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RESEARCH ARTICLE

Molecular detection of *Helicobacter pylori* in gastric biopsies and dental plaques of dyspeptic children

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Abstract

Background: Oral cavity is a significant possible reservoir of *H. pylori* as well as the gastric mucosa, and it contributes to the occurrence of diseases in both oral cavity and stomach.
Objective: The aim of this study was to evaluate the occurrence of *H. pylori* in dental plaque and gastric biopsy samples in dyspeptic children.
Subject & Methods: Dental plaques and gastric biopsies were collected from 55 children (15 girls and 40 boys; age range 2-14 years) who underwent upper gastrointestinal endoscopy in Gastroenterology Unit, Mansoura University Children Hospital (MUCH). *H. pylori* was detected in gastric biopsies by histopathological stains. DNA was extracted from frozen gastric biopsy and dental plaque specimens and polymerase chain reaction (PCR) was done for detection of *H. pylori* in both gastric biopsies and dental plaques.
Results: *H. pylori* was detected by histopathology in 30/55 (54.5%) children. *H. pylori* was detected by PCR in 25/55 (45.5%) and 18/55 (32.7%) of gastric biopsies and dental plaques, respectively. The presence of *H. pylori* in the dental plaque of children was significantly higher in children below 6 years and was more frequent in those who have dental caries.

Conclusions: Dental plaque may be a significant reservoir of *H. pylori*. Oral hygiene and removal of dental plaque must be performed along with antibiotic treatment of *H. pylori* infection.

INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative, spiral-shaped, microaerophilic bacterium (Marshal et al., 1984; Matysiak-Budnik and Megraud, 1997) that affects approximately half of the world's population (Fuccio et al., 2008).

Helicobacter pylori causes a life-long infection in humans, which is often asymptomatic but may result in chronic gastritis, peptic ulcers, gastric cancer, and mucosa associated lymphoid tissue (MALT) lymphoma (Parsonnet et al., 1994; Ernst and Gold, 2000; Pajares and Gisbert, 2006**).** It may be associated with several extra-gastroduodenal pathologies characterized by activation of inflammatory mediators and/or induction of autoimmunity (Realdi et al., 1999; Gasbarrini et al., 1999; Roussos et al., 2003). Moreover, *H pylori* infection has been incriminated in other syndromes as recurrent abdominal pain and iron deficiency anemia, but this remains to be confirmed (Me´graud et al., 2005).

H. pylori infection is almost always acquired in early childhood and usually persists throughout life unless a specific treatment is applied (Jones and Sherman, 1998). It was found in 90% of children with duodenal ulcers and in 25% of children with gastric ulcers (MacArthur et al., 1995; Huang et al., 1999).

Multiple routes of transmission of *H. pylori* infection have been postulated, including fecal-oral, gastro-oral, and oral–oral; however, the exact route of transmission of this bacterium is still unknown (Azevedo et al., 2009).

Various authors has reported the presence of *H. pylori* in the oral cavity as it provides an excellent microaerophilic environment and proposed that it could be the source for stomach infection and re-infection after an eradication therapy and could participate in the person to person transmission (Cave, 1997; Berroteran et al., 2002; Loster et al. 2006; Bürgers et al. 2008).

Dental plaque is a biofilm that is formed by a microbial community of multiple species and represents a strategy to allow survival and evolution of microbial co-aggregation in a dynamic equilibrium, in a favorable environment with selective advantages (Fux, 2005; Hall-Stoodley and Stoodley, 2005).

When the tooth's surface is cleaned, salivary proteins and glycoproteins are quickly deposited especially on the enamel surface resulting in formation of a thin, structureless membrane 0.5-1.0 mm in thickness termed the pellicle or acquired pellicle. Although the pellicle is bacteria free when formed, bacteria rapidly attach to its surface together with lymphocytes, leukocytes, desquamated epithelial cells and clumps of mucin. At first, only a few bacteria are present, but they rapidly grow into a thick plaque contains a variety of microorganisms with rods and filamentous organisms after a few days (Daniel et al., 2014).

