Mary de Assis Carvalho

Achados histopatológicos gástricos em crianças e adolescentes brasileiros com dispepsia e infecção por *Helicobacter pylori*

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Faculdade de Medicina - Campus de Botucatu

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Orientadora: Prof. Dra. Maria Aparecida Marchesan Rodrigues

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1. *Helicobacter pylori.* 2. Infecção por *Helicobacter pylori.*

Palavras-chave: Biópsia; Criança; Gastrite; *Helicobacter pylori*; Patologia.

Aos meus amados pais Azir e Maria das Dores,

Aos meus amados Nilton e Victor.

"As dificuldades, como as montanhas, aplainam-se quando avançamos por elas"

Émile Zola,

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Revisão da Literatura

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1. Dor Abdominal Crônica (DAC) em Crianças e Adolescentes

A Dor Abdominal Crônica (DAC) afeta aproximadamente 10 a 15% das crianças entre 4 e 14 anos (Apley, 1975; Liebman, 1978), sendo responsável por 2 a 4% de todas as consultas pediátricas. Está associada a elevado custo econômico e à redução substancial na qualidade de vida (Di Lorenzo et al., 2005).

A definição clássica de DAC por Apley & Naish (1958) ainda é a mais utilizada nos dias atuais, tanto em pesquisas científicas quanto em consultórios médicos: pelo menos 3 episódios de dor abdominal, em um período mínimo de 3 meses, com intensidade o suficiente para interferir nas atividades da criança. A DAC pode ser classificada em Funcional, quando não há evidências demonstráveis de condições patológicas anatômicas, inflamatórias, infecciosas, metabólicas ou neoplásicas, e em Orgânica, quando tais doenças estão implicadas. Na grande maioria das crianças a DAC é funcional, sendo que condições orgânicas estão presentes em um pequeno percentual (Di Lorenzo et al., 2005).

A DAC pode se apresentar, em um subgrupo de crianças, como síndrome dispéptica caracterizada por sintomas abdominais inespecíficos, contínuos ou intermitentes, geralmente associados à alimentação, relacionados a distúrbios do trato digestivo proximal e de duração superior a 2 meses (Barbara et al., 1989). Esses sintomas compreendem: vômitos, saciedade precoce, dor epigástrica pós-prandial, azia, estufamento abdominal, baixo ganho de peso e/ou anorexia. A prevalência da dispepsia em crianças e adolescentes varia de 0.27% a 10% e aumenta com a idade (Hyams et al., 2000).

Os sinais e sintomas de dispepsia presentes em crianças não são específicos, podendo ocorrer em doenças tais como: processos inflamatórios (parasitoses, esofagite, gastroenteropatia eosinofílica, infecção por *Helicobacter pylori,* doença de Crohn, doença hepática, pancreatite), doença biliar e intolerância à lactose (Boyle, 1991). Quando a dispepsia ocorre na ausência de doença demonstrável é chamada dispepsia funcional (Hyams et al., 2000), sendo os critérios diagnósticos atuais definidos pelos Critérios de Roma III, baseados em sintomas (Rasquin et al., 2006), para crianças e adolescentes de 4 a 18 anos. Os Critérios de

Roma III (Rasquin et al., 2006) estabeleceram que todos os seguintes critérios para o diagnóstico de dispepsia funcional devem estar presentes, pelo menos uma vez por semana por pelo menos 2 meses antes do diagnóstico: (1) dor ou desconforto persistente ou recorrente centrados no abdome superior (acima do umbigo); (2) não aliviado pela evacuação ou associado com mudança na frequência ou forma das fezes como acontece na síndrome do intestino irritável e (3) sem evidências de processo inflamatório, anatômico, metabólico ou neoplásico que possa explicar os sintomas.

Na literatura atual há poucos estudos que demonstrem que a anamnese e o exame físico sejam discriminatórios entre DAC orgânica e funcional, entretanto, critérios de abordagem ao paciente com DAC foram descritos, baseados em sinais e sintomas, que permitem ao pediatra identificar as crianças com maior probabilidade de etiologia orgânica subjacente. A AAP (American Academy of Pediatrics) e a NASPGHAN (North American Society for Pediatric Gastroenterology, Hepatology and Nutrition) sugerem a realização de exames laboratoriais na discriminação entre DAC funcional e orgânica, quando estiverem presentes sinais de alerta (Di Lorenzo et al., 2005; Rasquin et al., 2006) tais como: vômitos significativos (biliosos, protraídos, cíclicos), diarreia noturna, dor abdominal persistente em hemiabdome direito, febre inexplicada, história familiar de doença inflamatória intestinal crônica, de doença péptica ou doença celíaca, desaceleração do crescimento, sangramento gastrointestinal, massa abdominal palpável, hepatomegalia, esplenomegalia, dor no ângulo costo-vertebral ou sobre a espinha vertebral e anormalidades perianais. Os exames sugeridos incluem: hemograma, VHS e PCR, urina tipo I, urocultura, parasitológico seriado de fezes, pesquisa de sangue oculto nas fezes e radiografia simples de abdome.

Adicionalmente, para a discriminação entre dispepsia orgânica e funcional, Chelimsky & Czinn (2001) e See et al. (2001) descreveram sistema de pontuação para sintomas sugestivos de dispepsia orgânica em crianças, tais como: epigastralgia, dor retroesternal, relação com a alimentação, despertar noturno pela dor, dor ao acordar pela manhã, vômitos significativos (pelo menos 3x/mês), náusea crônica, saciedade precoce, eructações excessivas, regurgitação, anorexia, perda de peso, dor abdominal periumbilical, história familiar de úlcera, dispepsia ou síndrome do intestino irritável. Assim, diante de criança com sinais de alerta para

dispepsia orgânica, se a avaliação laboratorial inicial associada a testes adicionais apropriados não elucidarem o diagnóstico, a indicação de endoscopia digestiva alta para o diagnóstico diferencial seria o procedimento de escolha (Chelimsky & Czinn, 2001; See et al., 2001; Rasquin et al., 2006).

Dentre as causas de dispepsia orgânica na infância, a infecção pelo *Helicobacter pylori* destaca-se, em nosso meio, pela sua alta frequência (Oliveira et al., 1994; Solari et al., 1994; Souto et al., 1998; Ogata et al., 2001). Esta infecção está associada a processos inflamatórios no tubo digestivo alto tais como a gastrite crônica (assintomática ou sintomática), úlcera péptica (gástrica ou duodenal), carcinoma gástrico ou linfoma MALT (Drumm et al., 2000) e pode manifestar-se como DAC orgânica - padrão dispéptico.

2. *Helicobacter pylori*

2.1. Agente

O *Helicobacter pylori* (*H. pylori*) é uma bactéria Gram-negativa espiralada, móvel (flagelada) e microaerófila que coloniza as células epiteliais gástricas do homem (Marshall & Warren, 1984; Cave, 1996). Esta bactéria apresenta capacidade de estabelecer infecção da mucosa gástrica de hospedeiros humanos por décadas ou a vida toda (Marshall & Warren, 1984; Covacci et al., 1999; Chen et al., 2007). Foi classificado como carcinógeno do grupo I pela Agência Internacional para Pesquisa sobre o Câncer, sendo considerado como um fator de risco primário para câncer gástrico (NIH, 1994; Sipponem & Hyvarinen, 1993).

2.2. Fatores de Virulência e Colonização Bacterianos

O *H. pylori* possui múltiplos fatores de virulência que o fazem único em sobreviver indefinidamente ao ambiente hostil gástrico, mediante indução de efeitos tanto pró-inflamatórios quanto imunossupressores, resultando em ineficácia da resposta imune do hospedeiro (Robinson et al., 2007; Wilson & Crabtree, 2007; Amieva & El-Omar, 2008; Basso et al., 2010, Aebischer et al., 2010). Eles são:

- Enzima urease no citosol e superfície bacteriana, presente em todas as cepas de *H. pylori*, capaz de tamponar transitoriamente o ambiente ácido pela

degradação da ureia em amônia e dióxido de carbono. A urease também induz a dano epitelial e inflamação

- Flagelos para a motilidade e capacidade de controle da direção deste movimento por respostas quimiotáticas, essenciais para o *H. pylori* alcançar a região menos ácida do lago mucoso justaposto à superfície epitelial
- Expressão de variadas proteínas da membrana externa (OMPs) denominadas adesinas (especialmente BabA e SabA, além de outras), que conferem múltiplas possibilidades de ligação à superfície celular gástrica
- Produção de várias toxinas que ao alcançarem o citosol da célula epitelial gástrica via proteínas do Sistema de Secreção do Tipo IV (TFSS) ou via formação de poros, passam a controlar aspectos diversos da sua função. As mais estudadas são as proteínas CagA e VacA, que conferem virulência e estão envolvidas na patogênese da infecção pelo *H. pylori*:
    - CagA – citotoxina imunogênica codificada pelo gene cagA (gene associado à citotoxina) localizado na ilha de patogenicidade (cagPAI) do *H. pylori*, estando presente em aproximadamente 60% das cepas de *H. pylori*. É responsável por indução de várias vias de sinalização a jusante na célula epitelial, resultando em dissociação das células, perda da polaridade celular, reorganização do citoesqueleto, alongamento das células e progressão do ciclo celular, com efeitos pró-inflamatório e mitogênico, aumentando o risco de neoplasias. A cagPAI também contém outros genes responsáveis pela virulência, especialmente ligados à expressão de proteínas do TFSS e à indução de citocinas pró-inflamatórias, principalmente interleucina-8 (IL-8)
    - VacA (citotoxina vacuolizante) - produto da expressão do gene vacA. A VacA induz vacuolização citoplasmática por perturbar a maturação endossomal, aumenta a permeabilidade das células epiteliais formando poros na membrana celular, induz a liberação de citocromo C mitocondrial e apoptose celular, interfere com a fagocitose e a apresentação de antígenos e inibe a proliferação de células T, o que resulta em dano celular e inflamação. Está presente em todas as cepas de *H. pylori*, porém com heterogeneidades genotípicas que lhe conferem maior ou menor grau de virulência

- Expressão do TFSS, proteína de membrana do *H. pylori*, que funciona como agulhas que permitem a transferência de fatores bacterianos para dentro da célula gástrica, especialmente da CagA
- Mecanismos múltiplos de evasão e modulação do sistema imune para reduzir o reconhecimento bacteriano por parte do sistema imune inato, com rearranjos de seu DNA genômico que permitem que adapte sua expressão de antígenos de superfície. Estes incluem a geração de diversidade de lipopolissacarídeos e proteínas da membrana externa (OMPs) através de variação alélica, com mimetismo molecular de glicanos da superfície celular do hospedeiro e modificação de flagelos (Loughlin et al., 2003).

2.3. Transmissão e Epidemiologia

Os mecanismos mais prováveis de transmissão do *H. pylori* são a via oral-oral, gastro-oral e a fecal-oral, de pessoa para pessoa ou uma fonte comum de exposição (Drumm, 1993; Graham et al., 1994; Deltenre & De Koster, 2000). A cavidade bucal e o estômago são reservatórios do agente.

O *H. pylori* acomete cerca de 50% da população mundial, sendo uma das infecções crônicas mais comuns em humanos (Goodwin et al., 1997; Parsonnet, 1995; Ahuja & Sharma, 2002). Sua prevalência é variável entre países e entre grupos populacionais dentro do mesmo país, de acordo com a faixa etária, nível socioeconômico e grupo étnico estudado (Lindkvist et al., 1996; Mitchell et al., 1992).

A infecção pelo *H. pylori* é geralmente adquirida na infância pela ingestão da bactéria (Granstrom, et al., 1997; Mitchell et al., 1992; Rowland et al., 1999; Goodman & Correa, 2000; Rothenbacher et al., 2000; Torres et al., 2000; Bahu et al., 2003). É transmitida principalmente dentro das famílias com filhos, sendo a mãe e os irmãos as principais fontes de transmissão (Rothenbacher et al., 1999; Perez-Perez et al., 2004), e eventualmente entre os membros da família durante períodos de doença diarreica (Parsonnet et al., 1995). A idade de aquisição depende da prevalência do *H. pylori* e do nível socioeconômico da população estudada (Ozen et al., 2006; Malaty et al., 2002; Ohara et al., 2003; Camargo et al., 2004).

