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Helicobacter Pylori in Pediatrics

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ABSTRACT

This review includes the main pediatric studies published from April 2011 to march 2016. The important studies involving H. pylori genomes, especially those pertaining to genomic diversity, disease outcome, H. pylori population structure and evolution are reviewed. Genotypic variability in H. pylori strains influences the clinical manifestation of the infection. The antigen stool test is becoming the "gold standard" in prevalence studies, and according to the epidemiologic studies, the prevalence of H. pylori in childhood is not decreasing any more in the developed world. Studies showed conflicting results regarding the association between H. pylori infection and iron deficiency anemia. One study suggests that H. pylori eradication plays a role in the management of chronic immune thrombocytopenic purpura. The prevalence of H. pylori was higher in chronic urticaria patients and following H. pylori eradication, urticarial symptoms disappeared. An inverse relationship between H. pylori infection and allergic disease was reported. The resistance rate of H. pylori strains is high in children. Therefore, among other important issues concerning H. pylori in pediatrics, guidelines published by ESPGHAN and NASPGHAN last year also recommended culture and susceptibility testing before first-line treatment in areas with high or unknown antibiotic resistance rates.

Keywords: Helicobacter pylori, H pylori, infection, pediatrics

PATHOPHYSIOLOGY

Helicobacter pylori infection is the leading cause of gastric cancer worldwide. However, in children, H. pylori related malignancy is extremely rare and it is generally acquired in this period [1]. Various factors influence malignant potential including age of infection, bacterial genotype, host immune response, and host genetics. H. pylori genotypes associated with more severe inflammation of gastric mucosa in pediatric patients are cagA, vacAs1, and babA, and their detection could be of importance in areas with high risk of carcinoma. ***Sicinschi et al.***[1] used stool samples instead of gastric biopsy samples to genotype H. pylori virulence markers and found a high incidence of cagA and vacAs1 allele (in 66.1 and 91.7%, respectively) in asymptomatic children with H. pylori infection in a gastric cancer high risk area in Columbia. This could be concluded to be a contributing factor for the high incidence of gastric cancer in adults in this area. Meanwhile **Oliveira *et al.*** [2] described the prevalence of infection with cagA-positive strains in a group of children and adolescents in southern Brazil. The prevalence of cagA-positive H. pylori was 29.6%. There were no statistically significant differences in clinical or demographic characteristics or in the endoscopic and histologic features of the patients infected with cagA-positive strains as compared with those infected with cagA-negative strains.

Several studies were performed to identify Helicobacter pylori virulence factors that could be related to the evolution of disease. High positivity of virulence genes was found in dyspeptic or asymptomatic children [1–4]. Acute exposure to VacA initially triggers host autophagy to mitigate the effects of the toxin in epithelial cells. **Raju *et al.*** [5] identified a host autophagy gene (ATG16L1), susceptible for H. pylori infection and defined the mechanism by which the autophagy pathway is affected after H. pylori infection.

The virulence role of iceA allele was not clearly demonstrated until recently when a meta-analysis involving 50 relevant studies confirmed the importance of iceA1 allele in the development of peptic ulcer disease (PUD), especially duodenal ulcer [5]. On the other hand, connection between iceA allele and gastric cancer was not confirmed [6]. Lewis (Le) blood-group epitopes on the surface of H. pylori mimic structures present on human gastric

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surfaces and could be implicated in adverse autoimmune reactions of the host. Most studies in adults found that the majority of the H. pylori strains express type 2 Lex and/or Ley antigens, while pediatric isolates have the tendency to express also type 1 Leb antigen [7]. All Lex- and Ley expressing strains also had a functional cag pathogenicity island and vacAs1 allele. However, no association between bacterial virulence characteristics and the histopathologic observations was observed. Moreover, pediatric isolates have overwhelming presence of a1,6-glucan, yet another phenotypic characteristic that facilitates colonization and contributes to the antigenic diversity of H. pylori surface [7].

