

## Relationship between *Helicobacter pylori* detection and an increased risk of infection in childhood

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**Abstract:** The risk of developing gastric cancer is believed to be related to differences among *Helicobacter pylori* strains and the inflammatory responses mediated by host genetic factors. *H. pylori* infection is acquired at an early age and in the absence of appropriate antibiotic therapy, it generally persists for life. *Tp53* gene regulates the transcription of several cytokines and chemokines involved in innate immunity and its action may be influenced by the presence of different *H. pylori* strains. The present study aimed to detect *H. pylori* in pediatric patients, to access *Tp53* polymorphism at codon 72 and to correlate such findings with age and histopathological results. Three hundred and forty-two patients were analyzed. DNA from their gastric biopsies was extracted and the detection of *H. pylori* was performed through polymerase chain reaction assays, urease test and histopathologic examination. Allelic discrimination of SNP rs1042522 (*Tp53*) was performed by real-time polymerase chain reaction. Our results suggest a possible relationship between the presence of *H. pylori* and chronic gastritis in children and young patients, and showed a significant association between ageing and positivity for *H. pylori*. It was verified that patients aged  $\leq 10$  years were 1.3 times more likely to have infection by *H. pylori* when compared with those aged  $> 10$  years. Finally, no association was found between *Tp53* polymorphisms and the presence of *H. pylori*.

**Key words:** *Helicobacter pylori*, childhood, *Tp53* polymorphisms, PCR.

## INTRODUCTION

*Helicobacter pylori* is involved in the pathogenesis of a number of gastrointestinal diseases, including acute and chronic gastritis, peptic ulceration, gastric carcinoma and gastric lymphoma (1, 2). The risk of developing gastric cancer is believed to be related to differences among *H. pylori* strains and the inflammatory responses mediated by host genetic factors (3, 4).

*Tp53* tumor suppressor gene has a common polymorphism at codon 72, encoding either proline or arginine (P72 or R72, respectively) and a large number of epidemiological studies investigated the impact of this polymorphism

on cancer risk. Frank *et al.* (5), suggest that there may be a minor association between the P72 allele and increased cancer risk. In addition, the transcription of the *Tp53* gene is controlled by type I interferon signaling and the induction of *Tp53* participates in the host defense against viral infection. Moreover, *Tp53* regulates the transcription of several cytokines and chemokines involved in innate immunity and this can be influenced by the presence of different strains of *H. pylori* (5-7).

*H. pylori* infection is acquired at an early age (mostly before the age of 10), and in the absence of an antibiotic therapy, it generally persists for life (8). Studies have reported that the longer the

time interval for *H. pylori* detection, the higher the risk of developing cancer (9, 10). Eradication should be performed at this time because once the gastric mucosa is irreversibly harmed, treatment does not prevent carcinogenesis (8, 11).

It is unclear if the gastric mucosa or oral cavity could be considered permanent or transient reservoirs for *Helicobacter pylori*. The results obtained in the present study established that the microhabitats of *H. pylori* are the gastric mucosa and oral cavity using the polymerase chain reaction (PCR) (12, 13).

Studies using DNA fingerprinting confirmed that members of the same family tend to be infected by the same strain of bacteria, a fact that confirms the interpersonal transmission, and should therefore be considered in addition to the socioeconomic conditions of the population (8, 14, 15).

Other studies involving pediatric patients in developing countries show that the prevalence in children aged  $\leq 10$  years is above 50%. On the other hand, developed countries and those which have had an increase in their Human Development Index (HDI) show a reduction to around 10% in this age group (9, 16, 17). These results highlight the importance of detecting *H. pylori* in childhood. Based on these findings, the present study was developed aiming at detecting the *H. pylori* in pediatric patients, genotype the *Tp53* polymorphism at codon 72 and correlate with age and histopathological results.

## MATERIALS AND METHODS

### Patients and Samples

In this study, 342 dyspeptic patients (150 male and 192 female with a mean age 9.36 years) attended at Marília Medical School, Marília, SP, Brazil during the period between 2005 and 2011 were enrolled. All subjects were submitted to clinical examinations and only after previous medical indications they underwent endoscopic examination and investigation of peptic symptoms. All subjects had given their informed consent, and the study protocol was approved by the Ethics Committee of Sacred Heart University (n. 056/2005 and 223/2011).