Para a população geral a taxa de infecção é maior em países em desenvolvimento que em países industrializados (Graham, 1991; Zaitoun, 1995;

Luzza et al., 2001; Chen et al., 2002), sendo de aproximadamente 70-90% da população nos países em desenvolvimento e 25% em países industrializados (Dunn et al., 1997). Nos países industrializados, a taxa de aquisição do *H. pylori* tem diminuído substancialmente nas últimas décadas.

Nos países em desenvolvimento, quase 90% das crianças estão infectadas em torno dos 10 anos de idade (Blaser et al., 1995; Dimmick et al., 1997; Ernst, 1999; Gold et al., 2001, Hatakeyama & Brzozowski, 2006) enquanto nos países industrializados europeus ocidentais a infecção pelo *H. pylori* tem sido caracterizada por um aumento linear com a idade (Drumm, 1993; Ohara et al., 2003), com prevalência em torno de 10% em adolescentes (Koletzko et al., 2006).

No Brasil, estudos realizados em crianças dispépticas de três diferentes regiões geográficas demonstraram uma prevalência da infecção por *H. pylori* de 34% a 77%, utilizando sorologia, histologia, e cultura em crianças dispépticas submetidas à endoscopia (Oliveira et al., 1994; Solari et al., 1994; Souto et al., 1998; Ogata et al., 2001).

3. Patogenia da Infecção pelo *H. pylori*

3.1. Colonização da Mucosa Gástrica

Após ser ingerido, o *H. pylori* utiliza-se da atividade da urease para metabolizar ureia em dióxido de carbono e amônia, tamponando a acidez gástrica, e de respostas adaptativas ao estresse para tolerar temporariamente a acidez. A seguir movimenta-se ativamente (flagelado), penetra no muco gástrico viscoso na superfície da mucosa e alcança o pH mais neutro abaixo do muco, estabelecendo um contato íntimo com as células epiteliais (Eaton & Krakowka, 1994; Eaton et al., 1996).

Uma vez abaixo do muco, o *H. pylori* adere-se firmemente à membrana das células epiteliais gástricas utilizando-se de adesinas, (Clyne & Drumm, 1993). Assim, o *H. pylori* evita o seu clareamento mecânico pelo peristaltismo, utiliza a superfície celular como um sítio de replicação, transfere seus fatores de virulência pela indução de poros e TFSS, facilitando sua invasão e a vias de sinalização para a cascata

imune, induzindo ao dano celular (Amieva & El-Omar, 2008). Sabe-se atualmente que uma pequena proporção das bactérias invade tanto as células epiteliais como o cório da mucosa gástrica (Segal, 1997; Necchi et al., 2007).

A célula epitelial, induzida pelos fatores de virulência, passa a produzir quimiocinas, em especial a IL-8, se a cepa for cagAPAI, que recrutam e ativam as células dendríticas (DCs) (Ernst & Gold, 1999; Basso et al., 2010).

3.2. Resposta do Hospedeiro à Infecção

A resposta imune do hospedeiro ao *H. pylori* pode ser dividida em inata (inespecífica) e adaptativa (antígeno-específico).

3.2.1. Resposta Imune Inata

O sistema imune inato reconhece as moléculas bacterianas antigênicas, mediante tipos específicos de receptores Toll-like (TLRs) ou NOD-1 (Nucleotide-binding Oligomerization Domain), expressos pelas células apresentadoras de antígenos (APCs), tais como macrófagos e células dendríticas (DCs) (Di Tommaso et al., 1995; Smith et al., 2003; Gewirtz et al., 2004; Ishihara et al., 2004).

O contato do *H. pylori* com macrófagos e outras APCs, além das próprias células epiteliais gástricas, leva à secreção de grandes quantidades de citocinas e quimiocinas pró-inflamatórias tais como o Fator de Necrose Tumoral-α (TNF-α), Interleucina (IL)-1β, IL-8, IL-12, leucotrienos, e produtos de ativação do complemento. Estas citocinas agem na quimiotaxia local, induzindo a migração de neutrófilos, monócitos e linfócitos para a mucosa e definindo o tipo de resposta imune.

Associados aos neutrófilos, os macrófagos e células dendríticas também agem como células efetoras na morte bacteriana mediante fagocitose e produção de radicais livres de oxigênio e Óxido Nítrico (NO). O *H. pylori*, porém, desenvolveu mecanismos de escape ao reduzir a produção de NO das APCs por competir pelo substrato arginina (Gobert et al., 2001) e evitar a morte pós-fagocitose por impedir a fusão dos fagossomas com os lisossomos se a cepa for CagA positiva (Zheng & Jones, 2003). Por outro lado, o *H. pylori* pode induzir apoptose destas células, agravando a resposta inflamatória.

Nishi et al. (2003) demonstraram o envolvimento das células dendríticas no desenvolvimento dos folículos linfoides secundários na infecção por *H. pylori*, por induzirem o afluxo de células mieloides e foliculares dendríticas. A alta densidade de células infiltrantes está associada a maior gravidade inflamatória (Di Tommaso et al., 1995; Crabtree, 1996; Ernst & Gold, 1999).

3.2.2. Resposta Imune Adaptativa Mediada por Células

Os níveis elevados de citocinas pró-inflamatórias IL-12 e TNF-α, produzidas principalmente por DCs resultam em ativação e recrutamento de linfócitos com diferenciação para resposta imune T-helper (Th) na lâmina própria, predominantemente do tipo Th1. As células T CD4+ podem se diferenciar em múltiplas células efetoras e de memória, e o predomínio da resposta imune Th1 na mucosa gástrica se caracteriza pela indução de IFN-γ (interferon-γ) e IL-2. Estas citocinas parecem se correlacionar com maior intensidade de gastrite associada à infecção pelo *H. pylori* (Tummala et al., 2004; Wilson & Crabtree, 2007).

As células T reguladoras (Treg) desempenham um papel na modulação da inflamação na infecção por *H. pylori*, porém à custa de maior densidade de *Helicobacter pylori*. Raghavan et al. (2003) e Lundgren et al. (2003), demonstraram que as células Treg agem reduzindo a proliferação de células de memória Th1 e a produção de IFN-δ, limitando a resposta inflamatória à bactéria (Chen et al., 2001; Ismail et al., 2003).

3.2.3. Resposta Imune Humoral

O *H. pylori* suscita resposta humoral sistêmica e local de anticorpos específicos à infecção (Tosi & Czinn, 1990), com aumento do número de linfócitos B e plasmócitos na mucosa. Apesar da resposta imune humoral vigorosa, o *H. pylori* sobrevive e permanece relativamente inacessível aos anticorpos específicos ou de suas funções efetoras no seu nicho de proteção na mucosa gástrica.

Ocorre produção de sIgA (IgA secretora) anti-*H. pylori* na secreção gástrica, além da saliva e leite materno (Tummala et al., 2004). A IgA sérica pode ser detectada em menos de metade dos pacientes infectados e a IgM sérica é raramente encontrada. Estes achados são consistentes com uma infecção crônica geralmente adquirida na infância.

A resposta humoral imune sistêmica é composta principalmente de IgG, e a IgG anti-*H.pylori* circulante persiste em níveis constantes por anos durante a infecção. Níveis de subclasses IgG1, IgG2, IgG4 são normalmente elevados, enquanto os anticorpos IgG3 (associados com infecções agudas) não são detectados. Gonzalez-Valencia et al. (1996) e Berstad et al. (2001) relataram que a ligação de anticorpos IgG com *H. pylori* promove sua fagocitose in vitro por leucócitos polimorfonucleares.

Cepas de *H. pylori* são susceptíveis ao sistema Complemento ativado pela via clássica, mesmo na ausência de anticorpos específicos, ou pela via alternativa.

3.2.4. Autoimunidade

A resposta humoral ineficaz gerada contra o *H. pylori* e seus componentes podem contribuir para a patogênese das lesões inflamatórias na mucosa gástrica. Alguns dos anticorpos monoclonais direcionados contra o *H. pylori* podem fazer reação cruzada com o epitélio gástrico. Além disso, auto-anticorpos anti-mucosa gástrica associados ao *H. pylori* aparecem em um subgrupo de indivíduos infectados, cujo alvo principal é a bomba de prótons da célula parietal. Assim, a gastrite atrófica predominante no corpo gástrico associada ao *H. pylori* tem muitas características sobrepostas à gastrite autoimune, incluindo redução da massa de células parietais e hipocloridria associada (Israel & Peek, 2001).

3.3. Alterações na Fisiologia Gástrica mediada pela Inflamação

A produção de quimiocinas e citocinas associadas à resposta inflamatória ao *H. pylori* interfere com a regulação das respostas fisiológicas gástricas, especialmente quanto à secreção ácida.

No corpo gástrico a IL-1β e o TNF-α agem como potentes inibidores diretos da secreção ácida gástrica pela célula parietal e a IL-1β diminui a liberação de histamina pelas células enterocromafins. Em contraste, no antro gástrico as citocinas pró-inflamatórias IF-δ, IL-1, TNF-α, e IL-8 têm um efeito positivo sobre as células G, aumentando a concentração plasmática de gastrina. Ademais, na infecção pelo *H. pylori* perde-se o mecanismo de “feedback” negativo sobre a secreção de gastrina induzido pela somatostatina liberada pelas células D da mucosa gástrica. Questiona-

se se variações na resposta à gastrina estão associadas a cepas específicas (Wilson & Crabtree, 2007).

Assim, os indivíduos infectados com gastrite predominantemente antral mantêm secreção ácida normal ou elevada e são predispostos à úlcera duodenal. Em contraste, os indivíduos com gastrite predominante no corpo ou pangastrite desenvolvem hipocloridria e atrofia gástrica, que favorecem o desenvolvimento de úlcera gástrica e adenocarcinoma gástrico (Atherton, 2006). Alguns indivíduos com atrofia da mucosa podem desenvolver metaplasia intestinal, displasia e câncer gástrico do tipo intestinal (El-Omar et al., 1995; Uemura et al., 2001; Houghton et al., 2002).

Na maioria dos casos, a erradicação do *H. pylori* leva a resolução da inflamação, o que em muitos casos, pode resultar em redução no risco de câncer gástrico. Este achado sugere que mesmo em estágios mais avançados, a progressão da neoplasia depende da resposta imune à infecção.

Outras áreas relevantes para a carcinogênese gástrica são as alterações nos eventos do ciclo celular e vias de regulação da apoptose (Eguchi et al., 2004; Naumann & Crabtree, 2004), assim como na interação célula-célula e célula-matriz, induzidos pela infecção, especialmente em cepas CagA positivas (Crawford et al., 2003; Bebb et al., 2003).

4. Gastrite por *H. pylori*

4.1. Gastrite Aguda por H. pylori

Poucos estudos descreveram a infecção aguda em voluntários adultos (Sobala et al., 1991; Graham et al., 2004) demonstrando que esta é usualmente sintomática, com sintomas como desconforto epigástrico, náusea, mal-estar, eructações e halitose, com duração aproximada de 2 semanas. Histologicamente se caracteriza por um intenso exsudato neutrofílico e, em seguida infiltração gradual por todas as classes de células inflamatórias, principalmente linfócitos, que irá persistir. A infecção aguda é acompanhada por hipocloridria gástrica intensa e transitória,

provavelmente relacionada à ação da IL-1β. Não se sabe se a infecção aguda por *H. pylori* na infância é semelhante à dos adultos (Robinson et al., 2007).