Ikuse *et al*. [8] analyzed the expression of immune response factors in the H. pylori-infected gastric mucosa of children. Using microarray analysis, the total number of significantly upregulated and downregulated genes was 21 in the antrum and 16 in the corpus, when comparing patients with or without infection. Using real-time PCR, the expression of lipocalin-2, C-C motif chemokine ligand 18, C-X-C motif chemokine ligand (CXCL) 9, and CXCL11 was upregulated, while the expression of pepsinogen I and II was downregulated when comparing patients with or without infection. A better understanding of the immune response to H. pylori infection in children is important to develop an effective vaccine, as children are the main target of the vaccination. **Freire de Melo *et al.*** [9] evaluated IL-17 cell response to H. pylori and compared the gastric levels of Th17 and Treg-associated cytokines in children and adults. They concluded that Treg, instead of Th17, cell response to H. pylori infection predominates in children.

Muhsen *et al*. [10] examined the association between H. pylori infection, serum PG levels (as a measure of inflammation), and vibriocidal antibody seroconversion, following oral immunization with the live oral cholera vaccine CVD 103-HgR in children of various ages. The likelihood of vibriocidal antibody seroconversion following vaccination was significantly decreased in H. pylori- seropositive children aged 6 months–4 years with a PG II >8 lg/L, as well as in those with a low PG II level, compared to H. pylori-seronegative children. Helicobacter pylori-seropositive children aged 5–9 years with a serum PG I of 30 mg/L (indicating more severe gastritis) had higher odds of vibriocidal seroconversion than those with lower PG I levels. These data suggest that, as H. pylori gastritis progresses with increasing pediatric age, changes in gastric secretion may explain the differences in age-related immune responses to this live oral cholera vaccine [10].

EPIDEMIOLOGY AND TRANSMISSION

The prevalence of H. pylori infection in children has decreased over the last decade, but depending on many factors, it still varies all over the world. Acquisition of H. pylori infection in childhood reflects the social, environmental, and economic status of the community. Lower prevalence rates are reported in communities with higher socioeconomic status and generally better environmental conditions. A prevalence of 6% in Texas, USA [11], and 13% in Sardinia, Italy, was found [12] ,as well as 30.9% in Nigerians [13], 38% in school children in Mexico City [14], 30.8% in Cuban symptomatic children [15], and 78.1% in Sherpa residents in Nepal [16]. **Mohammed *et al.*** [17] studied a total of 303 children from the central and western regions of Saudi Arabia. The prevalence of H. pylori infection was 49.8%. Conditions positively associated with the presence of H. pylori were as follows: age above 10 years, a low-income level, more than eight persons in the household, bed sharing, and two infected parents [17].

Studies have shown that patients with inflammatory bowel disease (IBD) are less likely to be infected with H. pylori compared with non-IBD patients. The study by **Roka *et al*.** [18] confirms this inverse association between H. pylori and IBD.

The prevalence of H. pylori and different parasite infections was studied. A 3-fold higher risk of concomitant Giardia intestinalis and H. pylori infections was described in Uganda [19]. Ascaris lumbricoides seropositivity correlated with elevated IgE and anti-inflammatory Th2-IgG1 responses to H. pylori, while Toxoplasma gondii seropositivity was linked to elevated IgE, proinflammatory Th1-IgG2, IgG3, and IgG4 responses to H. pylori. These infections may have an impact on inflammatory responses to H. pylori and may partially explain differences in gastric cancer risk in Colombia [20].

Hirsch *et al.* [21] were able to detect H. pylori DNA by PCR in several plaque and root canal samples, and cultured H. pylori from two root canals, suggesting that root canals of endodontic-infected

deciduous teeth may be a reservoir for H. pylori and serve as a potential source of transmission.

Regarding transmission, intrafamilial infection is considered to be one of the main routes of transmission for H. pylori. **Osaki *et al*.** [22] assessed the genomic profiles of H. pylori isolates from family members by multilocus sequence typing (MLST) and identified the original strain infecting the index child. Mother-to-child transmission of H. pylori was demonstrated in four of five families, while transmissions from father to child and from sibling to sibling were demonstrated in two families and one family, respectively [22].

