academicJournals

Vol. 7(21), pp. 2505-2509, 21 May, 2013
DOI: 10.5897/AJMR12.1099
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Prevalence of *Helicobacer pylori* in saliva and dental plaque related to periodontal disease and gastritis

Myriam A. De La Garza-Ramos[1,2], Jesús A. Valdez-Gonzalez[1,2], Rogelio A. Elizondo-Pereo[3], Benito Pereyra-Alférez[4], Raúl G. Caffesse[5], Francisco González-Salazar[3,6]

[1]Facultad de Odontología, Universiad Autónoma De Nuevo León, Monterrey, México.
[2]Centro de Investigaciones y Desarrollo en Ciencias de la Salud, Universidad Autónoma de Nuevo Leon, Monterrey, México.
[3]División de Ciencias de la Salud, Universidad de Monterrey, Monterrey, México.
[4]Departamento de Biología Celular, Facultad de Biología Universidad Autónoma de Nuevo León, Monterrey, México.
[5]University of Texas Health Science Center at Houston, Houston, USA.
[6]División de Biología Celular y Molecular, Centro de Investigaciones Biomédicas del Noreste, Instituto Mexicano del Seguro Social. Monterrey, México.

Accepted 10 May, 2013

***Helicobacter pylori* is a major etiologic factor in the development of gastritis and peptic ulcer disease. Controversially, the oral cavity has been proposed as a reservoir for this bacterium. The aim of the present study was to determine the prevalence of *H. pylori* in saliva and dental plaque of adult patients affected by periodontal disease, with or without gastrointestinal symptoms, using polymerase chain reaction (PCR). Suitable PCR primers were designed using sequences of *H. pylori* DNA from the GenBank database. Chronic periodontal disease was clinically and radiologically diagnosed. The samples for PCR were obtained from periodontal pockets. Cell lysate was centrifuged and used as a DNA source for the PCR assays. The PCR products were analyzed in agarose gels and the sizes of the amplicons were estimated by comparison with DNA size markers. Two samples from each patient were obtained, one from dental plaque and the other from saliva. All salivary samples were negative to PCR isolation of *H. pylori*, while 13.3% of the plaque samples were positive. All patients with positive PCR isolation of *H. pylori* had been showing gastrointestinal symptoms. PCR isolation of *H. pylori* was directly related to the depth of the periodontal pocket.**

Key words: Periodontitis, gastritis, polymerase chain reaction, *Helicobacter pylori*.

INTRODUCTION

Helicobacter pylori is now recognized as a major etiologic factor in the development of chronic superficial gastritis and peptic ulcer disease in adults and children (NIH Consensus Conference, 1994). In 1994, *H. pylori* was classified as a group 1 carcinogen by the International Agency for Research on Cancer because of its association with gastric cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1994). *H. pylori* acquisition occurs predominantly during early childhood, and its incidence and prevalence is higher in developing countries (Thomas et al., 1999; Mitchell and Megraud, 2002; Malaty et al., 2002). Several risk factors have been associated with acquisition and transmission of *H. pylori* infection and they are mainly correlated with poor sanitary conditions and low socioeconomic status (Malaty et al., 2002; Chong et al., 2003; Malaty et al.,

*Corresponding author. Email: fgonz75@hotmail.com.

1996a).

In some studies, the prevalence rate of *H. pylori* infection was 49.7% in healthy children and it was 88% in adults (Muhsen et al., 2006; Malaty et al., 1996b). The oral cavity has been proposed as a reservoir for *H. pylori* (Watts, 2002), however, some authors have failed to isolate this bacterium in the oral cavity (De Sousa et al., 2006; Olivier et al., 2006). Also, researchers have isolated *H. pylori* in samples from the oral cavity without any gastrointestinal disease association (Chitsazi et al., 2006). This has created controversy about the reservoir role of the oral cavity in the progression of gastrointestinal disease.