4.2. Gastrite Crônica por H. pylori

A inflamação crônica, presente em todas as infecções por *H. pylori*, compreende toda a gama de tipos de células inflamatórias, sendo predominantemente linfocítica, porém com um componente persistente de neutrófilos, particularmente importantes na patogênese pela liberação de mediadores inflamatórios prejudiciais, tais como os radicais livres de oxigênio (ROS). Vários fatores de virulência levam à intensa e persistente liberação de citocinas pró-inflamatórias pelas células epiteliais, aumentando a infiltração de células inflamatórias no local da mucosa gástrica (Amieva & El-Omar, 2008).

A gravidade da inflamação gástrica é influenciada tanto pelos efeitos locais pró-inflamatórios do *H. pylori* no estômago quanto pela resposta imune local e sistêmica. A intensidade da inflamação e sua cronicidade no estômago aumentam o risco de doença associada ao *H. pylori*, mas a sua extensão parece ser um importante determinante do tipo de doença (Wilson & Crabtree, 2007).

5. Fenótipos Clínicos

A infecção por *H. pylori* resulta invariavelmente em gastrite crônica ativa e colonização persistente. A interação dos fatores de virulência bacteriana (com diversidade fenotípica e genotípica substancial), genética do hospedeiro e de fatores ambientais (McGee & Mobley, 2000; El Omar 2001; Megraud, 2001; Peek & Blaser, 2002; Tummala et al., 2004; Wilschanski, et al., 2007; Ladeira et al., 2008) determina fenótipos clínicos distintos tais como gastrite superficial assintomática, gastrite sintomática, úlcera duodenal e neoplasia gástrica (Blaser, 1992; Graham et al., 1994; Parsonnet et al., 1994; McGowan et al., 1996; Fallone et al., 1998; Israel & Peek 2001; Amieva & El- Omar, 2008).

Apenas uma pequena percentagem de indivíduos infectados (menos de 20%) desenvolvem sintomas (Sipponem et al., 1991; Elitsur & Yahav, 2005; Atherton et al., 2006; Kusters et al., 2006), sendo a maioria associados à gastrite. Estima-se que os

pacientes *H. pylori* positivos têm um risco de 10 a 20%, ao longo da vida, de desenvolver doença ulcerosa e um risco de 1-2% de desenvolver câncer gástrico (Kuipers et al., 1995; Kuipers, 1999; Ernst & Gold, 1999). A ocorrência de formas graves de apresentação é maior em adultos do que crianças e a precocidade da infecção parece aumentar o risco de carcinogênese na vida adulta.

Baseado em estudos de coorte conduzidos na Colômbia, Finlândia, Estônia e Japão, Correa & Miller (1998) propuseram um paradigma da carcinogênese gástrica que se tornou conhecida como a cascata de Correa. Neste modelo, gastrite crônica evolui progressivamente para gastrite crônica multifocal, metaplasia intestinal e atrofia gástrica, com consequente aumento do índice de mitose e maior possibilidade de mutações celulares. O risco de evolução para câncer gástrico é relacionado ao genótipo do *H. pylori* (Rugge et al., 1999) e à cronicidade da infecção.

Em crianças a infecção também é assintomática na maioria das vezes, entretanto, foi demonstrada piora na intensidade da inflamação gástrica de crianças infectadas assintomáticas após dois anos de seguimento, apesar de manterem inalterado o seu quadro clínico (Ganga-Zandzou et al., 1999).

Em crianças com sintomas, a infecção por *H. pylori* está frequentemente associada à gastrite crônica, ocasionalmente à úlcera péptica (Blecker et al., 1999) e muito raramente à neoplasia gástrica (Sherman et al., 2002; Koletzko et al., 2011). A relação causal entre infecção por *H. pylori* e doença duodenal ulcerosa em crianças (Huang et al., 1999) é indiscutível, porém a relação causal com DAC é ainda um tópico em debate. Embora as principais razões para o encaminhamento à endoscopia sejam a dor epigástrica ou abdominal crônica, com ou sem vômitos, nenhum destes sintomas se correlaciona especificamente com a infecção pelo *H. pylori* (Macarthur, 1999; Croffie et al., 2000; Hyams et al., 2000; Stringer et al., 2000; Czinn, 2005). No momento, as evidências sobre uma relação causal entre gastrite por *H. pylori* e DAC na infância, na ausência de doença ulcerosa péptica, são insuficientes (Malaty et al., 2002; Koletzko et al., 2011). Serão necessários novos estudos em subpopulações de crianças com DAC, especificamente com sintomas dispépticos sugestivos de doença orgânica para esclarecer tal relação.

As sociedades europeia e americana de gastroenterologia pediátrica e nutrição (ESPGHAN e NASPGHAN) (Koletzko et al., 2011) endossam a erradicação do *H. pylori* presente em criança submetida à Endoscopia Digestiva Alta (EDA) por apresentar DAC, sugestiva de origem orgânica, mesmo na ausência de úlcera documentada. Portanto, se uma criança com dispepsia necessitar realização de endoscopia, dever-se-á realizar biópsias gástricas para avaliar a presença de infecção pelo *H. pylori* (Chelimsky & Czinn, 2001; Drumm et al., 2000).

Estudos também sugerem que a infecção por *H. pylori* em crianças possa aumentar o risco de doenças diarreicas e manifestações extra intestinais, tais como deficiência de crescimento (Choe et al., 2000; Thomas et al., 2004), anemia, urticária crônica, púrpura de Henoch-Schönlein e púrpura trombocitopênica autoimune, porém também não há evidencias concretas de relação causal (Koletzko et al., 2011).

6. Métodos Diagnósticos

Muitos são os métodos para detectar a presença da infecção por *H. pylori*, cada um com vantagens e desvantagens, de tal forma que a escolha depende da aplicação (por exemplo, diagnóstico clínico versus estudo de epidemiologia) e da quantidade de erro aceitável (Dzierzanowska-Fangrat et al., 2006; Hirschl & Makristathis, 2007; Mégraud & Lehours, 2007).

6.1. Métodos Invasivos

Estes testes requerem endoscopia para a obtenção de biópsias da mucosa gástrica. Embora sejam muito específicos, a sensibilidade pode ser afetada pela distribuição focal da infecção no estômago (Gur et al., 1998).

Os testes invasivos são os de eleição no diagnóstico inicial da infecção pelo *H. pylori* e dependem da realização de EDA com biópsia gástrica e pesquisa indireta do agente, pelo teste rápido da urease, ou pesquisa direta do agente pela histopatologia ou por técnicas de detecção do gene do *H. pylori*.

O consenso pediátrico norte-americano e europeu (Koletzco et al., 2011) define como “status *H. pylori* positivo” se forem concordantes pelo menos dois testes

invasivos e “status *H. pylori* negativo” se forem concordantes todos de dois ou de três testes invasivos. Essas sociedades indicam a realização de EDA para o diagnóstico adequado da infecção pelo *H. pylori*.

6.1.1. Teste Rápido da Urease

Consiste na adição de amostra de biópsia gástrica diretamente em meio comercial, em gel ou líquido, contendo ureia e fenol vermelho, o qual muda da cor amarela para rosa se o pH subir acima de 6,0. Esta viragem do pH para alcalino, ocorre se a ureia no meio for metabolizada em amônia e dióxido de carbono pela urease do *H. pylori,* quando presente na amostra (resultado positivo) (Westblom & Bhatt 1999).

Madani et al. (2000) mostraram associação significativa entre a densidade do *H. pylori* através da histologia e possibilidade de um teste rápido da urease positivo. Goel et al. (2003) calcularam a sensibilidade e especificidade do teste rápido da urease lido em diferentes tempos e definiu que o momento ideal para a leitura do teste é de 4 h, mas outros trabalhos mostram ser o tempo ideal de 1 hora (Yousfi et al., 1997). O teste rápido da urease tem sensibilidade e especificidade um pouco maior do que a histopatologia (Madani et al., 2000).

Antagonistas dos receptores H2 e inibidores da bomba de prótons elevam o pH intragástrico podendo causar uma redução na atividade da urease, não relacionados a uma redução da carga bacteriana (Graham et al., 2004). Esse efeito pode reduzir a sensibilidade do exame histológico e do teste da urease para detecção do *H. pylori* em biópsias dos locais recomendados (Dickey et al., 1996), devendo-se recomendar a suspensão destes medicamentos pelo menos 1 mês antes do exame.

O teste rápido da urease tem uma sensibilidade geral de 75 a 100% e especificidade de 84 a 100% (Guarner et al., 2010).

6.1.2. Histopatologia

A histopatologia é o único método que pode detectar além do *H. pylori*, as lesões associadas com a infecção, e outras possíveis causas dos sintomas do

paciente. Apesar da alta sensibilidade da histopatologia, o local, o número e o tamanho das biópsias afetam a precisão do diagnóstico.

O Sistema Sydney para a classificação e graduação de gastrite e duodenite foi proposto por Misiewicz, em 1991, e, em seguida, atualizado por Dixon et al., em 1996. Este sistema enfatiza a importância de combinar informações topográficas, morfológicas e etiológicas em um esquema que ajuda a gerar diagnóstico reprodutível e clinicamente útil (Misiewicz, 1991; Dixon et al., 1996).

O Sistema de Sydney modificado (Dixon et al., 1996) fornece diretrizes quanto ao número e local de obtenção das biópsias gástricas (duas biópsias do antro, duas do corpo-fundo, e uma da incisura angularis), e da classificação histopatológica da gastrite por *H. pylori* em adultos. O número de biópsias indicado para adultos, em geral não é utilizado em estudos pediátricos, os quais geralmente obtêm quatro ou menos biópsias gástricas, sendo pelo menos uma destas utilizada para outras técnicas diagnósticas, tais como cultura, teste da urease e PCR.

As amostras enviadas para estudos histopatológicos são geralmente fixadas em formalina e incluídas em parafina. A seguir são coradas por uma variedade de colorações, incluindo a Hematoxilina e Eosina (H&E), colorações especiais (Giemsa ou pela prata, como Genta e Steiner), e imuno-histoquímica. Atualmente, as diretrizes sugerem que pelo menos duas colorações devam ser utilizadas: H&E para avaliar a inflamação e do Giemsa ou Genta para a detecção do *H. pylori*. Globalmente, o Giemsa é o corante preferencial para a detecção de *H. pylori* devido à sua simplicidade técnica, alta sensibilidade e baixo custo (Laine et al., 1997; Rotimi et al., 2000).

A imuno-histoquimica e as outras colorações especiais somente detectam a bactéria. A taxa de concordância entre as colorações pela prata e imuno-histoquímica é de 82% (kappa), sendo que a coloração imuno-histoquímica apresenta sensibilidade de 45% e especificidade de 96% na detecção do *H. pylori* (Guarner et al., 2010).

O estudo histopatológico com hematoxilina e eosina é o único método que pode detectar outras lesões associadas ao *H. pylori* (atrofia e metaplasia intestinal). O Sistema de Sydney modificado utiliza uma escala visual analógica com pontuação

semiquantitativa em leve, moderada e acentuada quanto à densidade de colonização por *H. pylori*, de infiltração por granulócitos (gastrite aguda), por células mononucleares (gastrite crônica), atrofia e metaplasia intestinal (Dixon et al., 1996). O fato de que indivíduos com pangastrite e atrofia multifocal serem mais propensos a desenvolver úlcera gástrica e câncer, aqueles com gastrite antral predominante apresentarem propensão a desenvolver úlcera duodenal indica uma associação entre o padrão de inflamação induzida pelo *H. pylori* e a evolução da doença. Esse padrão pode ser determinado pela virulência da cepa infectante ou de fatores do hospedeiro e ambientais.

A atrofia só pode ser avaliada em material de biópsia que é orientada corretamente, e a concordância diagnóstica entre os patologistas pode ser difícil de alcançar. Raramente se observa atrofia gástrica em crianças mas, alguns estudos mencionam a presença de atrofia e metaplasia intestinal em até 16% das crianças em determinadas populações (Guarner et al., 2003).