Patients were divided into two groups according to their age. The first group included patients aged  $\leq 10$  years and was composed of 193 patients (97 male and 96 female with a mean

age 6.53 years  $\pm$  2.67) and the second group was composed of 149 patients aged  $> 10$  years (53 male and 96 female with a mean age 12.94 years  $\pm$  1.71). During endoscopy, three gastric biopsy sections were taken from the antrum of each patient for detection of *H. pylori*. One was fixed in formalin, and assessed for the presence of *H. pylori* by histological examination, another section was used for rapid urease test and the third for molecular analysis.

### Detection of *Helicobacter pylori*

DNA from the gastric biopsies was extracted with the QIAamp® tissue kit (Qiagen, Germany) according to the manufacturer's recommendations. Additional fresh biopsies were placed in rapid urease test, using the TUPF kit (Laborclin, Brazil), according to the manufacturer's instructions. After six hours, the TUPF test was inspected for a change in color. The samples from antrum for histopathologic examination were fixed in formalin and stained with hematoxylin and eosin (HE) and Giemsa. The histological features of the gastric mucosa were recorded using the updated Sydney scoring system (18).

Primers Hpx1 (5'-CTGGAGARACTAAGYCCTCC-3') and Hpx2 (5' GAGGAATACTCATTGCGAAGGCGA-3') targeting the 16S ribosomal RNA gene of *H. pylori* were used according to the published data (13). PCR amplification was performed according to the method described previously (12, 13). In each experiment, positive (strain 26695) and negative (water) controls were included. The assay was considered positive when the PCR product was present.

### Genotyping of *Tp53* Polymorphism

Allelic discrimination of SNP rs1042522 (*Tp53*) (assay id: c\_2403545\_10) was performed by real time polymerase chain reaction (RT-PCR) on an Applied Biosystems 7500 Fast Real-Time PCR system (Applied Biosystems™, USA) using Taqman commercial probes (Applied Biosystems™, USA). Genotyping of *Tp53* followed the cycling program and conditions recommended by Applied Biosystems. The results were assessed taking into account the allelic discrimination and absolute quantitation in all samples; additionally, two negative controls per plate (non-template controls) were included. The interpretation was

performed with software Taqman Genotyper v1.0 (Applied Biosystems™, USA).

### Statistical Analyses

Statistical analyses were performed by chi-square test and Fisher's test. The significance level was set at a p value of < 0.05. Statistical analyses were performed using the statistical package SPSS 20.0 (Chicago, USA)

## RESULTS

### Presence of *H. pylori* and Genotyping of *Tp53* Gene

*H. pylori* was detected in biopsies of the antrum in 85 out of 342 (24.8%) patients by PCR assay. In addition, the histological analysis revealed the presence of *H. pylori* in 49 out of 286 subjects. Fifty-six patients did not have results by

histological analysis and the urease test detected *H. pylori* infection in 68 out of 342 (19.9%) patients.

In addition, examination of the histology of 286 biopsies found that 154 (53.8%) patients had chronic gastritis, four (1.4%) had esophagitis and 128 (44.8%) patients had normal mucosa without gastric alterations. Forty-seven of 154 patients with chronic gastritis were *H. pylori* positive. On the other hand, only two of 128 patients without gastric alterations were *H. pylori* positive. This data may suggest a possible relationship between the presence of *H. pylori* and chronic gastritis in children and young patients.

No statistically significant difference was observed between gender and positivity for *H. pylori* ( $p < 0.258$ ). However, when age was compared with positivity for *H. pylori* an important association ( $p < 0.026$ ) was observed;

**Table 1.** Detection of *Helicobacter pylori* by PCR and its possible association with the age of patients

PCR	Age (years)		Total
	≤ 10	> 10	
<i>H. pylori</i> negative	154 (79.8%)	103 (69.2%)	257(75.2%)
<i>H. pylori</i> positive	39 (20.2%)	46 (30.8%)	85 (24.8%)
<b>Total</b>	*193 (100%)	149 (100%)	342 (100%)

\*  $p < 0.026$

**Table 2.** Genotype distribution of *Tp53* polymorphisms and *H. pylori* detection in childhood

PCR	Genotype			Total
	CC	CG	GG	
<i>H. pylori</i> negative	120 (75.5%)	115 (77.2 %)	22 (64.7 %)	257(75.2 %)
<i>H. pylori</i> positive	39 (24.5%)	34 (22.8%)	12(35.3%)	85 (24.8%)
<b>Total</b>	159 (100%)	149 (100%)	34 (100%)	342 (100%)

the results showed that patients aged ≤ 10 years were 1.3 times more likely to have PCR positive when compared with patients aged > 10 years (Table 1).