CLINICAL MANIFESTATION

Gastrointestinal Manifestations

Helicobacter pylori infection is well known as the main cause of peptic ulcer disease (PUD). Nonspecific abdominal complaints such as pain, cramps and nausea are very common in pediatric population of various organic diseases and, more often, of functional gastrointestinal disorders. **Dore *et al.*** [12], in a cross-sectional sero-epidemiologic study, found that nausea or vomiting and diarrhea were significantly associated with H. pylori infection (OR 2.2 and 2.1, respectively), but not with abdominal pain or heartburn. Perforated ulcer is rare, but several cases of peritonitis secondary to duodenal perforation have been described [23-24].

Extraintestinal Manifestations

Several studies have shown an association between iron deficiency anemia (IDA) status and H. pylori infection. In the presence of H. pylori infection, they probably develop IDA faster than adults. In H. pylori-associated atrophy, hypochlorhydria has a role in ID through changes in the physiology of iron-complex absorption. **Darvishi *et al.*** [25] conducted a case–control study to evaluate the association between H. pylori infection and IDA among preschool children (age range: 40–75 months) in Iran. In total, 81.3% of children with IDA and 14.3% of nonanemic controls were seropositive for H. pylori ($p < .0001$) [25]. Hematologic parameters returned to normal 3 months after H. pylori eradication, with disappearance of lymphocytic gastritis, in a 12-year-old premenstrual girl with refractory ID anemia and focal intestinal metaplasia [26].

The association between H. pylori infection and chronic immune thrombocytopenic purpura (cITP) is not well established in children, although the cure of thrombocytopenia has been described in approximately half of H. pylori-eradicated adult patients [27]. **Xiong et al.** [28] carried out a meta-analysis to evaluate this possible association in Chinese children and showed that 49.27% of ITP children had evidence of H. pylori infection compared with 23.39% of the control group. Moreover, H. pylori eradication therapy was able to reduce the recurrence of ITP [28].

There are conflicting results regarding the role of H. pylori in children's growth. **Kopacova *et al*.** [29] led a multicenter study corresponding well to the geographic distribution of the Czech population. They evaluated vital signs and body indices in H. pylori-positive and H. pylori-negative persons. The overall prevalence of H. pylori infection was 5.2%. Chronic H. pylori infection appeared to be associated with short stature in children. H. pylori infection did not influence blood pressure, body weight, or body mass index either in adults or in children and adolescents [29].

The recent increase in asthma and allergy seems to be associated with a decrease in the H. pylori infection prevalence, with some studies reporting a negative relationship. A significantly lower borderline H. pylori seropositivity was found in children with wheezing compared with nonwheezers; however, no association between H. pylori serological status and allergic rhinitis, atopic dermatitis, or asthma was found by **Holster *et al*.** [30]. In a meta-analysis performed by **Wang *et al.*** [31], little evidence was found for an inverse association between asthma and H. pylori infection both in children and in adults. In a prospective, randomized study, **Akelma *et al.*** [32] included all pediatric and adult patients who presented themselves at the allergy outpatient clinic for CU. Following H. pylori eradication, urticarial symptoms recovered in 15 of 18 adults (83.3%) and 10 of 10 (100%) children ($p = .172$). The authors suggest that CU patients with unknown etiology should be routinely screened for H. pylori, even if they do not present GI symptoms, and that those with H. pylori-positive results receive eradication treatment [32]. In a meta-analysis of 16 studies involving 965 CU and 325 controls, the prevalence of H. pylori was higher in CU patients than in controls [27].

DIAGNOSIS

Testing for H. pylori in children should be performed in properly selected patients (Table 1)

and with an adequate diagnostic procedure. As no single test is accurate enough for detection of H. pylori, current guidelines recommend endoscopy with gastric biopsies and confirmation of infection with two different tests: either histopathology and rapid urease test or a culture [33].