Os métodos de histopatologia têm uma sensibilidade geral de 66 a 100% e especificidade de 94 a 100% (Guarner et al., 2010), dependendo em parte do quadro clínico, em parte, da densidade de colonização e, em parte, da experiência do histopatologista. Em geral, o diagnóstico histológico pode ser feito em cerca de 90% dos casos (Cutler et al., 1995; el-Zimaity et al., 1996).

6.1.3. Cultura

A cultura é o único método com especificidade de 100% (padrão de referência), mas a sensibilidade, de 55 a 96% (Guarner et al., 2010), varia de acordo com a experiência de laboratório. È um método trabalhoso devido à natureza do microrganismo, cuja principal vantagem está em permitir estudos de sensibilidade para determinar a escolha do antibiótico em pacientes com resistência ao tratamento (Nakamura, 2001).

6.1.4. Técnica de Hibridização Fluorescente In Situ (FISH) e Reação em Cadeia da Polimerase (PCR)

Apesar de kits comerciais para FISH e PCR estarem agora disponíveis, sua utilização ocorre principalmente em instituições acadêmicas (Guarner et al., 2010). Estas técnicas moleculares precisas são utilizadas na detecção do DNA do *H. pylori*

e de fatores de virulência específicos, podendo ajudar na detecção inclusive de resistência bacteriana à claritromicina (Moosavian et al., 2007).

O método de PCR tem uma sensibilidade de 96 a 100% e especificidade de 94 a 100% e o FISH sensibilidade de 92 a 94% e especificidade próxima a 100% (Guarner et al., 2010). Assim, para o diagnóstico da infecção, acredita-se que a PCR realizada em amostras de biópsia não seja muito superior a outras técnicas invasivas combinadas (Ashton-Key et al., 1996; Guarner et al., 2010). Por outo lado, a principal vantagem da PCR na detecção do *H. pylori* está na possibilidade de sua realização em amostra de suco gástrico, que pode ser coletado através de uma sonda nasogástrica, com sensibilidade de 96% e especificidade de 100% (Westblom et al., 1993).

6.2 Métodos Não Invasivos

Há vários testes não invasivos para o diagnóstico de infecção pelo *H. pylori* (pesquisa de anticorpos séricos, na saliva, na placa dentária e na urina, pesquisa de antígeno fecal, teste respiratório com ureia marcada com carbono 13 ou 14, PCR ou cultura de fezes e suco gástrico), porém em geral, de menor sensibilidade e especificidade quando aplicados a crianças em relação a adultos, especialmente antes dos dois anos de idade (Gold et al., 2001). Os testes não invasivos para o diagnóstico de infecção por *H. pylori* são somente úteis para finalidades epidemiológicas e no seguimento de um paciente que tenha tido a infecção já diagnosticada por EDA. Entretanto, a força de tarefa europeia tem uma opinião diferente. No grupo etário pediátrico, como não há nenhuma manifestação clínica específica da infecção por *H. pylori*, Drumm et al. (2000), indicam testes não invasivos para selecionar crianças com sintomas dispépticos, definindo as *H. pylori* positivas e consequentemente indicar sua erradicação terapêutica. Este posicionamento é especialmente válido em locais com alta prevalência da infecção, com fatores de risco para desenvolvimento de úlcera e câncer gástrico, nos quais, o *H. pylori* está implicado.

Considerando-se:

- a DAC como um problema frequente em clínicas ambulatoriais de gastroenterologia pediátrica;

- a infecção por *H. pylori* como potencial agente etiológico de DAC em crianças e adolescentes, especialmente em países em desenvolvimento;
- a controvérsia entre estudos que relacionam a DAC e a infecção pelo *H. pylori*;
- a dispepsia crônica como um subgrupo da DAC e possivelmente como forma de apresentação da infecção pelo *H. pylori*;
- as possíveis complicações da infecção pelo *H. pylori* associadas à aquisição precoce;
- a importância de se realizar estudos que avaliem as características clínicas e histológicas da infecção por *H. pylori* em crianças brasileiras;

Objetivos

7. Objetivos:

Este estudo visou avaliar em crianças e adolescentes com DAC e dispepsia crônica, submetidas à endoscopia digestiva alta:

- a frequência de crianças com gastrite associada ao Helicobacter pylori;
- os achados histopatológicos em estômago, duodeno e esôfago;
- a comparação dos dados clínicos e histopatológicos entre os grupos Helicobacter pylori positivo e Helicobacter pylori negativo;
- a correlação de dados clínicos e histopatológicos no esôfago, estômago e duodeno entre os grupos Helicobacter pylori positivo e Helicobacter pylori negativo.

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8. Referências Bibliográficas

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Article

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Gastric histopathological findings in Brazilian dyspeptic children and adolescents with *Helicobacter pylori* infection

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Patologia da Faculdade de Medicina de Botucatu – UNESP, como parte dos requisitos para obtenção do título de Doutor.

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Resumo

Objetivo: Avaliar as características histopatológicas da mucosa gástrica, duodenal e esofágica de crianças e adolescentes brasileiros infectados por *Helicobacter pylori (H. pylori)* e submetidas à endoscopia digestiva alta para investigar dispepsia crônica. As correlações entre variáveis clínicas e histopatológicas também foram analisadas.

Materiais e Métodos: Foi realizado estudo prospectivo de biópsias do trato gastrointestinal superior (mucosa gástrica, duodenal e esofágica) em crianças e adolescentes de 4 a 17 anos de idade. A análise histopatológica foi realizada com coloração por Hematoxilina & Eosina, as lesões gástricas e duodenais foram classificadas de acordo com o Sistema Sydney atualizado e a intensidade da esofagite foi graduada. O estudo incluiu um grupo de crianças infectadas por *H. pylori* (Hp+), com resultados positivos para dois testes (teste da urease e histologia) e um grupo de não infectadas (Hp-). Utilizou-se teste de Mann-Whitney, Qui-Quadrado, exato de Fisher e correlação de Spearman, sendo $p<0,05$ considerado significativo.

Resultados: Foram estudadas 185 crianças dispépticas (idade 9.5±2.7 anos), 63,2% (117/185) do sexo feminino, 96 (51,8%) *H. pylori* positivos (Hp+) e 89 (48,2%) *H. pylori* negativos (Hp-). O grupo Hp+ foi significativamente mais velho (9.9±2.8 anos) do que o grupo Hp- (9.0±2.6 anos) ($p=0,02$). Não houve diferença entre a proporção de sintomas entre os grupos Hp+ e Hp-. Não foram encontradas úlcera gástrica ou duodenal durante o procedimento endoscópico. Gastrite crônica ativa moderada/grave esteve presente em 70,5% das biópsias do antro e em 45,2% das biópsias do corpo no grupo Hp+, com intensidade significativamente maior no antro do que no corpo ($p<0,05$). A pontuação para densidade de *H. pylori* foi significativamente maior ($p=0,005$) nas biópsias do antro do que no corpo. Houve uma associação positiva significativa entre a densidade de colonização pelo *H. pylori* e o grau de inflamação crônica/ativa ($p<0,0001$) em biópsias tanto do antro quanto do corpo. Não houve correlação significativa entre idade e intensidade de infiltrado polimorfonuclear/mononuclear ou densidade do *H. pylori* no antro e corpo do grupo Hp+. A distribuição topográfica da gastrite em 84 pacientes do grupo Hp+, com biópsias de ambos antro e corpo, foi: 32,1% predominantemente antral, 5,9%

predominante no corpo e 61,9% com pangastrite. A distribuição topográfica da gastrite não diferiu entre as crianças mais jovens e mais velhas no grupo Hp+. Metaplasia intestinal não foi encontrado no grupo Hp+, nem atrofia gástrica ou duodenite crônica significativos. A pontuação da esofagite foi significativamente maior ($p<0,05$) no grupo Hp- (1,43±0,84) do que no Hp+ (1,07±0,91). Uma correlação negativa significativa ($p <0,05$) foi observada entre o escore de esofagite e os achados histopatológicos gástricos, tais como infiltrado de células polimorfonucleares e mononucleares em antro e infiltrado mononuclear no corpo.

Conclusões: Os resultados do presente estudo demonstram a alta prevalência de infecção por *H. pylori* em crianças e adolescentes brasileiras dispépticas, associada a grau moderado/grave de inflamação gástrica, envolvendo tanto o antro quanto o corpo, e com intensa densidade de Hp. Estes achados morfológicos são consistentes com a hipótese de patologia gástrica progressiva associada ao *H. pylori*, o que pode representar grande risco para complicações futuras.

Palavras-chave: Biópsia; Criança; Gastrite; *Helicobacter pylori;* Patologia.

Abstract

Objective: To evaluate the histopathological features of the gastric, duodenal and esophageal mucosa from Brazilian children and adolescents infected with *Helicobacter pylori (H. pylori)*, submitted to upper gastrointestinal endoscopy to investigate chronic dyspepsia. The correlations between clinical and histopathological variables were also analyzed.

Materials and Methods: A prospective study of biopsies from the upper gastrointestinal tract (gastric, duodenal and esophageal mucosa) was performed on children and adolescents 4 to 17 years-old. The histopathological analysis was performed on Hematoxylin-and-Eosin-stained slides, the gastric and duodenal lesions were graded according to the updated Sydney System and the intensity of esophagitis was recorded. The study included one group of *H. pylori* infected children (Hp+), with positive results for two tests (rapid urease test and histology) and one uninfected group (Hp-). Mann-Whitney, Chi-square, Fisher exact test and Spearman rank correlation were performed and $p<0.05$ considered significant.

Results: We studied 185 dyspeptic children (age 9.5±2.7 years), 63.2% (117/185) female, 96 (51.8 %) *H. pylori* positive (Hp+) and 89 (48.2%) *H. pylori* negative (Hp-). *H. pylori*-positive were significantly older (9.9±2.8 years) than Hp- (9.0±2.6 years) ($p=0.02$). There was no difference among the proportion of symptoms between Hp+ and Hp- groups. Gastric or duodenal ulcers were not found during the endoscopic procedure. Moderate/severe chronic active gastritis was present in 70,5% of antrum biopsies and in 45,2% of corpus biopsies in the Hp+ group, with significantly higher grading in antrum than in corpus ($p<0.05$). The scores for *H. pylori* density were significantly higher ($p=0.005$) in the antrum biopsies than in the corpus. There was a significant positive association between the density of *H. pylori* colonization and the degree of active/chronic inflammation ($p<0.0001$) in both antral and corpus biopsies. There was no significant association between age and polymorphonuclear, mononuclear cell infiltration or *H. pylori* density in antrum and corpus in Hp+ group. The topographic distribution of gastritis on the 84 patients from the Hp+ group, with both antral and corpus biopsies, was: 32.1% antral-predominant, 5.9% corpus-predominant and 61.9% with pangastritis. The topographic distribution of gastritis did not differ between the younger and older children in the Hp+ group. Intestinal

metaplasia was not found in the Hp+ group, nor significant gastric atrophy or chronic duodenitis. The esophagitis score was significantly higher ($p<0.05$) in the Hp- group (1.43±0.84) than in the Hp+ (1.07±0.91). A significant negative correlation ($p<0.05$) was observed between the esophagitis score and the gastric histopathological findings, such as polymorphonuclear and mononuclear cell infiltration in antrum and mononuclear infiltrate in corpus.

Conclusions: The results of the present study demonstrate the high prevalence of *H. pylori* infection among dyspeptic Brazilian children and adolescents associated with moderate/severe degrees of gastric inflammation, involving both antrum and corpus, and with marked Hp density. These morphological findings are consistent with the hypothesis of a *H. pylori*-associated progressive gastric pathology, which may represent a major risk for future complications.

Key words: Biopsy; Child; Gastritis; *Helicobacter pylori*; Pathology.

