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BRIEF ARTICLE

Study of *Helicobacter pylori* genotype status in saliva, dental plaques, stool and gastric biopsy samples

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Abstract

AIM: To compare genotype of *Helicobacter pylori* (*H. pylori*) isolated from saliva, dental plaques, gastric biopsy, and stool of each patient in order to evaluate the mode of transmission of *H. pylori* infection.

METHODS: This cross-sectional descriptive study was performed on 300 antral gastric biopsy, saliva, dental plaque and stool samples which were obtained from patients undergoing upper gastrointestinal tract endoscopy referred to endoscopy centre of Hajar hospital of Shahrekord, Iran from March 2010 to February 2011. Initially, *H. pylori* strains were identified by rapid urease test (RUT) and polymerase chain reaction (PCR) were applied to determine the presence of *H. pylori* (*ureC*) and for genotyping of voculating cytotoxin gene A (*vacA*) and cytotoxin associated gene A (*cagA*) genes in each specimen. Finally the data were analyzed by using statistical formulas such as Chi-square and Fisher's exact tests to find any significant relationship between these genes and patient's diseases. $P < 0.05$ was considered statistically significant.

RESULTS: Of 300 gastric biopsy samples, 77.66% were confirmed to be *H. pylori* positive by PCR assay while this bacterium were detected in 10.72% of saliva, 71.67% of stool samples. We were not able to find it in dental plaque specimens. The prevalence of *H. pylori* was 90.47% among patients with peptic ulcer disease (PUD), 80% among patients with gastric cancer, and 74.13% among patients with none ulcer dyspepsia (NUD) by PCR assay. The evaluation of *vacA* and *cagA* genes showed 6 differences between gastric biopsy and saliva specimens and 11 differences between gastric and stool specimens. 94.42% of *H. pylori* positive specimens were *cagA* positive and all samples had amplified band both for *vacA s* and *m* regions. There was significant relationship between *vacA s1a/m1a* and PUD diseases ($P = 0.04$), *s2/m2* genotype and NUD diseases ($P = 0.05$). No statically significant relationship was found between *cagA* status with clinical outcomes and *vacA* genotypes ($P = 0.65$). The evaluation of *vacA* and *cagA* genes showed 6 differences between gastric biopsy and saliva specimens and 11 differences between gastric and stool specimens.

CONCLUSION: Regard to high similarity in genotype of *H. pylori* isolates from saliva, stomach and stool, this study support the idea which fecal- oral is the main route of *H. pylori* transmission and oral cavity may serve as a reservoir for *H. pylori*, however, remarkable genotype diversity among stomach, saliva and stool samples showed that more than one *H. pylori* genotype may exist in a same patient.

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Key words: *Helicobacter pylori*; Gastric biopsy; Saliva; Dental plaque; Stool

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is the organism responsible for diseases such as atrophic gastritis, chronic gastritis, duodenal ulcers, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer[1]. *H. pylori* is distributed worldwide and is found in developing countries in particular. For instance, more than 90% of Iranian individuals are infected with *H. pylori*[2]. Although there is much information about *H. pylori* infection, several aspects of the pathogenesis and epidemiology of this organism remains unclear[3]. The transmission route of *H. pylori* infection has been the topic of several studies. Most infections are probably acquired in childhood, mainly *via* oral-oral or fecal-oral routes[4], however, the exact mode(s) of transmission is still unknown.

