeprazole for Helicobacter pylori eradication in children and adolescents. Arq Gastroentrol. 2001;38:203-6.
117. Cardinali LCC. Infecção por Helicobacter pylori em crianças sintomáticas de Belo Horizonte: validação de dois testes não-invasivos e determinação do padrão de susceptibilidade a antimicrobianos [dissertação]. Belo Horizonte: Universidade Federal de Minas Gerais; 2002.
118. Versalovic J, Shortridge D, Kibler K, Griffy MV, Beyer J, Falmm RK, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in Helicobacter pylori. Antimicrob Agents Chemother. 1996;40:477-80.
119. Chisholm SA, Owen RJ, Teare EL, Saverymuttu S. PCR-based diagnosis of Helicobacter pylori infection and real- time determination of clarithromycin resistance directly from human gastric biopsy samples. J Clin Microbiol. 2001;39:1217-20.
120. van der Ende A, van Doorn LJ, Rooijakkers S, Feller M, Tytgat GN, Dankert J. Clarithromycin-susceptible and -resistant Helicobacter pylori isolates with identical randomly amplified polymorphic DNA-PCR genotypes cultured from single gastric biopsy specimens prior to antibiotic therapy. J Clin Microbiol. 2001;39:2648-51.
121. Trebesius K, Panthel K, Strobel S, Vogt K, Faller G, Kirchner T, et al. Rapid and specific detection of Helicobacter pylori macrolide resistance in gastric tissue by fluorescent in situ hibridisation. Gut. 2000;46:608-14.
122. Juttner S, Vieth M, Miehlke S, Schneider-Brachert W, Kirsch C, Pfeuffer T, et al. Reliable detection of macrolide-resistant Helicobacter pylori via fluorescence in situ hybridization in formalin-fixed tissue. Mod Pathol. 2004;17:684-9.
123. Russmann H, Feydt-Schmidt A, Adler K, Aust D, Fisher A, Koletzko S. Detection of Helicobacter pylori in paraffin-embedded and in shock-frozen gastric biopsy samples by fluorescent in situ hybridization. J Clin Microbiol. 2003;41:813-5.
124. Feldman M, Burton ME. Histamine$_2$-receptor antagonists: standard therapy for acid-peptic diseases. 1. N Engl J Med. 1990;323: 1672-80.
125. Israel DM, Hassall E. Omeprazole and other proton pump inhibitors: pharmacology, efficacy, and safety, with special reference to use in children. J Pediatr Gastroenterol Nutr. 1998;27:568-79.
126. Hassall E, Israel D, Shepherd R, et al. Omeprazole for treatment of chronic erosive esophagitis in children: a multicenter study of efficacy, safety, tolerability and dose requirements. International Pediatric Omeprazole Study Group. J Pediatr. 2000;137:800-7.
127. Silva MG, Milwad G. Endoscopia pediátrica. Rio de Janeiro: Medsi/ Guanabara Koogan; 2004.
128. Carvalho E, Nita MH, Paiva LM, Silva AA. Hemorragia digestiva. J Pediatr (Rio J). 2000;76 Suppl 2:S135-46.

Correspondence:
Dulciene M. M. Queiroz
Laboratório de Pesquisa em Bacteriologia, Fac. de Medicina, UFMG
Av. Alfredo Balena, 190/40
CEP 30130-100 – Belo Horizonte, MG – Brazil
Tel./Fax: +55 (31) 3274.2767
E-mail: dqueiroz@medicina.ufmg.br