Introduction

Introduction

Helicobacter pylori (*H. pylori*) is a fastidious, spiral-shaped Gram-negative microaerophilic flagellate bacterium that permanently colonizes the gastric mucosa with the capacity to establish long term infection in the human stomach (Marshall & Warren 1984; Covacci et al., 1999). *H. pylori* infects more than 50% of the world's population, the rate of infection being higher in developing countries (Graham, 1991; Ahuja & Sharma, 2002). It is generally acquired in childhood (Mitchell et al.,1992; Granstrom, et al., 1997; Goodman & Correa, 2000; Perez-Perez et al., 2004; Torres et al., 2000) and in developing countries, almost all children are infected by the age of 10 (Drumm, 1993; Bardhan, 1997).

H. pylori almost invariably causes chronic gastritis in both children and adults (Drumm, 1993; Sipponem & Hyvarinen, 1993; Basso et al., 2010). The clinical results range from asymptomatic gastritis in the majority of cases to symptomatic gastritis, peptic ulcers, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Sipponem & Hyvarinen, 1993; McGowan et al., 1996; Chelimsky & Czinn, 2001). However, worse clinical outcomes appear most frequently in adults (Bonamico et al., 1997).

Several bacterial and host related factors contribute to determining the outcome of *H. pylori* infection and the development of a sustained gastric inflammatory and immune response to infection appears to be pivotal for the development of disease (Wilson & Crabtree, 2007; Aebischer et al., 2010). Different types of *H. pylori* gastritis link to different clinical outcomes; mild pangastritis often leads to no serious gastrointestinal disease, antrum-predominant gastritis is associated with duodenal ulcer and corpus-predominant gastritis and multifocal atrophy with gastric ulcer or cancer (Rugge & Genta, 2005; Amieva & El-Omar, 2008). Since there is also accumulating evidence supporting the association between the duration of *H. pylori* infection and the clinical outcome in adults (Blaser et al., 1995; Bedoya et al., 2003; Kato et al., 2006), the infected children should be an important concern in populations with high prevalence of *H. pylori* infection. Besides the duodenal and gastric disorders due to *H. pylori* infection, there is inversely associated Barrett's metaplasia in adults (Sonnenberg et al., 2010) but the relationship between *H. pylori* and gastro-esophageal reflux disease (GORD) in

children is controversial (Özçay et al., 2002; Levine et al., 2004; Daugule et al., 2007; Moon et al., 2009; Abdollahi et al., 2011), with both positive and negative correlation.

The most reliable method for diagnosing *H. pylori* infection is directly from endoscopic biopsies (Guarner et al., 2010) and major reasons for endoscopy referral of children include chronic epigastric or abdominal pain, with or without vomiting (Blecker et al., 1996; Vandenplas & Blecker, 1998; Wewer et al., 1998; Thakkar et al., 2009). Whether *H. pylori* gastritis causes abdominal pain in the absence of peptic ulcer disease is still debatable (Koletzko et al., 2011), but studies are required to determine if subsets of children with abdominal pain can be identified in whom symptoms and signs are caused by *H. pylori* infection.

The Updated Sydney System (Dixon et al., 1996) is used for grading gastritis and duodenitis in both adults and children. The histopathological changes secondary to the infectious process of *H. pylori* are well defined in adults, but the findings are less conclusive in children (Meining et al., 1996; Riddell 1999; Cohen et al., 2000; Langner et al., 2009; Jaramillo et al., 2010). Children, in general, experience less inflammation than adults and display an inflammatory process whose intensity and type vary depending on their geographic location (Jaramillo et al., 2010). There are different histological expressions of *H. pylori*-associated inflammation in children compared to adults which suggests that specific pediatric aspects of *H. pylori*-associated gastritis may be envisaged (Meining et al., 1996; Riddell 1999). So, we aimed to evaluate the histopathological features of the gastric, duodenal and esophageal mucosa of infected *H. pylori* children and adolescents submitted to upper gastrointestinal endoscopy to investigate chronic dyspepsia.

Materials and Methods

Materials and Methods

1. Patients

A total of 471 consecutive children and adolescents attended at the Outpatient Pediatric Gastroenterology Clinic of the Department of Pediatrics of Botucatu Medical School, Botucatu, São Paulo, Brazil, and were followed for chronic abdominal pain of more than three months duration, according to Apley's criteria (Apley & Naish, 1958), in a 5-year period, from January 2006 to December 2010, in a prospective study. This study included a subset of 185 patients, with chronic dyspeptic syndrome (epigastric pain, postprandial fullness, retrosternal pain, early satiety, upper abdominal distention, nausea, retching, belching, and vomiting for at least two days per week, for a period of at least three months) suggestive of organic underlying disease, according to the criteria of Chelimsky & Czinn (2001), submitted to upper gastrointestinal endoscopy. The children considered eligible for the study were between 4 to 17 years old, had normal hematology, urine analysis and culture, had routine stool examination performed three times without any ova or cysts and had normal plain radiograph of abdomen. Patients with a history of gastric surgery, active gastrointestinal bleeding, duodenal ulcer, gastric ulcer were excluded from the study. None of the patients had received anti-secretory drugs (H2 receptor blockers and proton pump inhibitors), bismuth compounds, recent use of non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics or immunosuppressive drugs, in the preceding four weeks. A standard clinical protocol was applied in the day of the upper gastrointestinal endoscopy. Antropometric data were assessed according to WHO growth standards (WHO, 2010).

2. Endoscopy

During endoscopy from each patient, multiple biopsy specimens were collected (one esophageal biopsy sample from the 20% proximal part of the esophagogastric junction, two from the antrum, one from the corpus, and one from the proximal duodenum).

3. Rapid Urease Test

One biopsy from the antrum was used for an immediate rapid urease test (Renilab Uretest®). Briefly, biopsies were directly inoculated onto the commercial

agar gel, which was considered to be positive when the color changed from yellow to pink within 4 h, in according to the manufacturer's instruction.

4. Histopathological evaluation

Biopsy specimens were fixed in 10% neutral buffered formalin solution, processed for histology, and embedded in paraffin. Several serial longitudinal 4-μm sections from each specimen were cut, stained with Hematoxylin-Eosin for evaluation of gastric, duodenal and esophagus inflammation, and one was analyzed with the modified Giemsa method for assessment of *H. pylori* colonization.

All histopathology slides were reviewed by authors (MAMR and MAC), who were unaware of the clinical information and endoscopic findings of each patient and jointly examined all the specimens and reached a consensus on the score of each of the considered histological variables. Only cases with two adequately sized biopsies and with at least one gastric biopsy were accepted for histological assessment.

For histopathologic evaluation of gastric and duodenal biopsies, Hematoxylin-and-Eosin-stained slides were graded using the visual analog scale (Appendix 1) according to the updated Sydney System (Dixon et al., 1996). Intensity of inflammation (mononuclear cell infiltration), activity (neutrophil infiltration), and atrophy were classified into grade 0 (absence); 1 (mild); 2 (moderate) and 3(marked). The density of *H. pylori* colonization was assessed by modified Giemsa method in antral and corpus biopsy specimens and graded as 0 (absent in all of the histological samples); 1 (few and isolated bacteria revealed only in scattered foveolae); 2 (easily detected as isolated bacteria) and 3 (present as colonies and/or widely stratified on the surface epithelial layer). Additionally, the presence or absence of intestinal metaplasia and lymphoid follicles were noted.

Classification of histological changes was as follows: normal mucosa was defined as one with absence of acute or chronic inflammatory infiltrate as well as epithelial or glandular lesions; active chronic gastritis/duodenitis as one with polymorphonuclear and lymphoplasmocytic infiltrate with epithelial lesions, with or without glandular involvement; and non-active chronic gastritis/duodenitis as one with lymphoplasmocytic infiltrate without polymorphonuclear infiltrate or epithelial lesions.

4.1. Gastritis topographic distribution

The phenotypic pattern of pangastritis was defined if inflammation was distributed throughout the stomach, with little or no difference between antrum and corpus. The antral-predominant gastritis was defined if there was (1) a moderate to severe inflammation in the antrum and (2) a normal to mild inflammation in the corpus. The corpus-predominant gastritis was considered if there was (1) a moderate to severe inflammation in the corpus and (2) a normal to mild inflammation in the antrum.

4.2. Gastritis grading

Grading represent the semi quantitative assessment of the combined severity of mononuclear and granulocytic inflammation scored in both antral and corpus biopsy samples. Grading is a measure of the severity of the inflammatory lesions and express the cumulative intensity of the inflammatory cells (lymphocytes and granulocytes) within the lamina propria graded as according to the visual analogue scales of the Updated Sydney System. The final grade of inflammation resulting from the combination of the grades of the inflammatory lesions in antral and corpus mucosa were applied according to Rugge & Genta (2005). Grades (Appendix 2) ranged from 0 (absence of inflammatory cells in any of the specimens) to IV (a very dense infiltrate in all the biopsy samples).

4.3. Definition of *H. pylori* status

The patients were considered *H. pylori* positive (Hp+) if the rapid urease test and the histologic examination of the gastric specimens were both positive and noninfected, *H. pylori* negative (Hp-) when both tests gave negative results. If the patient had only one positive test, then the patient was not included in the study.

4.4. Esophagus biopsy

The histopathological diagnosis of esophagitis was performed according to Knuff et al. (1984) and Leape et al. (1981), as recommended by the European Society for Pediatric Gastroenterology Hepatology and Nutrition (Vandenplas, 1994). The scores were graded as 0, Ia, Ib, Ic, II, III, IV and V (Appendix 3). To simplify the comparison between the methods, hyperplasia of basal cells (Ia), lengthening of the papillae (Ib) and dilatation of intraepithelial vessels (Ic), were grouped and defined as grade I.

5. Statistical analysis

The statistical analysis was performed using GraphPad Prism version 4.0, 2003. Calculation of the mean, standard deviation, median, and 95% Confidence interval was performed for all quantitative variables. Qualitative variables were described by counts (N) and percentages (%). The nonparametric Mann-Whitney test was used for statistical comparisons between quantitative variables; the Chi-square or Fisher's exact tests were used for statistical comparisons between categorical variables. Tests of association between different variables were performed using the Spearman rank correlation. All statistical tests were two-sided and values of $p<0.05$ were considered statistically significant.

The study (protocol number OF.642/2006-CEP) was approved by the Hospital Ethical Committee.

Results

Results

1. Patient characteristics and clinical data

A total of 185 symptomatic children and adolescents with chronic abdominal pain and clinical criteria for chronic organic dyspepsia (age range 4–17 years, mean age 9.5±2.7 years) of whom 63.2% (117/185) were female and resided in the same geographical area were studied. Of the total, 96 (51.8 %) were *H. pylori* positive (Hp+) and 89 (48.2%) were *H. pylori* negative (Hp-). The groups studied were homogeneous, except for age at endoscopy. Hp+ patients were significantly older at endoscopy than Hp- (9.9±2.8 vs. 9.0±2.6 years). The results of demographics and clinical characteristics are summarized in Table 1.

All the patients had chronic abdominal pain and chronic dyspepsia as including criteria. The most common presenting symptoms were epigastric pain, non epigastric abdominal pain, vomiting, retrosternal pain and anorexia respectively 87%, 13%, 39%, 53% and 40% for Hp+ group and 85%, 15%, 52%, 54% and 32% for Hp- group. There was no difference among the proportion of symptoms between Hp+ and Hp- groups (Appendix 4). From the 89 patients on the Hp- group, 77.5% had a final diagnosis of gastroesophageal reflux disease, 20.2% functional dyspepsia and 2.3% eosinophilic gastroenteropathy. Gastric or duodenal ulcers were not found during the endoscopic procedure.

2. *H. pylori* Infection and histopathology

The histopathological assessment was performed in all 185 cases. Biopsies specimens were available from antrum in 183 patients, from corpus in 171, from antrum and corpus in 169, from duodenum in 118 and from esophagus in 181 patients. There was no difference for number of gastric, duodenal and esophageal biopsies among Hp+ and Hp- groups.