In a study by **Masumi *et al.*** [34] a stool antigen test (SAT) using a monoclonal antibody against native catalase was compared to a real-time PCR. The SAT showed an 89.5% sensitivity and 100% specificity. Only 10 of 151 patients were PCR positive with a negative SAT, but the results of a UBT conducted on these patients were in agreement with the results of the SAT [34]. In another prospective study, **Abdulqawi *et al.*** [35] compared the accuracy of three invasive diagnostic tests (rapid urease test, histology, and culture) and one noninvasive test (IgG serology) in Egyptian children and concluded that the association of urease test and histology with serology leads to greater accuracy in the diagnosis of the H. pylori infection.

In children, especially in the youngest, the usefulness of the diagnostic test based on the detection of H. pylori-specific IgG antibodies (serum, urine, whole blood, saliva) is controversial due to their low sensitivity. **Okuda *et al.*** [36] evaluated the accuracy of two urinary IgG antibodies tests (Urine-HpELISA test and Rapid urine-HpAb) obtaining sensitivity and specificity of 91.9% and 96.9% for Urine-HpELISA and 78.4% and 100% for Rapid urine-HpAb and recommended these methods as simple, low cost, rapid, and reliable for screening of H. pylori.

Regarding noninvasive tests, recently published meta-analysis on the performance of the ^{33} C-urea breath test (^{33} C-UBT) showed relatively good accuracy especially in children older than 6 years of age (sensitivity 96.6%, specificity 97.7%) [37].

Histopathologic studies are still important to identify mucosal lesions. Carvalho *et al.* [38] analyzed histopathologic lesions in 96 Brazilian children with H. pylori infection. 70.5% had moderate-to-severe chronic active gastritis. Intestinal metaplasia was not found, and gastric atrophy was not significant. 61.9% had pangastritis, and H. pylori density was higher in the antrum than in the corpus [36].

Table 1: When to search for Helicobacter pylori infection (based on ESPGHAN/NASPGHAN recommendations[33]

A. Testing for H. pylori infection required:
When searching for the cause of gastrointestinal symptoms suggestive of organic disease, serious enough to justify upper endoscopy
B. Testing for H. pylori infection to be considered: In children with first-degree relatives with gastric cancer
In children with refractory iron deficiency anemia in which other causes have been ruled out
C. Testing for H. pylori not justified by current evidence: In functional abdominal pain
In otitis media, upper respiratory tract infections, periodontal disease, food allergy, sudden infant death syndrome (SIDS), idiopathic thrombocytopenic purpura, short stature
SPGHAN: European Society for Paediatric Gastroenterology, Hepatology and Nutrition. NASPGHAN: North American Society for Paediatric Gastroenterology.
Hepatology and Nutrition;

TREATMENT

The updated evidence-based guidelines from the joint societies of ESPGHAN and NASPGHAN for H. pylori infection in children published in 2011[33], recommend eradication treatment in children with PUD and suggest to consider triple therapy with a PPI and amoxicillin and imidazole or clarithromycin; or with a bismuth salts, amoxicillin and imidazole; or sequential therapy.

Children have more difficulty than adults in eradicating H. pylori infection and very often the routine therapeutic combinations do not achieve 80% eradication rates. In a review of 10 randomized trials performed in different countries, **Zullo *et al.*** [39] found that sequential therapy achieved significantly higher eradication rates compared with the 7- and 10-day standard triple therapies, even in clarithromycin and metronidazole resistance H. pylori strains. However, the success rate of the

sequential regimen tends to be lower in recent studies compared with previous trials[39]. **Horvath *et al.*** [40] reviewed the randomized controlled trial comparing sequential therapy with standard triple therapy for H. pylori eradication involving 857 children. They found that sequential therapy was superior to the 7-day standard triple therapy, but not significantly better than the 10-day or 14-day triple therapies.