H. pylori has been found in saliva, dental plaques and feces, which shows that oral and fecal cavities are probably involved in *H. pylori* transmission[5]. The role of *H. pylori* in the oral cavity remains controversial since the detection rate of the bacterium in the mouth is very diverse, ranging between 0% and 100%[6]. Different typing methods have been proposed for the study of correlations between *H. pylori* isolates from different anatomical sites for epidemiological purposes[7]. Genotyping using some well-known virulence marker genes, such as the cytotoxin associated gene A (*cagA*) and vocuolating cytotoxin gene A (*vacA*), are considered as one of the best approaches[8]. The *cagA* gene is located at the end of the *cag* pathogenicity island (PAI) and has been proposed as a marker for the *cag* PAI, and the presence of certain *cagA* alleles (e.g., *cagA1a* in East Asian strains) have been associated with severe clinical outcomes[9]. The *vacA* gene is present in virtually all strains of *H. pylori* but it is polymorphic, comprising variable signal regions (type *s1* or *s2*) and mid-regions (type *m1* or *m2*). Type *s1/m1 vacA* causes more epithelial cell damage than type *s1/m2*, whereas type *s2/m2* and the rare *s2/m1* are non-toxic due to the presence of a short 12-residue hydrophilic extension on the *s2* form[10,11]. The s-region is classified into *s1* and *s2* types and the m-region into *m*1 and *m*2 types. The *s*1 type is further subtyped into *s1a*, *s1b* and *s1c* subtypes, and the *m1* into *m1a* and *m1b* subtypes. The mosaic combination of sand m-region allelic types determines the particular cytotoxin and, consequently, the pathogenicity of the bacterium[12,13]. Recently, several studies have examined the presence of *H. pylori* in saliva, dental plaque, gastric biopsies and stool, but few studies have evaluated the relationship between genotypes *of H. pylori* isolated from these specimens in a single patient. Therefore, we aimed to compare *H. pylori cagA* and the *vacA* allelic status among strains isolated from saliva, dental plaque, gastric biopsies and stool samples in the same patient with dyspepsia manifestations in order to evaluate the mode of transmission of *H. pylori* infection.

MATERIALS AND METHODS

Patients and samples

Samples were obtained over a year (March 2010 to February 2011) from patients with gastroduodenal diseases that were referred to the endoscopy center of Hagar Hospital of Shahrekord, Iran.

Prior to sampling, the questionnaire, including medical history and demographic data, were recorded for each patient. All studied patients signed an informed consent form before endoscopy and declared their willingness to allow the application of their anonymous data for research purposes. Gastric biopsies, saliva, dental plaques and stool samples were collected from each patient. Saliva and dental plaque sampling was done in the morning before undergoing endoscopy. All patients were asked to wash their mouth with normal saline prior to saliva and dental plaque sampling. Saliva samples, in a volume of 2-3 mL, were collected using sterile toothpicks and filter paper. Dental floss was used to remove the dental plaque from the interdental spaces and both samples were transported in sterile flasks containing digestion buffer [100 mmol NaCl, 10 mmol Tris-HCl (pH 8.0), 250 mmol ethylenediaminetetraacetic acid (EDTA) (pH 8.0) and 1% sodium lauryl sarcosine] on the day of sampling and were stored at -70 ℃ until DNA extraction[6]. For each patient, two biopsy specimens from the antrum were taken using a disinfected endoscope. One was used for screening of *H. pylori* positive specimens by a rapid urease test (RUT). The second piece from RUT-positive patients was placed in 1 mL of sterile phosphate buffer saline solution. Stool was collected in a container with a screw cap and was transported immediately to the biotechnology research center of Islamic Azad University, Shahrekord Branch for molecular analysis.

Rapid urease test

One biopsy piece from each patient was inoculated immediately after collection into 1.5 mL to 2 mL of urea broth (Merck, Germany). It was incubated at 37 ℃ in the incubator for 1.5 h. The change in color of the broth from yellow to pink was taken as a positive test.

Genomic DNA extraction and polymerase chain reaction

DNA was extracted from biopsies and stool specimens using a Genomic DNA Purification kit (Fermentas, Ger-

Table 1 Primers used for polymerase chain reaction analysis of voculating cytotoxin gene A and cytotoxin associated gene A