2.1. Gastric histopathology

The gastric biopsy scores in the Hp+ group and Hp- group considering polymorphonuclear, mononuclear, density of *Helicobacter pylori* and atrophy for antrum and corpus are summarized in Table 2. Upon histological Updated Sydney System examination, moderate and severe active antral gastritis, chronic antral

gastritis, active corpus gastritis and chronic corpus gastritis were more frequent for Hp+ group than for Hp- group, with strong significant statistical difference ($p<0.0001$).

The comparison of histopathological parameters between the antrum and corpus of stomach in Hp+ group according to Updated Sydney System is described in Table 3. For Hp+ group the infiltration of polymorphonuclear cells was significantly higher in the antrum than in the corpus. There was also a significantly higher intensity of mononuclear infiltration in the antrum than in the corpus. Moderate/severe chronic active gastritis was present in 70.5% of antrum biopsies and in 45.2% of corpus biopsies, with significantly higher grading in antrum than in corpus. The scores for *H. pylori* density were significantly higher in the antrum biopsies than in the corpus. Moderate/severe scores for *H. pylori* density were present in 67.3% (64/95) of antrum biopsies and in 54.1% (46/85) of corpus biopsies. Only two patients had mild or moderate atrophy in the antrum and six in the corpus. For Hp- group, polymorphonuclear infiltration was absent in 100% of patients in both antrum and corpus and mononuclear infiltration was found in only 8/86 (9.3%) children in antrum and 4/86 (4.6%) in corpus.

The topographic distribution of gastritis on the 84 patients from the Hp+ group, with both antral and corpus biopsies, was: 32.1% antral-predominant, 5.9% corpus-predominant and 61.9% with pangastritis. The topographic distribution of gastritis did not differ ($p=0.4216$) between the younger and older children in the Hp+ group.

Lymphoid follicles were found in 29/95 (30%) of antrum biopsies and in 16/85 (18%) of corpus biopsies in Hp+ children, and were present in 8/88 (9%) patients in the antrum and in 4/86 (4.6%) in the corpus in the Hp- group. Lymphoid follicles were significantly more prevalent in biopsies from *H. pylori* infected children both in antrum and corpus ($p<0.05$). Intestinal metaplasia was not found in any child in the present series of patients.

2.2. Overall gastritis grading

Gastritis grading I, II, III and IV of 84 patients in Hp+ group, with both antrum and corpus biopsies, were respectively 23.8%, 22.6%, 21.4% and 32.2%. Considering that the median age of the Hp+ group was 9.7 years old and the median duration of dyspepsia was 1 year, there was no difference on gastritis grading (Figure

1) according to younger or older age at endoscopy (≤9.7 vs. >9.7 years, p=0.0730) or to duration of dyspepsia (≤1 vs. >1 year, p=0.0749) in infected patients. However, when considering these overall inflammation scores of gastritis grading there was a significant negative association with esophagitis score (p=0.0073, r=-0.2941).

2.3. Duodenal and esophageal histopathology

2.3.1. Duodenal biopsies

Of the 118 patients for which duodenal and antral biopsies were available, 49.2% were Hp+ (58/96) and 50.8% were Hp- (60/89). All Hp+ patients were defined as no/mild active duodenitis and 87% no/mild chronic duodenitis. Of Hp- group 97% were no/mild acute duodenitis and 85% were no/mild chronic duodenitis.

2.3.2. Esophageal biopsies

Esophagus biopsies from Hp+ were 93/96 and from Hp- were 88/89 patients. The esophagitis score of Knuff et al. (1984) and Leape et al. (1981) was significantly higher (p=0.06) in the Hp- group (1.43±0.84) than in the Hp+ (1.07±0.91) (Appendix 5).

3. Correlation

3.1. Correlation between clinical data and gastric histopathological findings

There was no significant association between age and polymorphonuclear, mononuclear cell infiltration or *H. pylori* density in antrum and corpus in children infected with *H. pylori*. No significant relationship between the duration of dyspepsia and the polymorphonuclear, mononuclear cell infiltration or *H. pylori* density in antrum and corpus was found in children infected with *H. pylori*.

3.2. Correlation between *H. pylori* density and gastric histopathological findings

There was a significant positive association between the density of *H. pylori* colonization and the degree of active/chronic inflammation (p<0.0001) in both antral and corpus biopsies, but there was no significant relationship (p>0.05) between *H. pylori* density in the antrum and polymorphonuclear cell infiltration in the duodenum.

3.3. Correlation between esophagitis score and gastric histopathological findings

A significant negative relationship ($p<0.05$) was observed between the esophagitis score and the gastric histopathological findings such as polymorphonuclear and mononuclear cell infiltration in antrum and mononuclear infiltrate in corpus. However, no correlation was observed between esophagitis score and active inflammation in corpus or *H. pylori* density in both antrum and corpus.

Discussion

Discussion

This prospective study was based on histopathological findings of the gastric, duodenal and esophageal biopsies of 185 Brazilian children and adolescents submitted to upper gastrointestinal endoscopy for chronic organic dyspepsia. We evaluated the potential association between *H. pylori* infection and clinical and histological findings in the upper gastrointestinal tract. It should be emphasized that the number of cases included in the present study is large enough to be representative.

The recently published guidelines from ESPGHAN and NASPGHAN (Koletzko et al., 2011) for *Helicobacter pylori* infection in children had highlighted large gaps in knowledge regarding *H. pylori* infection and abdominal pain in children and emphasized that there is no convincing data to support *H. pylori* as a cause of abdominal pain. However, whether *H. pylori* gastritis causes abdominal pain in the absence of peptic ulcer disease is still debatable (Koletzko et al., 2011). In studies from countries where the prevalence of *H. pylori* infection is high, it has been reported that *H. pylori* infection is a causative agent of various gastrointestinal symptoms such as chronic abdominal pain (Shamaly et al., 2000; Nijevitch et al., 2001; Ozen et al., 2001). Therefore, we have chosen to study a subset of children with chronic abdominal pain with dyspeptic syndrome suggestive of organic underlying disease. However, in the present study the dyspeptic symptoms did not discriminate between the nonulcer Hp+ group and Hp- group, as found by Kalach et al. (2005).

Several expert panels on *H. pylori* infection in children have established endoscopy with gastric biopsy as the “gold standard” for the diagnosis of this disease in children (Gold et al., 2000; Sherman et al., 2002; Koletzko et al., 2011). Of the tissue based methods, rapid urease tests have slightly higher sensitivity and specificity than histopathology to detect presence of *H. pylori*. The sensitivity of rapid urease test ranges from 80 to 95% while the specificity range is 95–100% (Guarner et al., 2010). However, histopathology can detect the organic lesions associated with the disease, including gastritis and other conditions that could be the cause of the child’s symptoms (Guarner et al., 2010). The specificity of histopathology is 95–98% because of the morphological characteristics of *H. pylori* and its typical location on

the luminal surface of the epithelial cells. When *H. pylori* is present, careful examination almost always reveals infection, irrespective of the stains used. Nevertheless, the modified Giemsa was our method of choice because it is sensitive, cheap, easy to perform, and reproducible, facilitating the distinction of *H. pylori* from other very rare intragastric bacteria (Laine et al., 1997; Rotimi et al., 2000). Ogata et al. (2001) and Koletzko et al. (2011) suggested that among invasive methods, an association between the rapid urease test and histology constituted the best choice for the detection of *H. pylori*, as used in this study.

Guidelines for the histopathologic grading of *H. pylori* associated gastritis in adults is provided by the updated Sydney System classification for gastritis (Dixon et al., 1996) which suggest two biopsies from the antrum, two from the fundus, and one from the cisura angularis. However, for ethical reasons, most published pediatric studies on updated Sydney System classification obtain four or less gastric biopsies and at least one of these biopsies is used for rapid urease test (El-Zimaity et al., 1999; Goel et al., 2003).

The prevalence of *H. pylori* infection in children from developing countries is greater than those observed in children from industrialized countries (Blaser et al., 1995; Dimmick et al., 1997; Ernst, 1999; Gold et al., 2001, Hatakeyama & Brzozowski, 2006). In Brazil, studies performed on dyspeptic children from three different geographic regions showed a *H. pylori* prevalence of 34% to 77%, using serological, histological, and culture analysis in dyspeptic children submitted to endoscopy (Oliveira et al., 1994; Solari et al., 1994; Souto et al., 1998). A previous Brazilian study reported a decrease in the prevalence of *H. pylori* infection in symptomatic children and adolescents submitted to upper gastrointestinal endoscopy within a ten year period. The prevalence was 60.4 percent in the first year of the study (1993) and 30.4 percent at the end of the study (Kawakami et al., 2008). In our series of 185 dyspeptic children submitted to upper gastrointestinal endoscopy we have observed a high prevalence of infected patients (51,8%), probably due to regional characteristics of the population studied and to the inclusion criteria adopted, which considered eligible for the analyses only children who had dyspeptic symptoms suggestive of organic disease. In a meta-analysis of 18 studies involving endoscopy and biopsy evaluations in children with recurrent abdominal pain, *H. pylori* infection was determined in 2% to 63% of the patients (Thakkar et al., 2007). In an earlier

study, a 54% prevalence rate of *H. pylori* infection among Israeli children with recurrent abdominal pain had been reported by Gram stain and urease tests (Heldenberg et al., 1995). In studies from Czech Republic, Turkey and Poland approximately half of the children with upper gastrointestinal symptoms had active *H. pylori* infection (Gzyl et al., 1999; Ozen et al., 2001; Sökücü et al., 2002; Sedlackova et al., 2003), which indirectly points to the role of the bacterium in the development of symptoms.

In the present study mean age of the *H. pylori* positive group was significantly higher than that of the *H. pylori* negative group, although the duration of dyspepsia did not differ between the groups. This is an indication that they are two distinct groups of disease. *H. pylori* infection is generally acquired at any time between infancy and late childhood and persists as an asymptomatic infection for decades in most individuals (Parsonnet, 1995; Blazer et al., 1995; Rothenbacher et al., 2000; Nizami et al., 2005). We have found a slight predominance of girls (63%) in our study and this could be due to the fact that chronic abdominal pain is more often observed in girls (Tindberg et al., 1999).

H. pylori has been recognized as an important etiologic factor in the development of chronic gastritis and peptic ulcer disease in adults and in children (NIH Consensus Conference, 1994). All of the components of the inflammatory response to *H. pylori* infection described in adults are present in children (Drumm et al., 1987) and the gastric mucosal damage may be progressive from childhood until adulthood; hence, it is important to study *H. pylori* infection in children (Go, 2002). Host and bacterial factors dictate eventual significant disease phenotypes and the age of acquisition of the bacteria has been proposed as an important factor for the later adverse outcomes (Rothenbacher et al., 2000; Torres et al., 2000). Early *H. pylori* infection is associated with a high risk for the development of peptic ulcer and gastric cancer (Torres et al., 2000; Bedoya et al., 2003; Munoz et al., 2007), probably because of the more intense inflammation and the development of atrophic gastritis (Parsonnet, 1995; Blaser et al., 1995; Kato et al., 2006). In most developing countries, the higher prevalence of *H. pylori* infection in children contributes to chronic infection in the majority of young adults and the elderly population (Pounder & Ng, 1995).

In our study there was a high prevalence of pangastritis identified in both younger and older children infected with *H. pylori*. This suggests that *H. pylori* tends to colonize the vast majority of the gastric mucosa after the initial antral infection (Wyatt, 1995). In almost two thirds of the overall study population, features of gastritis had spread out beyond the antrum and involved the gastric corpus. Our data are in agreement to the Chilean children studied by Guiraldes et al. (2002) and with the Brazilian children studied by Langner et al. (2009), but contrasts to previous results that reported preponderance of antral-predominant gastritis in children infected with *H. pylori* (Cohen et al., 1989). These differences might be related to the virulence of *H. pylori* strains and/or to a massive exposure to *H. pylori* earlier in the life of these young patients and to a prolonged duration of the infection (Guiraldes et al., 2002).