There are conflicting results about the role of dental plaque as a source of infection for *H. pylori* with a detection rate ranging from 0% to > 90%. Most studies have failed to isolate *H. pylori* by culture from dental plaque of patients undergoing endoscopy (Bickley et al., 1993; Bernander et al., 1993; Khandaker et al., 1993; Banatvala et al., 1994). Studies employed culture methods may have underestimated the prevalence of *H. pylori* in the oral cavity. This may be explained by the presence of viable but non-culturable coccoid *H. pylori* organisms (Bod et al., 1993).

The current study was performed to determine the simultaneous presence of *H. pylori* in both dental plaque and gastric mucosa in children suffering from digestive pathologies.

MATERIALS AND METHODS

Patients and Sampling

This study was performed over a period of 6 months from May 2013 to October 2013 and included 55 children (15 girls and 40 boys) with age ranged from 2-14 years (median age 3 years). Children underwent upper gastrointestinal endoscopy in Gastroenterology Unit, Mansoura university Children Hospital due to gastric problems.

Inclusion criteria included unexplained vomiting, repeated abdominal pain, weight loss and signs of organic gastro-duodenal pathology. Exclusion criteria were anti-acid treatment, or antibiotics.

Written informed consent was obtained from every patient' parents and ethical approval was acquired from Local Ethical Committee prior to initiation of the study.

Dental Plaque Samples

Dental plaque samples were collected before endoscopy using sterile curettes. Plaque samples were scraped, put in sterile physiological saline and stored at -20˚C for molecular analysis.

Gastric Biopsies

During endoscopy, two antral gastric biopsy sections were taken from the stomach of each patient. One biopsy was analyzed by histological methods and the other section was stored at -20˚C for molecular analysis.

Histological Evaluation

Gastric biopsy samples were fixed in 10% formalin and sent to the Pathology Laboratory, where they were embedded in paraffin wax, cut at 5 µm thickness, and stained with Giemsa and hematoxylin and eosin. Samples were considered positive when curved or spiral form bacilli associated with the mucosa surface and within the mucus layer, with histological changes consistent with leukocyte infiltration were detected.

DNA Extraction

DNA was extracted from dental plaque and gastric samples using GeneJET™ Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Briefly, samples were disrupted into small pieces, vortexed, lysed using digestion solution and proteinase K and incubated at 56˚C until complete lysis. RNase A solution, lysis solution and 50% ethanol were added prior to samples loading into GeneJET™ Genomic DNA Purification columns. Then, samples were washed with wash buffers, eluted in elution buffer and stored at -20˚C for PCR.

PCR Analysis

PCR was targeted *16s rRNA* gene of *H. pylori* using a forward primer, 5′GTGTGGGAGAGGTAGGTGGA3′, and a reverse primer, 5′TGCGTTAGCTGCATTACTGG3′ (Thermo Scientific).

PCR master mix contained 2.5 mM $MgCl_2$, 2.5 µM of each dNTPs, one unit of Taq polymerase enzyme, 1X Taq Buffer (invitrogen), 10 pM of forward and reverse primers and 5 µl DNA sample. Thermal cycler Peltier-Effect cycling MJ Research was set to 35 amplification cycles of initial denaturation at 94˚C for 2 min, denaturation 94˚C for 45s, annealing at 53˚C for 45s, extension at 72˚C for 45s and final extension at 72˚C for 7 min. PCR products were visualized by electrophoresis in 1.8% agarose gel strained with ethidium bromide, and examined using ultraviolet light to detect a band of 225 bp (Chaudhry et al., 2011).

Each sample was performed in duplicates. Positive control contained DNA extracted from *H. pylori* strain and negative control using PCR grade water were included in each run. Master mix preparation, sample handling, PCR run, and gel electrophoresis were held in separate rooms to prevent cross-contaminations.

Statistical Analysis

The data were analyzed using SPSS software (Version 17.SPSS Inc, United States) and *P* values were estimated using Chi-square and *F* test to detect any significant relationship. $P < 0.05$ was considered statistically significant.