Polymerase chain reaction (PCR) has been used to isolate *H. pylori* in previous studies (Olivier et al., 2006; Chitsazi et al., 2006; Umeda et al., 2003). The prevalence of *H. pylori* isolation using PCR was 42 and 35.1% in patients with and without periodontal disease, respectively (Umeda et al., 2003). The aim of this study was to determine the prevalence of *H. pylori* in the saliva and dental plaque of adult patients with periodontal disease, and determine its association with gastrointestinal symptoms of gastritis.

MATERIAL AND METHODS

Subjects, sampling, and bacterial isolates

Subject population

A total of 60 clinical samples were obtained from 30 untreated patients. Patients were over 35 years old and were recruited from the Clinic of Periodontics, Faculty of Dentistry at the Autonomic University from Nuevo Leon, Mexico. Salivary and dental plaque samples were obtained and analyzed from each patient. Informed consent was obtained from each individual.

Clinical measurements and sample collection. Periodontal disease was determined by clinical and radiographic examination. We measured the level of attachment loss and probed the pocket depth in all teeth. If the pocket depth was more than 4 mm with a conventional North Caroline periodontal probe (Hu-Friedy Chicago, IL, USA) in a least six sites, the patient was considered to have periodontal disease.

Patients that had used systemic antibiotics in the previous six months or had any systemic disease were excluded. Information about gastrointestinal symptoms was obtained from patients by direct interview. All oral specimens were inoculated onto brain heart infusion agar plates (Merck, Darmstadt, Germany) supplemented with 5% sheep blood and cultured in anaerobic conditions using Gaspack ® (Becton Dickinson Franklin Lakes, NJ, USA). All patients with symptoms of gastritis underwent upper endoscopy to confirm the diagnosis and underwent a rapid urease test for the presence of *H. pylori* in the gastric chamber. The presumptive positive culture isolates of *H. pylori* were identified in vitro by urease and catalase activities. The control strain was *H. pylori* ATCC 43504, 43649, which was donated by Dr. Stanley Holt.

DNA extraction

Suspensions of the bacterial strain were prepared in 100 µl of sterile double-distilled water and they were obtained by the inoculation of a single bacterial colony from the infusion of agar plates with a standard loop. A loop full of cells was transferred to water and the mixture was boiled for 10 min at 94°C to lyse the cells. The resulting cell lysate was centrifuged briefly (30 s at 10,000 rpm; Eppendorf model 5415C centrifuge (Eppendorf, Hamburg, Germany) and 19 µl of the supernatant was used as the DNA sample for the PCR mixture.

Oligonucleotide PCR primer design

Suitable primers were designed using 16s rRNA gene sequences of *H. pylori* available in the GenBank database. Multiple comparisons were made with related organisms using the FASTA and Clustall Wallis programs. Oligonucleotides were synthesized using a synthesizer of DNA (Microsyn 1450A; Systec Inc.) with the reagents and conditions specified by the manufacturer. We designed two primers; the first was named LH-F (5´-TAGATTATGTGCCTCTTAGTT-3´; position 123 to 146, GenBank accession AE000511:0) and the next was named LH-R (5´-AGGAGGTGATCCAACCGC-3´; position 2431 to 2446 GenBank accession AE000511:0). A Blast search was performed to check the specificity of the primer sequences. To test the sensitivity, we used dilutions of the DNA extracted from ATCC43504, 43629 *H. pylori* strains. The specificity of the primers and PCR conditions were evaluated by testing ATCC43504, 43629 *H. pylori* strains as positive controls, as well as related bucal flora *Porphyromonas gingivalis* ATCC 33277T, *Streptococcus intermedius* ATCC 27335T, *Prevotella intermedia* ATCC 25611T, and more distantly *Escherichia coli*, *Proteus vulgaris*, *Bacillus thuringiensis* and *Bacillus subtilis* as negative bacteria.