The main histopathological findings on the Hp+ group in our study were moderate/severe chronic active gastritis identified in 70.5% of antrum biopsies and in 45.2% of corpus biopsies, with significantly higher grading in antrum than in corpus. These findings of marked inflammation were associated to high scores of *H. pylori* colonization in both the antrum and corpus samples. Similar to the data published for the adult population, the antrum demonstrated the highest sensitivity rate for the detection of Hp organisms (Plebani et al., 1996). Among the studies conducted in children on intensity and type of inflammatory response and density of colonization, contradictory results have been reported; most studies report a poor inflammatory response (Gottrand et al., 1997; Lynch et al., 1999; Guiraldes et al., 2002; Camorlinga-Ponce et al., 2003; Lobo Gatti et al., 2003; Ozawa et al., 2005; Jaramillo et al., 2010), whereas others suggest a strong inflammatory response (Recavarren-Arce et al., 1995, Bedoya et al., 2003; Kato et al., 2006; Sgouras et al., 2009).

Our study is in agreement with the findings of Recavarren-Arce et al. (1995) in Peru, Bedoya et al. (2003) in Colombia, Kato et al. (2006) in Japan, and with the results of Sgouras et al. (2009) in Greece, who reported high prevalence of *H. pylori* infection associated to an intense inflammatory response. Our children probably come from early infected populations with more virulent *H. pylori* strains and higher risk of gastric cancer, which is related to greater degrees of gastric mucosal damage and greater density of *H. pylori* colonization (Bedoya et al., 2003). By contrast, other studies reported poor gastric inflammatory response (Gottrand et al., 1997; Lynch et al., 1999; Guiraldes et al., 2002; Camorlinga-Ponce et al., 2003; Ozawa et al., 2005;

Jaramillo et al., 2010). Differences in the characteristics of the predominant *H. pylori* strains (less virulent), in the genetics of the host and in the environment may account for the mild inflammatory infiltrate observed in those populations.

Some studies have reported that mononuclear but not polymorphonuclear infiltration was higher in the antrum than in the corpus (Lynch et al., 1999; Ozawa et al., 2005; Jaramillo et al., 2010). However, in the present study, both mononuclear and polymorphonuclear infiltration were higher in the antrum than in the corpus. This is in agreement to the results of Recavarren-Arce et al. (1995), Bedoya et al. (2003) and Kato et al. (2006). The association of high degree of *H. pylori* colonization with a more severe inflammation in the antrum, but not in the corpus, emphasizes that the antrum may be the primary site of *H. pylori* colonization in children.

The Updated Sydney System remains essential for the recognition of gastric inflammatory disease. However, most pathologists find them too cumbersome to use in their routine diagnostic activities. Rugge & Genta (2005) proposed a reporting system for chronic gastritis in grading that offer a more immediate perception of the overall condition of the gastric mucosa, combining severity of both mononuclear and granulocytic inflammation. Our analysis of grading for Hp+ group revealed a superior number of cases with the highest overall grading of gastritis (score IV), although not statistically significant.

Interestingly, our histopathological analysis did not show significant amount of atrophy nor intestinal metaplasia, in spite of the degree of inflammation, which is in agreement with previous studies (Campbell et al., 2001; Guiraldes et al., 2002). The presence of gastric mucosal atrophy associated with *H. pylori* gastritis is still controversial in children, with prevalence varying between 0% and 72% according to different studies (Ricuarte et al., 2005; Dimitrov & Gottrand, 2006), probably due to different criteria for detecting these complications.

Only few cases of the Hp- children had slight mononuclear cells in in the antrum (9.3%) and corpus (4.6%), but none of the uninfected children presented active gastritis.

There was significantly higher esophagitis in the Hp- group than in the Hp+ group, and we have observed a significant negative correlation between *H. pylori*

infection and the histopathological finding of inflammation in the esophageal mucosa. In adults, the majority of data suggests a negative association between *H. pylori* infection and reflux esophagitis (Raghunath et al., 2003; Cremonini et al., 2003) and with Barret's esophagus (Sonnenberg et al., 2010). This topic is still controversial in the pediatric population, with limited studies, some showing similar inverse correlation in children (Abdollahi et al., 2011), others demonstrating a positive correlation (Daugule et al., 2007; Moon et al., 2009) and others no relationship (Emiroglu et al., 2010). *H. pylori* has a profound impact on the gastric mucosa and to a lesser extent on gastric physiology (gastrin, somatostatin, and acid secretion), whereas gastroesophageal reflux disease is the result of an increased esophageal exposure to gastric acid (Raghunath et al., 2003). The negative association with the severity of esophagitis in adults has added weight to the theory of a protective role of *H. pylori* infection, especially by decreasing gastric acid secretion, which can occur when the gastritis spreads out beyond the antrum and involves the gastric corpus, as occurred in the majority of our *H. pylori* positive children. In these children, with predominant nonatrophic pangastritis, there could be an important role for interleukin-1 beta in mediating a hypochlorydric response to *H. pylori* (Furuta et al., 2002). However, it remains unclear whether our findings represent a real association or rather a bias generated by distinct pathologic entities on the two groups of children, which share common clinical symptoms.

In conclusion, the results of the present study show high prevalence of *H. pylori* infection among Brazilian dyspeptic children and adolescents, with significant degrees of inflammation of the gastric mucosa associated to high density of *H. pylori* colonization involving both antrum and corpus. These findings are consistent with the hypothesis of a *H. pylori*-associated progressive gastric pathology and are probably due to early acquisition of the infection or to the virulence of *H. pylori* strains, which should be better defined.

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Tables, Figures and Appendix

Table 1. Demographics and clinical characteristics of *Helicobacter pylori* positive and negative children and adolescents.

	Hp+ (n=96)	**Hp- (n=89)**
	Mean±Standard Deviation (95% Confidence Interval of the Mean)	
Gender M/F n (%M)*	35/61 (36%)	33/56 (37%)
Age at endoscopy (years)	9.9±2.8 (9.4 - 10.5)#	9.0±2.6 (8.5 – 9.6)#
Duration of dyspepsia (years)	1.7 ± 1.7 (1.3 – 2.1)	1.5 ± 1.2 (1.2 - 1.7)
Age of mothers (years)	33.2 ± 6.7 (31.7 - 34.6)	33.0 ± 5.9 (31.7 - 34.4)
Age of fathers (years)	37.9 ± 7.8 (36.1 - 39.7)	36.3 ± 6.6 (34.7 - 37.8)
Number of children at home	2.4 ± 1.1 (2.1 - 2.6)	2.4 ± 0.9 (2.1 - 2.6)
Crowding index (co-residents per room)	1.06 ± 0.47 (0.95 – 1.16)	1.02 ± 0.43 (0.93 – 1.12)
Height z score	-0.18±1.05 (-0.40-0.03)	0.03±1.11 (-0.22-0.28)
Body Mass Index z score	0.55±1.16 (-0.18-0.3)	0.32±1.26 (0.03-0.61)

*Number of feminine/masculine gender (% of masculine gender);
#p<0.05

Table 2. Comparison of antral and corpus histopathological parameters between *Helicobacter pylori* positive and negative children and adolescents according to Updated Sydney System.

	Antrum			Corpus		
Hystopathological parameters	**Hp+ (n=96)**	**Hp- (n=89)**	**p**	**Hp+ (n=96)**	**Hp- (n=89)**	**p**
Number of biopsies (n)	95	88	>0.05#	85	86	>0.05#
Polymorphonuclear Score						
Mean±SD	1.98±0.84	0	<0.0001*	1.54±0.85	0.01±0.10	<0.0001*
0/1 (n)	29	88	<0.0001#	49	86	<0.0001#
2/3 (n)	66	0		36	0	
Mononuclear Score						
Mean±SD	2.08±0.84	0.10±0.34	<0.0001*	1.63±0.88	0.08±0.35	<0.0001*
0/1 (n)	26	87	<0.0001#	44	84	<0.0001#
2/3 (n)	69	1		41	2	
***Helicobacter pylori* Score**						
Mean±SD	2.00±0.86	0	<0.0001*	1.64±0.82	0	<0.0001*
0/1 (n)	31	89	<0.0001#	39	89	<0.0001#
2/3 (n)	64	0		46	0	
Atrophy Score						
Mean±SD	0.02±0.14	0.04±0.20	0.3580*	0.14±0.55	0.01±0.10	0.0508*
0/1 (n)	95	88	-	81	86	0.0508#
2/3 (n)	0	0		4	0	
Lymphoid follicles (n)	29	8	0.0004#	16	5	0.0103#
Intestinal metaplasia (n)	0	0	-	0	0	-

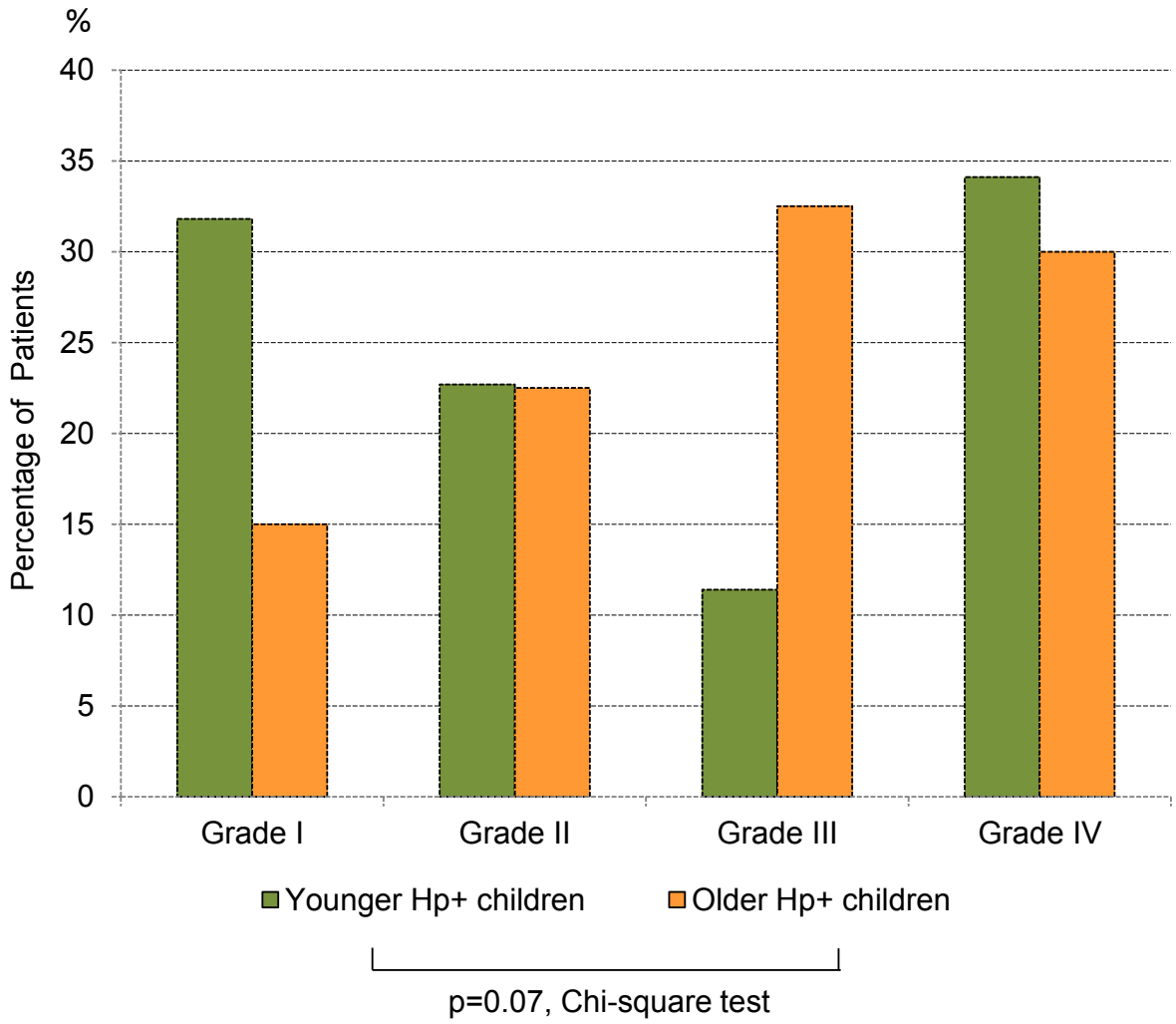
**H. pylori* positive versus *H. pylori* negative patients, using Mann Whitney test;
#H. pylori positive versus *H. pylori* negative patients, using Fisher exact test;
SD= Standard Deviation.