Although most of the studies worldwide confirmed an increase in macrolide resistance and metronidazole resistance either decreased or remained stable. When susceptible antimicrobials were used, eradication occurred in 78.7 versus 37.5% when the treatment included a resistant drug [41]. In a prospective multicenter European study, primarily comprised of adults, **Megraud *et al.*** [42] **found** a 31.8% resistance rate to clarithromycin and 25.7% to metronidazole in the 311 H. pylori isolates from children from eight countries included in the study. The increase in clarithromycin resistance in many countries (especially in Western/ Central and Southern Europe) has prohibited its empirical use in standard therapeutic regimens. **Ogata *et al.*** [43] in Brazilian children and adolescents, reported a high metronidazole (40%), clarithromycin (19.5%), and amoxicillin (10.4%) resistance rate and 18.2% of multiple resistance. All H. pylori strains were susceptible to furazolidone and tetracycline, and they proposed the use of these two antimicrobials, both associated with amoxicillin, in future eradication regimens.

The increasing number of children infected with resistant H. pylori strains promotes evaluation of new treatment protocols. Unfortunately, some of the second-line antibiotics, such as tetracycline, are not approved for use in children. In a multicenter trial, **Schwarzer *et al.*** [44] showed that high dose therapy with amoxicillin, metronidazole and esomeprazole during 2 weeks was a good treatment option in children infected with double- resistant strains. Furthermore, several recently published articles confirmed the efficacy of sequential therapy in children and found it even more efficacious than standard triple-therapy regimen, especially in areas with low clarithromycin resistance [42–44].

Several meta-analyses suggested that probiotics improve H. pylori eradication and/or reduce the treatment's side effects. Probiotics could help stave off complications by decreasing the bacterial density in gastric mucosa and prevent the reinfection by inhibiting the adherence of the bacteria to gastric epithelial cells. Although not all of the studies support this beneficial effect in children. **Tolone *et al.*** [45], in a randomized study including 68 H. pylori-infected children, reported significantly fewer treatment side effects after adding a commercial multistrain probiotic and bovine lactoferrin to a 7-day standard triple therapy compared to those observed with the triple therapy alone. In a prospective study, **Yang *et al.*** [46] found low Bifidobacterium microflora in the gut of H. pylori-infected children. They concluded that probiotic-containing yogurt offers the benefit of restoring the fecal Bifidobacterium spp./ Escherichia coli ratio, and of suppressing the H. pylori load with an increment of serum IgA and pepsinogen II levels and a reduction in serum IL-6 level, in these children [47].

RECURRENCE

The rate of recurrence of H. pylori infection is higher in developing than in developed communities. Strain genotyping before and after treatment is necessary to distinguish between them. The reinfection rate in children varied between 2% and 10%, being more frequent in developing countries. Intrafamilial transmission could be the major risk factor associated with reinfection in children [49]. **Candelli *et al.*** [50] reported a higher prevalence of H. pylori infection in young patients with diabetes than in the control group. Three years after a standard eradication treatment, the reinfection rate in the patients with diabetes was higher than in the control group [51], due to the higher susceptibility of patients with diabetes to develop infections. Age and socioeconomic status are also related to H. pylori reinfection in these patients.

CONCLUSION

Helicobacter pylori infection differs in children compared to infected adults in respect to prevalence and pathophysiology, diagnostic tests accuracy and applicability, and antibiotic resistance rates. Although many uncertainties still prevail and there is lack of randomized pediatric trials, recently published studies provide further insight into the clinical implications of H. pylori infection, enabling development of the most recent diagnostic and therapeutic guidelines for children.

REFERENCES

1- **Sicinschi LA, Correa P, Bravo LE, Peek RM Jr, Wilson KT, Loh JT *et al.* (2012):** Non-invasive

genotyping of Helicobacter pylori cagA, vacA, and hopQ from asymptomatic children. Helicobacter,17:96–106.

2- **Oliveira JG, Ferreira CH, Camerin AC, Rota CA, Meurer L, Silveira TR (2014):** Prevalence of infection with cagA-positive Helicobacter pylori strains among children and adolescents in southern Brazil.Arq Gastroenterol.,5:180–5.

3- **Smith SI, Fowora MA, Lesi OA, Agbebaku E, Odeigah P, Abdulkareem FB *et al.* (2012):** Application of stool-PCR for the diagnosis of Helicobacter pylori from stool in Nigeria- a pilot study.Springerplus,1:78.