RESULTS

Among 55 patients, *H. pylori* was detected by histopathology in 30/55 (54.5%) of children. In PCR analysis, *H. pylori* was detected in 25/55 (45.5%) of gastric samples and in 18/55 (32.7%) of dental plaques was (Table 1).

The positivity of *H. pylori* in gastric and dental samples (Table 2) were statistically significant (P = 0.0001) for gastric biopsies and for dental plaques (P = 0.022).

Among all *H. pylori* positive PCR in dental plaque, there were 10 (55.6%) who had dental caries, compared with 9 (24%) of children without *H. pylori* infection. There was a significant difference in presence of *Helicobacter* in dental plaques (p = 0.012) in children below 6 years than those above 6 years especially in those with dental caries (p=0.034).

There was no significant difference in children who are positive PCR in dental plaque and PCR negative children regarding residence, sex or family history (Table 3).

Table (1): Results of different diagnostic tests for H. pylori in gastric biopsies and dental plaques.

	H. pylori Positive n (%)	*H. pylori* Negative, n (%)
Histopathology	30 (54.5%)	25 (45.5%)
PCR in gastric biopsy	25 (45.5%)	30 (54.5%)
PCR in dental plaque	18 (32.7%)	37 (67.3%)

Table (2): Results of PCR from dental plaque and gastric biopsy specimens.

	Positive *H. pylori* in gastric Histopathology (30)	Negative *H. pylori* in gastric Histopathology (25)	P value
PCR positive gastric biopsies	25	0	0.0001
PCR positive dental plaques	14	4	0.022

Table (3): Numbers of children with and without H. pylori in dental plaques according to, age, sex, family history, residence and presence of dental caries.

	PCR positive dental plaques (18)	PCR negative dental plaques (37)	P value
Age < 6 years	17	23	0.012
Sex (male)	11	29	0.208
Dental caries	10	9	0.034
Residence (rural)	18	32	0.160
Family history	1	9	0.140

DISCUSSION

H. pylori is a Gram negative bacterium, influences nearly half of the world's population and is associated with many gastric disorders such as peptic ulcer diseases and gastric cancers. Additionally it is implicated in pathogenesis of liver diseases in hepatitis C infected patients leading to bad prognosis ***(Marshal et al., 1984; El-Masry et al., 2010; Momtaz et al., 2012).***

Prevalence and pattern of *H. pylori* infection differ markedly between developed and developing countries. In developing countries, there is a high prevalence (70%-90%) of infection that starts early and frequently during childhood and persists nearly all life if untreated, in contrary to the developed countries where the infection begins later at adulthood with low frequency due to socioeconomic, industrialization and hygienic levels ***(Bardhan, 1997; Amer et al., 2013)***.

The current study revealed that *H. pylori* was diagnosed in 54.5% of dyspeptic children with histopathology in agreement with Fayed *et al.* who detected *H. pylori* infection in 55% of Egyptian pediatrics aged 6 months-14 years with gastrointestinal troubles ***(Fayed et al., 2010)***. Similarly, Falsafi and his colleagues reported *H. pylori* from 57% of children suffering from persistent gastrointestinal symptoms in Iran using biopsy-based tests ***(Falsafi et al., 2009)***.

Variable percentages of *H. pylori* infection have been postulated in literatures. In Egypt, 91% of patients with gastric diseases had *H. pylori* infection; 100% were positive by PCR targeting *ureA* gene, 83% were positive by gastric histopathology and 79% were positive by the rapid urease test ***(Amer et al., 2013)***.

On the other hand, 36.5% of dyspeptic patients had *H. pylori* isolated by culture. In that study, gastric histopathology and rapid urease test detected lesser number of *H. pylori*, while *ureC* PCR was sensitive as the culture ***(Lage et al., 1995)***. The differences in *H. pylori* detection level in literatures may be due to different used diagnostic tools, the chosen diagnostic gold standard and type of population ***(Bardhan, 1997)***.