Table 3. Comparison of histopathological parameters between the antrum and corpus of stomach in *Helicobacter pylori* positive children and adolescents according to Updated Sydney System.

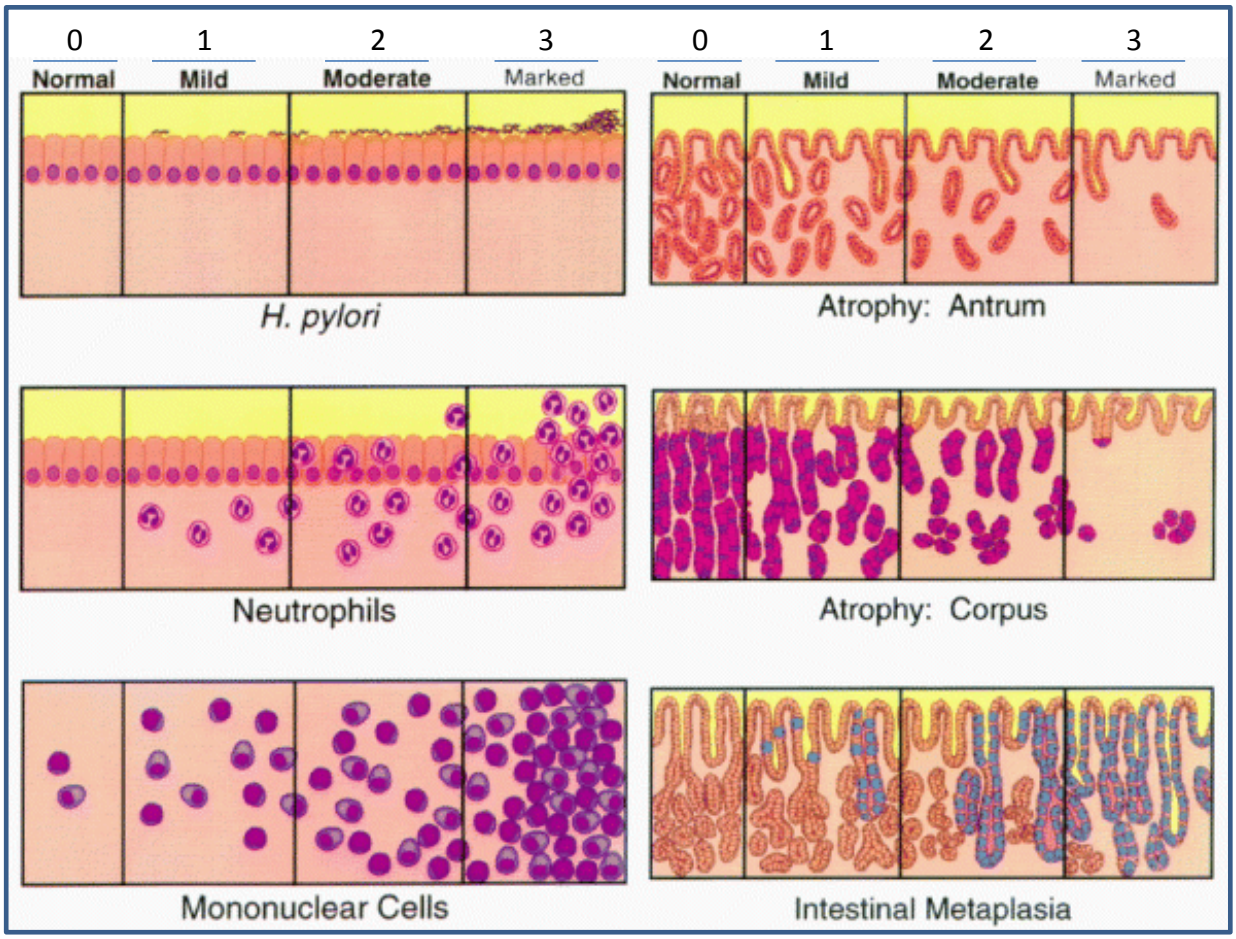
Hystopathological parameters	Hp+ (n=96)		
	Antrum	Corpus	p
Number of biopsies (n)	95	85	>0.05#
Polymorphonuclear Score			
Mean±SD	1.98±0.84	1.54±0.85	0.0005*
0/1 (n)	29	49	
2/3 (n)	66	36	
Mononuclear Score			
Mean±SD	2.08±0.84	1.63±0.88	0.0007*
0/1 (n)	26	44	
2/3 (n)	69	41	
***Helicobacter pylori* Score**			
Mean±SD	2.00±0.86	1.64±0.82	0.0075*
0/1 (n)	31	39	
2/3 (n)	64	46	
Atrophy Score			
Mean±SD	0.02±0.14	0.14±0.55	0.1025*
0/1 (n)	95	81	
2/3 (n)	0	4	
Lymphoid follicles (n)	29	16	0.0852#
Intestinal metaplasia (n)	0	0	-

**H. pylori* positive versus *H. pylori* negative patients, using Mann Whitney test;
 # H. pylori positive versus *H. pylori* negative patients, using Fisher exact test;
SD= Standard Deviation.

Figure 1. Gastritis grading distribution of the younger and older *H. pylori* positive group, according to Rugge & Genta, 2005.



Appendix 1. Visual analog scale according to the Updated Sydney System (Dixon et al., 1996).



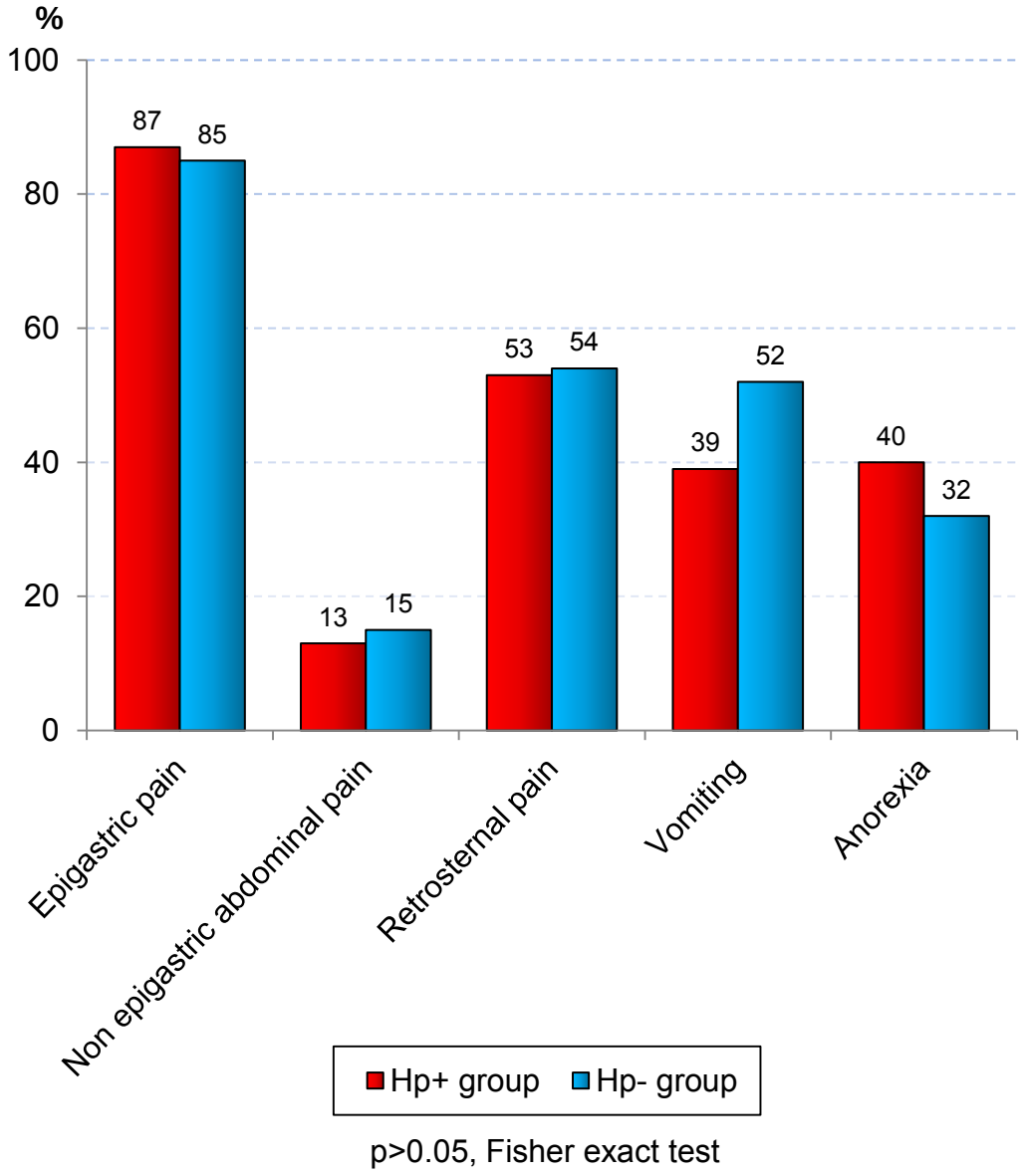
Appendix 2. Overall inflammation gastritis grading according to Rugge & Genta (2005), resulting from the combined severity of mononuclear and granulocytic inflammation scores in both antral and corpus biopsy samples.

		CORPUS			
		No Inflammation (G0)	Mild Inflammation (G1)	Moderate Inflammation (G2)	Severe Inflammation (G3)
ANTRUM	No Inflammation (G0)	GRADE 0	GRADE I	GRADE II	GRADE II
	Mild Inflammation (G1)	GRADE I	GRADE II	GRADE II	GRADE III
	Moderate Inflammation (G2)	GRADE II	GRADE II	GRADE III	GRADE IV
	Severe Inflammation (G3)	GRADE II	GRADE III	GRADE IV	GRADE IV

Appendix 3. Esophagitis classification according to Knuff et al. (1984) & Leape et al. (1981).

Grade	Histology criteria
0	Normal
Ia	Basal zone hyperplasia
Ib	Elongated stromal papillae
Ic	Vascular ingrowth
II	Polymorphs in the epithelium ± lamina propria
III	Polymorphs with ephitelial defect
IV	Ulceration
V	Abnormal columnar epithelium

Appendix 4. Presenting symptoms of *Helicobacter pylori* positive and negative children and adolescents.



p>0.05, Fisher exact test

Appendix 5. Comparison of esophagitis scores between *H. pylori* positive and negative children and adolescents according to Knuff et al. and Leape et al.

Hystopathological parameters	Hp+ (n=96)	Hp- (n=89)	p
Number of esophageal biopsies	93	88	
Esophagitis Score Mean±SD	1.07±0,91	1.43±0,84	0.006*
Esophagitis Score 0 (n)	35	20	
1 (n)	16	10	0.0192#
2 (n)	42	58	

*****_H. pylori_ positive versus _H. pylori_ negative patients, using Mann Whitney test;
#_H. pylori_ positive versus _H. pylori_ negative patients, using Chi-Square test;
SD= Standard Deviation**.**