PCR amplification

The template DNA was added to 50 µl of a reaction mixture containing 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 1.5% [vol/vol] Triton X-100), 1.5 mM MgCl2, 200 µM concentrations of each deoxynucleoside triphosphate and 50 pmol of each primer. Amplification was performed in a Perkin-Elmer Thermocycler 2400 (Perkin-Elmer, Applied Biosystems, and Foster City, Calif.) As the first step of a denaturing process begins with 5 min exposure to 95°C, 0.3 □l of (5 U/□l) of Taq DNA polymerase Epicentre, from a Master Amp Taq PCR core kit, (Epicentre® Biotechnologies, Madison, Wisconsin, USA), were subsequently added. Then, 30 cycles of the next cycle program were performed: denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 70°C for 1 min, and a final extension step at 70°C for 10 min. The PCR products were analyzed with 1.5% (wt/vol) agarose gels in Tris acetic acid-EDTA pH8 buffer (TAE) and the sizes of the amplicons were estimated by comparison with 100-bp DNA size markers (Promega Corporation, Madison, Wisconsin, USA).

RESULTS

We analyzed the samples from 30 patients (17 males [56.7%] and 13 females [43.3%], mean age 50.1+ 11.03 years [Table 1]). Sixty samples were obtained; that is, two from each patient. One sample was dental plaque and the other was a saliva sample. None of the 30 Salivary samples were positive for *H. pylori* in the PCR analysis, while 4 of the dental plaque samples (13.3%) were positive. All patients with positive PCR isolation of *H. pylori* in the dental plaque had gastritis by endoscopy

Table 1. Patient symptoms and *H. pylori* isolation by PCR.

Patient	Gender	Age	Gastritis	PCR	Urease	PD*
ADE	M	37	+	-	-	
FGA	F	45	+	-	-	
PSD	M	49	+	+	+	+
LAF	M	72	+	-	-	
EGR	F	38	+	-	-	+
RBG	M	61	+	+	+	+
ATZ	M	44	+	+	-	
DMS	M	52	+	-	-	
BRT	F	58	+	-	-	
DEM	M	45	+	+	+	+
SRB	F	49	+	-	-	
MMA	F	37	+	-	-	
JGG	M	56	+	-	-	
MGR	F	63	-	-	-	+
GPR	F	60	-	-	-	
JVV	M	75	-	-	-	
BMC	M	36	-	-	-	
RPC	F	41	-	-	-	
GMT	F	46	-	-	-	+
HHT	M	67	-	-	-	
CGT	M	54	-	-	-	
FGS	M	49	-	-	-	+
GRS	F	37	-	-	-	
JSA	M	40	-	-	-	
RVT	F	56	-	-	-	
SGF	M	42	-	-	-	
MGD	M	43	-	-	-	
RCE	F	38	-	-	-	+
MMT	F	64	-	-	-	
BCC	M	49	-	-	-	

*PD, Periodontal disease.

and 75% had a urease rapid test in the gastric biopsy (Table 1). Of all the analyzed patients, 66.7% were asymptomatic and 33.3% showed gastrointestinal symptoms confirmed by endoscopy as gastritis.

Both groups that is, asymptomatic and symptomatic patients, were compared. None of the 17 asymptomatic patients (without gastritis) were positive for the presence of *H. pylori* in the PCR dental plaque test, while 4/13 (30.7%) of the symptomatic gastritis patients showed PCR positivity (Table 1). A Fischer exact test showed an association between the PCR positivity for *H. pylori* and symptomatic patients ($p = 0.02$). Another important finding was related to the depth of the pockets. 4.7% of the samples from patients with 4-5 mm pockets were positive for *H. pylori* compared to 33.3% of the samples from patients with 6-7 mm pockets ($p < 0.02$; Table 2). When determining the association of the isolates by PCR according to age and gender, no significant differences were found using a Fisher's exact test. Subsequently, by grouping patients with and without gastritis, the proportion of males was 61.5 and 52.9%, respectively. In the group of patients with gastritis, all patients with isolates were male 4/8 (50%), but a Fisher's exact test showed this was not significant ($p=0.07$). The mean age of patients with gastritis was 49.46 + 10.3 y and the mean age for asymptomatic patients was 50.58 + 11.5 y ($p=0.51$). The mean age of patients with *H. pylori* isolation was 49.75 + 7.8 y and it was 50.15 + 11.5 y for the patients without *H. pylori* ($p=0.41$).