Region	Primer	Sequence (5'-3')	Size and location of PCR product
s1a	*vacA s1a*-F	CTC TCG CTT TAG TAG GAG C	213 bp
	VA1-R	CTG CTT GAA TGC GCC AAA C	(843-1055)
s1b	SS3-F	AGC GCC ATA CCG CAA GAG	187 bp
	VA1-R	CTG CTT GAA TGC GCC AAA C	(869-1055)
s1c	*vacA s1c*-F	CTC TCG CTT TAG TGG GGY T	213 bp
	VA1-R	CTG CTT GAA TGC GCC AAA C	(843-1055)
s2	SS2-F	GCT AAC ACG CCA AAT GAT CC	199 bp
	VA1-R	CTG CTT GAA TGC GCC AAA C	(433-631)
m1a	VA3-F	GGT CAA AAT GCG GTC ATG G	290 bp
	VA3-R	CCA TTG GTA CCT GTA GAA AC	(2741-3030)
m1b	VAm-F3	GGC CCC AAT GCA GTC ATG GA	291 bp
	VAm-R3	GCT GTT AGT GCC TAA AGA AGC AT	(2741-3031)
m2	VA4-F	GGA GCC CCA GGA AAC ATT G	352 bp
	VA4-R	CAT AAC TAG CGC CTT GCA	(976-1327)
cagA	*cagA*-U	GGA ATA CCA AAA ACG CAA AAA CCA	300 bp
	cagA-L	CCC CAC AAT ACA CCA GCA AAA CT	
ureC (*glmM*)	GlmM1-R	GCTTACTTTCTAACACTAAC-GCGC	296 bp
	GlmM1-F	GGATAAGCTTTTAGGGGTGT-TAGGGG	

PCR: Polymerase chain reaction; *cagA*: Cytotoxin associated gene A; *vacA*: Voculating cytotoxin gene A.

many) according to the manufacturer's instructions. To prepare DNA from saliva and dental plaque, one volume of the digestion buffer and 100 g/mL proteinase K were added to the saliva samples and incubated at 55 ℃ for 3 h. DNA was extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with 3 mol sodium acetate and 0.7 mL volume of isopropanol. Rinsed and dried DNA pellets were dissolved in Tris-EDTA (TE) buffer (Tris 10 mmol, EDTA 1 mmol and pH 8.0)[8]. The concentration and quality of DNA preparations were determined spectrophotometrically by measuring absorbance at 260 nm and 280 nm by agarose gel electrophoresis. The DNA preparations were stored at -20 ℃. The presence of *ureC* and *cagA* and the genotypes of *vacA* alleles (*s1a*, *s1b*, *s1c*, *m1a*, *m1b* and *m2*) were determined by polymerase chain reaction (PCR). The primer sequences are shown in Table 1[8,11,14].

DNA samples from *H. pylori* (D0008, Genekam, Germany) were used as positive controls for *ureC, cagA* and *vacA* genes, and sterile distilled water was used as a negative control. All PCR mixtures were prepared in a volume of 25 μL containing 1X PCR buffer, 0.4 μmol of each primer, 0.3 U Taq DNA polymerase and 2 μL DNA sample[5]. The mixture was placed in a thermocycler (Eppendrof Mastercycler 5330; Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany), and PCR products were visualized by electrophoresis in a 1.5% agarose gel, strained with ethidium bromide, and examined under ultraviolet illumination.

Statistical analysis

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

RESULTS

The study population consisted of 300 patients; 143 men and 157 women with mean age 46 ± 17 years. The patients were classified at the time of endoscopy and histopathology as having peptic ulcer disease (PUD: $n = 63$), gastric cancer (GC: $n = 5$) and none ulcer dyspepsia (NUD: $n = 232$) regardless of *H. pylori* status. Based on RUTs, 271 (90.33%) patients were positive for *H. pylori* while 233 (77.66%) patients had positive PCR results by using specific primers (*ureC*) looking for *H. pylori* DNA in their gastric specimens. *H. pylori* was detected in 25 (10.72%) of saliva and 167 (71.67%) of stool samples but we were not able to detect this bacterium in the dental plaques of studied patients.