In our study, gastric histopathology identified *H. pylori* in 54.5% of cases; 83.3% of them (45.5% of all cases) were positive by PCR targeting *16s rRNA* gene. Additionally, PCR detected 46.7% and 16% of *H. pylori* in dental plaques of positive and negative cases of gastric histopathology, respectively.

A similar pattern was observed in a comparative study for diagnostic markers of *H. pylori* where *H. pylori* was diagnosed from 33.3% of dyspeptic adults; 76% were positive by histopathology, 50% were PCR positive and 28.5% were positive by culture. The authors concluded that PCR was the most sensitive diagnostic tool for *H. pylori* but its specificity was 75%, whereas culture was the most specific tool with sensitivity 85.4% ***(Ramis et al., 2012)***.

Variability in positivity of diagnostic tools of *H. pylori* has been documented in many contexts. PCR diagnosed 59% of *H. pylori* infection in adults Brazilian dyspeptic patients, while histopathology detected only 27% of infections ***(Rasmussen et al., 2010)***. Similarly PCR was superior to histopathology in diagnosis of *H. pylori* infection associated with gastric problems in adults ***(Assumpcao et al., 2010)***.

Molecular methods are rapid and highly accurate tools for *H. pylori* diagnosis and PCR can be used in treatment follow up ***(Lage et al., 1995; Khalifehgholi et al., 2013)*** but it is affected by type of used primers, the load of organism and presence of inhibitors ***(Falsafi et al., 2009)***. Histopathology is a good gold standard for accurate *H. pylori* diagnosis but can be biased subjectively by the pathologist ***(Aktepe et al., 2011; Khalifehgholi et al., 2013)***. Moreover, any diagnostic test is affected by the biopsies' collection sites due to irregular distribution of *H. pylori* in gastric mucosa ***(Ramis et al., 2012)***.

Consequently, each *H. pylori* diagnostic tool has its pros and cons and choice of any of them depends on patient's age, availability, accessibility, merits and demerits of the tool and the need to endoscopy ***(Ramis et al., 2012; Garza-Gonzalez et al., 2014)***.

No consensus is present about characters of each diagnostic tool and none of them is a gold standard alone for diagnosis. Due to the absence of a common gold standard for diagnosis and limitation of diagnostic tools, the positivity of 2 or more of the invasive biopsy based tools, as it is preferable over the non invasive tools, is the current gold standard for diagnosis despite this may lead to some errors ***(Aktepe et al., 2011; Ramis et al., 2012; Khalifehgholi et al., 2013)***.

Role of oral cavity as a source for gastric *H. pylori* infection is a matter of debate. Detection of *H. pylori* in dental plaque varied greatly in publications. Using PCR, Eskandari and his colleagues diagnosed *H. pylori* in 34% and 6% of gastric and dental plaque samples, respectively from chronic periodontitis patients. All patients (6%) with dental *H. pylori* had gastric *H. pylori* infection ***(Eskandari et al., 2010)***. 88.4% of patients with digestive pathologies had *H. pylori* detected by gastric histopathology and 35% of them had *H. pylori* in oral samples (dental plaque and saliva) identified by PCR. Additionally, *H. pylori* was detected by PCR in only 5.5% of oral samples with negative gastric *H. pylori* infection ***(Medina et al., 2010)***.

Similarly, *H. pylori* was detected in 56.5% of dyspeptic patients with gastric histopathology; 93% of them was positive also by PCR targeting *16s rRNA* gene. 42% of those patients had oral *H. pylori* reported by PCR and there was no oral colonization with *H. pylori* in gastric *H. pylori* negative patients ***(Mapstone et al., 1993)***. Additionally, simultaneous presence of *H. pylori* was diagnosed in oral cavity (saliva and/or dental plaque) and gastric biopsies of 71.2% adult patients with gastric problems. 28.8% of those patients had gastric *H. pylori* infection with no oral colonization, while 50% of patients without gastric *H. pylori* infection had oral colonization with it ***(Rasmussen et al., 2010)***.