4- **Goncalves MH, Silva CI, Braga-Neto MB, Fialho AB, Fialho AM, Queiroz DM, Braga LL. (2013)**: Helicobacter pylori virulence genes detected by string PCR in children from an urban community in northeastern Brazil. J Clin Microbiol.,51:988–9.

5- **Raju D, Hussey S, Jones NL (2012)**: Crohn disease ATG16L1 polymorphism increases susceptibility to infection with Helicobacter pylori in humans. Autophagy,8:1387–8.

6- **Shiota S, Watada M, Matsunari O, Iwatani S, Suzuki R, Yamaoka Y (2012)**: Helicobacter pylori iceA, clinical outcomes, and correlation with cagA: a meta-analysis. PLoS ONE,7:e30354.

7- **Altman E, Chandan V, Harrison BA, Panayotopoulou EG, Roma-Giannikou E, Li J *et al.* (2012)**: Helicobacter pylori isolates from Greek children express type 2 and type 1 Lewis and alpha1,6-glucan antigens in conjunction with a functional type IV secretion system. J Med Microbiol.,61:559–66.

8- **Ikuse T, Ohtsuka Y, Kudo T, Hosoi K, Obayashi N, Jimbo K *et al.* (2012)** :Microarray analysis of gastric mucosa among children with Helicobacter pylori infection. Pediatr Int.,54:319–24.

9- **Freire de Melo F, Camargos Rocha AM, Aguiar Rocha G, De Assis Batista S, Porto Fonseca de Potro L, Dinis Carvalho S *et al.* (2012):** A regulatory instead of an IL 17 T response predominates in Helicobacter pylori-associated gastritis in children. Microbes Infect.,14:341–7.

10-**Muhsen K, Lagos R, Reymann MK, Graham DY, Pasetti MF, Levine MM (2014**): Age-dependent association among Helicobacter pylori infection, serum pepsinogen levels and immune response of children to live oral cholera vaccine CVD 103-HgR. PLoS ONE,9:e83999.

11-**Patterson T, Straten E, Jimenez S (2012)**: The prevalence of Helicobacter pylori antibody in different age groups in Central Texas. Clin Lab Sci.,25:102–6.

12-**Dore MP, Fanciulli G, Tomasi PA, Realdi G, Delitala G, Graham DY, Malaty HM (2012):** Gastrointestinal symptoms and Helicobacter pylori infection in school-age children residing in Porto Torres, Sardinia, Italy. Helicobacter;17:369–73.

13-**Etukudo OM, Ikpeme EE, Ekanem EE (2012)** :Seroepidemiology of Helicobacter pylori infection among children seen in a tertiary hospital in Uyo, southern Nigeria. Pan Afr Med J.,12:39.

14-**Duque X, Vilchis J, Mera R, Trejo-Valdivia B, Goodman KJ, Mendoza ME *et al.* (2012):** Natural history of Helicobacter pylori infection in Mexican schoolchildren: incidence and spontaneous clearance. J Pediatr Gastroenterol Nutr.,55:209–16.

15-**Llanes R, Millan LM, Escobar MP, Gala A, Capo V, Feliciano O *et al.* (2012):** Low Prevalence of Helicobacter pylori Among Symptomatic Children from a Hospital in Havana, Cuba. J Trop Pediatr.,58:231–4.

16-**Sherpa TW, Sherpa KT, Nixon G, Heydon J, Dovey S (2012):** The prevalence of Helicobacter pylori infection in Sherpa residents of the Upper Khumbu, an isolated community in Eastern Nepal.N Z Med J.,125:30–7.

17-**Hasosah M, Satti M, Shehzad A *et al.* (2015):** Prevalence and risk factors of Helicobacter pylori infection in Saudi children: a three-year prospective controlled study. Helicobacter,20:56–63.

18-**Roka K, Roubani A, Stefanaki K, Panayotou I, Roma E, Chouliaras G (2014):** The prevalence of Helicobacter pylori gastritis in newly diagnosed children with inflammatory bowel disease. Helicobacter,19:400–5.