DISCUSSION

As reported in previous studies (De Flora and Bonanni, 2011; Dai et al., 2011), *H. pylori* is related to gastrointestinal disease that is, mainly to gastritis and gastric cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1994). We performed this study to associate *H. pylori* in dental plaque with gastritis because

Table 2. Deep pocket depth and *H. pylori* isolation by PCR.

Patient number	Deep pocket		PCR isolation
	4-5 mm	6-7 mm	
1	+		
2	+		
3		+	+
4	+		
5		+	
6		+	+
7	+		+
8	+		
9		+	
10		+	+
11	+		
12	+		
13	+		
14		+	
15	+		
16	+		
17	+		
18	+		
19		+	
20	+		
21	+		
22		+	
23	+		
24	+		
25	+		
26	+		
27	+		
28		+	
29	+		
30	+		

some researchers have suggested that plaque may act as a reservoir for the bacteria (Watts, 2002). Also, there is controversy over the possibility that infected people could develop gastric infection and gastrointestinal symptoms (Navabi et al., 2011). We found a lower percentage (30%) of *H. pylori* in symptomatic gastritis patients than reported in previous studies using PCR isolation (Umeda et al., 2003). This may be due to PCR technique variations. Umeda et al. (2003) performd nested PCR, while we used standard PCR. We found important differences compared to previous studies because none of the Salivary samples were positive for *H. pylori* (Sugimoto et.al., 2009; De Sousa et al., 2006; Olivier et al., 2006; Chitsazi et al., 2006; Umeda et al., 2003; Tiwari et al., 2005). This may be due to a lower concentration of the bacteria in salivary samples. Some authors have suggested that other oral bacteria may cause false positive results. We feel that our PCR technique should be improved in order to detect lower counts of bacteria in salivary samples.

No asymptomatic patient showed the presence of *H. pylori* in plaque or salivary samples, which is in contrast with the findings of previous studies (Fernández-Tilapa et.al., 2011). We think that these people are really free of *H. pylori*, however, another possibility is that the prevalence of *H. pylori* in this population is low. By increasing the sample size, we might be able to find individuals with positive isolations. Finally, we consider the possibility that bacterial counts were actually low and, thus, the PCR technique should be improved to detect low counts of bacteria in plaque samples.

When gender associations were analyzed, we found that males were the major proportion of cases with *H. pylori* isolation compared to females. In other studies, the proportion of males was the same as females for isolation in the oral cavity and females were the majority showing isolation in the gastric cavity (Berroteran et al., 2002). A strong association between *H. pylori* isolation and the presence of gastric symptoms was demonstrated in our

study. We found that more than 30% of the patients with gastritis have *H. pylori* in their dental plaque. *H. pylori* isolation was dependent on the depth of the pocket and this was dependent on the severity of the periodontal disease. The greater the depth of the pocket, the greater the likelihood of *H. pylori* isolation by PCR. We demonstrated that 75% of our patients with *H. pylori* positive isolation had periodontal disease, while only 3.3% of the patients without periodontitis showed *H. pylori* isolation by PCR. Patients without gastrointestinal symptoms did not show a positive PCR result for isolation of *H. pylori*. We conclude that *H. pylori* isolation by PCR in patients with periodontal disease and gastritis was demonstrated. In our study, we were not able to detect *H. pylori* in Salivary samples by PCR.

REFERENCES

Berroteran A, Perrone M, Correnti M, Cavazza ME, Tombazzi C, Goncalvez R, Lecuna V (2002). Detection of Helicobacter pylori DNA in the oral cavity and gastroduodenal system of a Venezuelan population. J. Med. Microbiol. 51(9): 764-70.

Chitsazi, Fattahi E, Farahani RM, Fattahi S (2006). Helicobacter pylori in the dental plaque: is it of diagnostic value for gastric infection? Med. Oral Patol. Oral Cir. Bucal. 11(4): 325-328.

Chong SK, Lou Q, Zollinger TW, Rabinowitz S, Jibaly R, Tolia V, Elitsur Y, Gold BD, Rosenberg A, Johnson A, Elkayam O, Rosenthal P, Gilger M, Li BU, Peacock JS (2003). The seroprevalence of *Helicobacter pylori* in a referral population of children in the United States. Am. J. Gastroenterol. 98(10): 2162- 2168.