The previous data together with our study revealed obviously that, oral *H. pylori* is higher in patients suffering from gastric pathologies than normal individuals. The high association between *H. pylori* in oral cavity (dental plaque and saliva) and stomach reflects that oral cavity may be a major reservoir for gastric re-infection via reflux from stomach leading to oral colonization and gastric re-infection after eradicative treatment, however the reverse can occur. So, to eradicate *H. pylori* infection, antibiotic treatment must be aided with plaque removal and oral hygiene. Additionally, oral screening for *H. pylori* can help in diagnosis of gastric infection ***(Mapstone et al., 1993; Eskandari et al., 2010; Medina et al., 2010; Rasmussen et al., 2010)***.

Moreover, detection of *H. pylori* by quantitative PCR in 35% of healthy children less than 5 years augmented the possible role of dental plaque as a second reservoir for *H. pylori* infection and a route of transmission ***(Valdez-Gonzalez et al., 2014)***.

Furthermore, *H. pylori* was identified in saliva from 11% of patients all of them with gastric *H. pylori* infection with no *H. pylori* in dental plaques. High homology was detected between *H. pylori* from saliva and gastric samples reflecting the possible role of saliva as a reservoir for gastric infection. The authors attributed the low oral *H. pylori* colonization to good oral hygiene, very low number of bacteria to be detected and role of reflux as a mechanism for oral colonization ***(Momtaz et al., 2012)***.

In contrary, Hardo *et al*. concluded that dental plaque had no role as a reservoir for infection or a method of transmission of gastric *H. pylori* infection as no *H. pylori* was detected in dental plaque of patients with gastric *H. pylori* infection ***(Hardo et al., 1995)***. Additionally, dental plaque colonization by *H. pylori* was very low as quantified by competitive PCR and was independent on gastric colonization, so *H. pylori* may be a part of normal flora but is not necessary leads to gastric infection ***(Song et al., 2000)***.

Contradictive data about the relationship between oral and gastric *H. pylori* infections could be due to different studied populations, different sampling, samples' contamination, variable diagnostic techniques and oral contamination from gastro-oesophageal reflux at endoscopy time ***(Hardo et al., 1995; Valdez-Gonzalez et al., 2014)***.

In our study there was a high association between dental *H. pylori* colonization and dental caries in children in accordance with Liu *et al*. where there was a close relationship between oral (saliva and dental plaque) *H. pylori* and dental caries in children aged 3-6 years ***(Liu et al., 2008)***. Similarly, oral *H. pylori* was higher in patients with oral pathologies delaying the eradication of *H. pylori* and facilitating the gastric re-infection ***(Medina et al., 2010)***. Consequently, oral *H. pylori* may be considered a risk factor for oral diseases as dental caries and gastric *H. pylori* infection. So oral hygiene, dental caries treatment and eradication of *H. pylori* from oral cavity could lead to controllable dental caries, reduce gastric *H. pylori* re-infection and prevent *H. pylori* oral transmission ***(Liu et al., 2008; Assumpcao et al., 2010)***.

In this study, dental *H. pylori* infection was highly significant in children less than 6 years in agreement with Liu *et al*. ***(Liu et al., 2008)***. It has been postulated that *H. pylori* infection is acquired in early childhood may be from first few months and persists for life with no spontaneous clearance if untreated ***(Bardhan, 1997)***. Moreover, infection with virulent *H. pylori* was not uncommon in children (20%) and was positively correlated with those aged 6 months to 14 years, possibly due to their higher exposure to the pathogen ***(Fayed et al., 2010)***.

In conclusion, *H. pylori* is commonly associated with childhood and oral colonization with it can be considered a risk factor for gastric infection, re-infection and oral diseases. Standardization of *H. pylori* diagnostic markers will aid achieving a consensus about the role of oral *H. pylori*; is it a result or promoter for gastric infection? Finally, good oral hygiene plus eradicative antibiotic treatment will reduce the risk of *H. pylori* infection and oral screening for the organism can prevent its transmission and decrease its prevalence, morbidity and mortality. More research is required on a large scale of patients together with a standard diagnostic gold marker will clarify the active role of oral *H. pylori*.

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