19-**Ankarklev J, Hestvik E, Lebbad M, Lindh J, Kaddu-Mulindwa DH, Andersson JO *et al.* (2012)**: Common coinfections of Giardia intestinalis and Helicobacter pylori in non-symptomatic Ugandan children. PLoS Negl Trop Dis.,6:e1780.

20-**Courtney E, Whary MT, Ihrig M, Bravo LE, Correa P, Fox JG. (2012)** Serologic evidence that Ascaris and Toxoplasma infections impact inflammatory responses to Helicobacter pylori in Colombians. Helicobacter;17:107–15.

21-**Hirsch C, Tegtmeyer N, Rohde M, Rowland M, Oyarzabal OA, Backert S. (2012)** Live Helicobacter pylori in the root canal of endodontic-infected deciduous teeth. J Gastroenterol; 47:936–40.

22-**Osaki T, Konno M, Yonezawa H, Hojo F, Zaman C, Takahashi M, Fujiwara S, Kamiya S. (2015)** Analysis of intra-familial transmission of Helicobacter pylori in Japanese families. J Med Microbiol;64:67–73.

23-**Schwartz S, Edden Y, Orkin B, Erlichman M. (2012)** Perforated peptic ulcer in an adolescent girl. Pediatr Emerg Care;28:709–11.

24-**Ndour O, Bansouda J, Fall AF, Alumeti DM, Diouf C, Ngom G, Ndoye M. (2012)** [Peritonitis following gastroduodenal ulcer perforation disease in children: report of 4 cases]. Arch Pediatr;19:1065–9.

25-**Darvishi M, Ziari K, Mohebi H, Alizadeh K. (2015)** Association between iron deficiency anemia and Helicobacter pylori infection among children under six years in Iran. Acta Med Iran;53:220–4.

26-**Santalha MF Jr, Costa E, Miguel N, Vizca_ıno R, Barbot J, Pereira F. (2013)** Iron deficiency anemia due to lymphocytic gastritis with Helicobacter pylori infection in childhood: case report. J Pediatr Hematol Oncol;35(4):321–2.

27-Gu H, Li L, Gu M, Zhang G. (2015) Association between Helicobacter pylori infection and chronic urticaria: a meta-analysis. Gastroenterol Res Pract 2015:486974.

28-Xiong LJ, Tong Y, Wang ZL, Mao M. (2012) Is Helicobacter pylori infection associated with Henoch-Schonlein purpura in Chinese children? a meta-analysis. World J Pediatr;8:301–8.

29-Kopacova M, Koupil I, Seifert B, Fendrichova MS, Spirkova J, Vorisek V, Rejchrt S, Douda T, Tacheci I, Bures J. (2014) Blood pressure and stature in Helicobacter pylori positive and negative persons. World J Gastroenterol;20:5625–31.

30-Holster IL, Vila AM, Caudri D, den Hoed CM, Perez-Perez GI, Blaser MJ *et al.* (2012) The impact of Helicobacter pylori on atopic disorders in childhood. Helicobacter;17:232–7.

31-Wang Q, Yu C, Sun Y. (2013) The association between asthma and Helicobacter pylori: a meta-analysis. Helicobacter;18:41–53.

32-Akelma AZ, Cizmeci MN, Mete E, Tufan N, Bozkurt B. (2015) A neglected cause for chronic spontaneous urticaria in children: Helicobacter pylori. Allergol Immunopathol (Madr);43:259–63.

33-Koletzko S, Jones NL, Goodman KJ, Gold B, Rowland M, Cadranel S *et al.* (2011) Evidence-based guidelines from ESPGHAN and NASPGHAN for Helicobacter pylori infection in children. J Pediatr Gastroenterol Nutr;53:230–43.

34-Okuda M, Osaki T, Kikuchi S, Ueda J, Lin Y, Yonezawa H, Maekawa K, Hojo F, Kamiya S, Fukuda Y. (2014) Evaluation of a stool antigen test using a mAb for native catalase for diagnosis of Helicobacter pylori infection in children and adults. J Med Microbiol;63:1621–5.