Dai YC, Tang ZP, Zhang YL (2011). How to assess the severity of atrophic gastritis. World J. Gastroenterol. 17(13): 1690-1693.

De Flora S, Bonanni P (2011). The prevention of infection-associated cancers. Carcinogenesis 32(6): 787-95.

De Sousa L, Vasquez L, Velasco J, Parlapiano D (2006). Isolation of Helicobacter pylori in gastric mucosa, dental plaque and saliva in a population from the Venezuelan Andes. Invest Clin. 47(2):109-116.

Fernández-Tilapa G, Axinecuilteco-Hilera J, Giono-Cerezo S, Martínez-Carrillo DN, Illades-Aguiar B, Román-Román A (2011) vacA genotypes in oral cavity and Helicobacter pylori seropositivity among adults without dyspepsia. Med. Oral Patol. Oral Cir. Bucal. 16(2):175-80.

IARC (1994). Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 61:1-241.

Malaty HM, El-Kasabany A, Graham DY, Miller CC, Reddy SG, Srinivassan SR, Yamaoka Y, Berenson GS (2002). Age at acquisition of *Helicobacter pylori* infection: a follow-up study from infancy to adulthood. Lancet 359(9310): 931-935.

Malaty HM, Kim JG, Kim SD, Graham DY (1996b). Prevalence of Helicobacter pylori infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults. Am J Epidemiol. 143(3): 257-262.

Malaty HM, Paykov V, Bykova O, Ross A, Graham DP, Anneger, JF, Graham DY (1996a). Helicobacter pylori and socioeconomic factors in Russia. Helicobacter. 1(2): 82-87.

Mitchell H, Megraud F (2002). Epidemiology and diagnosis of Helicobacter pylori infection. Helicobacter. 323(910): 8-16.

Muhsen Kh, Athamna A, Athamna M, Spungin-Bialik A, Cohen D (2006). Prevalence and risk factors of Helicobacter pylori infection among healthy 3- to 5-year-old Israeli Arab children. Epidemiol. Infect. 134(5): 990-996.

Navabi N, Aramon M, Mirzazadeh A (2011). Does the presence of the Helicobacter pylori in the dental plaque associate with its gastric infection? A meta-analysis and systematic review. Dent. Res. J. (Isfahan)., 8(4):178-82.

NIH Consensus Conference (1994). Helicobacter pylori in peptic ulcer disease.. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. JAMA 272(1): 65-69.

Olivier BJ, Bond RP, van Zyl WB, Delport M, Slavik T, Terhaar Sive Droste JS, Lastovica A, van der Merwe SW (2006). Absence of Helicobacter pylori within the oral cavities of members of a healthy South African community. J. Clin. Microbiol. 44(2): 635-636.

Sugimoto M, Wu JY, Abudayyeh S, Hoffman J, Brahem H, Al-Khatib K, Yamaoka Y, Graham DY (2009). Unreliability of results of PCR detection of Helicobacter pylori in clinical or environmental samples. J. Clin. Microbiol. 47(3): 738-42.

Thomas JE, Dale A, Harding M, Coward WA, Cole TJ, Weaver LT (1999). Helicobacter pylori colonization in early life. Pediatr. Res. 45(2): 218-223

Tiwari SK, Khan AA, Ahmed KS (2005). Rapid diagnosis of Helicobacter pylori infection in dyspeptic patients using salivary secretion: a non-invasive approach. Singapore Med. J. 46(5): 224-228.

Umeda, Kobayashi H, Takeuchi Y, Hayashi J, Morotome-Hayashi Y, Yano K, Aoki A, Ohkusa T, Ishikawa I (2003). High prevalence of *Helicobacter pylori* detected by PCR in the oral cavities of periodontitis patients. J. Periodontol. 74(1): 129-134.

Watts T (2002). Management of Helicobacter pylori infection. Dental plaque is a potential reservoir of Helicobacter pylori. BMJ 324(7337): 614-615.