We did not find any relation between *Tp53* (rs1042522) polymorphisms and the presence of *H. pylori*. Genotype frequencies are presented in Table 2.

## DISCUSSION

There are few studies involving pediatric patients in the literature investigating the detection of *H. pylori* in gastric biopsies and its association with age, route of transmission and *Tp53* polymorphisms. The present study suggests a possible relationship between the presence of

*H. pylori* and chronic gastritis in childhood. The analyses verified that 47 of 154 patients with chronic gastritis were *H. pylori* positive and only two of 128 patients without gastric alterations were *H. pylori* positive. These results are similar to those found by Gold (19), and Sherman *et al.* (20), thus suggesting an association between the presence of *H. pylori* and antral gastritis, and duodenal ulcers throughout childhood.

Pacifico *et al.* (17) in his review paper identifies, through a Medline search, studies published in English over the past two decades finding strong evidence of an association between *H. pylori* infection and antral gastritis and duodenal ulcer in children. The present study also shows a new and important relationship demonstrating that patients aged < 10 years were 1.3 times more likely to test positive for *H. pylori* when compared with patients aged > 10 years (Table 1). Goodman and Correa (21) suggest that early childhood is a high risk period for infection with *H. pylori* and the prevalence appears to increase rapidly until the early teen years. The forms of acquisition and transmission of *H. pylori* in children remains unclear. Fecal-oral, oral-oral, and gastro-oral routes have all been implicated in the transmission of the bacteria. Intra-familial clustering has been described in some studies (21-23)

Rothenbacher *et al.* (24), have suggested that mothers play a pivotal role in the transmission of *H. pylori* within a household. Nahar *et al.* (25) reported a strong relationship between *H. pylori* isolates from mothers and the young children, which suggests that transmission from mother to child occurs predominantly at a young age. Close and intimate contact between mother and, in particular, young children thus appears to be a key route responsible for the transmission of *H. pylori*. Possible behavioral risk factors for the transmission of *H. pylori* from mother to child may be the common use of spoons, sharing the teat of feeding bottles, and chewing or tasting of the child's food (25).

Sinha *et al.* (26) found *H. pylori* in maternal saliva and in the water in which soothers (pacifiers) were cleaned, which suggests possible routes of mother-to-child oral transmission. Kivi *et al.* (27) also state that the presence of infected family members is an important risk factor for children. These data suggest that a prophylactic treatment should be considered to all family members

since the chronic colonization by *H. pylori* can influence the development of gastric tumors and diseases.

The role of *Tp53* in innate immunity and the inflammatory response is now well established. Whereas the impact of the codon 72 polymorphism on cancer risk appears to be minor, there are compelling examples in the literature of an effect of this polymorphism on diseases associated with inflammation (5). In this study, it was not found an association of *Tp53* (rs1042522) polymorphisms with the presence of *H. pylori*, similar results were found by Palli *et al.* (28), who verified no association between the presence of *Tp53* mutations and various risk factors, including *H. pylori* infection, smoking habits, social class, education, and family history of gastric cancer.

Recently Jing *et al.* (29) suggested that the p53 codon 72 polymorphism modulates susceptibility to oral cancer in Chinese Han patients. On the other hand, Salehi and Hadavi (30) suggested that the TP53 codon 72 polymorphism do not confer susceptibility to prostate cancer in the Iranian population. The literature is controversial about association of presence of *Tp53* mutations, risk factors and type of cancer. It is worth mentioning that the infection rates and *Tp53* mutations can vary dramatically depending on the geographic region and race which may directly influence results.

In conclusion, no association was found between *Tp53* polymorphisms and the presence of *H. pylori*. Moreover, no statistically significant difference was observed between gender and positivity for *H. pylori*. However, when comparing age with positive results for *H. pylori*, a significant association was observed and results showed that patients aged  $\leq 10$  years were 1.3 times more likely to have positive PCR when compared with patients aged > 10 years.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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## ETHICS COMMITTEE APPROVAL

The present study was approved by the Ethics Committee of Sacred Heart University, Bauru, SP, Brazil, under the following numbers: 056/2005 and 223/2011.

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