35-Abdulqawi K, El-Mahalaway AM, Adelhameded A, Abdelwahab AA. (2012) Correlation of serum antibody titres with invasive methods for rapid detection of Helicobacter pylori infection in symptomatic children. In J Exp Pathol;93:295–304.

36-Okuda M, Kamiya S, Booka M, Kikuchi S, Osaki T, Hiatani T *et al.* (2013) Diagnostic accuracy of urine-based kits for detection of Helicobacter pylori antibody in children. Pediatr Int;55 (3):337–41.

37-Leal YA, Flores LL, Fuentes-Panana EM, Cedillo-Rivera R, Torres J. (2011) 13C-urea breath test for the diagnosis of Helicobacter pylori infection in children: a systematic review and metaanalysis.Helicobacter;16:327–37.

38-Carvalho MA, Machado NC, Ortolan E, Rodrigues MA. (2012) Upper gastrointestinal histopathological findings in children and adolescents with nonulcer dyspepsia with Helicobacter pylori infection. J Pediatr Gastroenterol Nutr;55:523–9.

39-Zullo A, Hassan C, Ridola L, De Francesco V, Vaira D. (2013) Standard triple and sequential therapies for Helicobacter pylori eradication:an update. Eur J Intern Med;24:16–9.

40-Horvath A, Dziechiarz P, Szajewska H. (2012) Meta-analysis: sequential therapy for Helicobacter pylori eradication in children. Aliment Pharmacol Ther;36:534–41.

41-Montes M, Villalon FN, Eizaguirre FJ, Delgado M, Mu~noz-Seca IM, Fern_andez-Reyes M, P_erez-Trallero E. (2015) Helicobacter pylori infection in children. Antimicrobial resistance and treatment response. Helicobacter;20:169–75.

42-Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM *et al.* (2013) Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut;62:34–42.

43-Ogata SK, Godoy AP, Da Silva Patricio FR, Kawakami E. (2013) High Helicobacter pylori resistance to metronidazole and clarithromycin in Brazilian children and adolescents. J Pediatr Gastroenterol Nutr;56(6):645–8.

44-Schwarzer A, Urruzuno P, Iwanczak B, Martinez-Gomez MZ, Kalach N, Roma-Giannikou E, *et al.* (2011) New effective treatment regimen for children infected with a double-resistant Helicobacter pylori strain. J Pediatr Gastroenterol Nutr;52: 424–8.

45-Bontems P, Kalach N, Oderda G, Salame A, Muyshont L, Miendje DY, *et al.* (2011) Sequential therapy versus tailored triple therapies for Helicobacter pylori infection in children. J Pediatr Gastroenterol Nutr;53:646–50.

46-Erdur B, Ozturk Y, Gurbuz ED, Yilmaz O. (2012) Comparison of sequential and standard therapy for Helicobacter pylori eradication in children and investigation of clarithromycin resistance. J Pediatr Gastroenterol Nutr. [Epub ahead of print].

47-Albrecht P, Kotowska M, Szajewska H. (2011) Sequential therapy compared with standard triple therapy for Helicobacter pylori eradication in children: a double-blind, randomized, controlled trial. J Pediatr;159:45–9.

48-Tolone S, Pellino V, Vitalli G, Lanzafame A, Tolone C.(2012) Evaluation of Helicobacter pylori eradication in pediatric patients by triple therapy plus lactoferrin and probiotics compared to triple therapy alone. Ital J Pediatr;38:63–7.

49-Yang Y, Sheu B. (2012) Probiotics-containing yogurts suppress Helicobacter pylori load and modify immune response and intestinal microbiota in the Helicobacter pylori –infected children. Helicobacter;17:297–304.

50-Candelli M, Rigante D, Schiavino A, Gabrielli M, Crea F, Minguell del Lungo L *et al.* (2012) High reinfection rate of Helicobacter pylori in young type 1 diabetic patients: a three-year follow-up study. Eur Rev Med Pharmacol Sci;16:1468–72.

51-Moya DA, Crisinger KD. (2012) Helicobacer pylori persistence in children: distinguishing inadequate treatment resistant organism and reinfection. Curr Gastroenterol Rep;14:236–42.