According to gastric specimen results, the prevalence of *H. pylori* was 90.47% (57 of 63) among patients with PUD, 80% (4 of 5) among patients with GC, and 74.13% (172 of 232) among patients with NUD by PCR assay. Generally, of 233 *H. pylori* positive isolates from gastric biopsy specimens, 220 samples (94.42%) were *cagA* positive and all samples had amplified bands both for *vacA* s and m regions. Overall, 114 (48.92%) samples had *vacA s1a*, 32 (13.73%) had *vacA s1b*, 52 (22.31%) had *vacA s1c* and 35 (15.00%) had *vacA s2* alleles, whereas the frequency of *m1a*, *m1b* and *m2* alleles were 76 (32.61%), 13 (5.57%) and 144 (61.80%), respectively. There was a significant relationship between *vacA s1a*/*m1a* and PUD diseases ($P = 0.04$) and the *s2*/*m2* genotype and NUD diseases ($P = 0.05$). No statically significant relationship was found between *cagA* status with clinical outcomes and *vacA* genotypes ($P = 0.65$). There was a statistically significant correlation between *H. pylori s2*/*m2* genotypes and the development of NUD ($P = 0.05$) and among *s1a*/*m1a* and PUD outcomes ($P = 0.04$).

Of 25 saliva samples positive for *H. pylori*, all were *cagA* positive while 18 (72.00%) samples had *s1a*/*m2*, 5 (20.00%) samples had *s1a*/*m1a*, 2 (8.00%) samples had *s2*/*m2* genotypes and all of the samples were *cagA* positive (Table 2). There was no association between genotypes of *H. pylori* from saliva with clinical outcomes ($P > 0.05$).

In stool samples, of 167 positive strains, the *cagA* gene was positive in 162 (97.00%) specimens. One hundred twenty (71.85%) had *s1a*/*m2*, 22 (13.17%) had *s2*/*m2*, 14 (8.38%) had *s1a*/*m1a*, 3 (1.79%) had *s1c*/*m2*, 3 (1.79%) had *s1c*/*m1a*, 2 (1.19%) had *s1b*/*m2*, 2 (1.19%) had *s1b*/*m1a* and *s1a*/*m1b* genotypes (Table 2). There was a significant relationship between NUD manifestation and the *s2*/*m2* genotype of *H. pylori* from stool samples ($P = 0.04$).

Table 2 The frequency of cytotoxin associated gene A and voculating cytotoxin gene A genotypes in gastric biopsy, saliva and stool samples

	cagA *n* (%)	*vacA* *n* (%)											
		s1a/m1a	*s1a/m1b*	*s1a/m2*	*s1b/m1a*	*s1b/m1b*	*s1b/m2*	*s1c/m1a*	*s1c/m1b*	*s1c/m2*	*s2/m1a*	*s2/m1b*	*s2/m2*
Gastric biopsy	220 (94.42)	36 (15.45)	9 (3.86)	60 (25.75)	7 (3)	5 (2.14)	13 (5.57)	17 (7.29)	5 (2.14)	39 (16.73)	12 (5.15)	0	30 (12.87)
Saliva	25 (100)	5 (20)	-	18 (72)	-	-	-	-	-	-	-	-	2 (8)
Stool	162 (97)	14 (8)	2 (1.19)	120 (71.85)	2 (1.19)	-	2 (1.19)	3 (1.79)	-	3 (1.79)	-	-	22 (13.17)

cagA: Cytotoxin associated gene A; *vacA*: Voculating cytotoxin gene A.

Table 3 The list of patients with incompatible *Helicobacter pylori* voculating cytotoxin gene A genotypes

Patient number	Gastric biopsy strain	Saliva strain	Stool strain
1	*s1a/m1a*	*s1a/m2*	*s2/m2*
2	*s1a/m1a*	*s1a/m2*	-
3	*s2/m1a*	*s1a/m2*	-
4	*s1c/m2*	*s1a/m2*	*s1c/m2*
5	*s2/m2*	*s1a/m2*	-
6	*s2/m2*	*s1a/m2*	*s2/m2*
7	*s1a/m1a*	*s1a/m1a*	*s2/m2*
8	*s1a/m2*	*s1a/m2*	*s2/m2*
9	*s1a/m2*	-	*s1a/m1a*
10	*s1a/m1b*	-	*s1b/m2*
11	*s2/m2*	*s2/m2*	*s1a/m2*
12	*s2/m2*	-	*s1a/m2*
13	*s2/m2*	-	*s1a/m2*
14	*s1a/m2*	*s1a/m2*	*s1a/m1a*
15	*s2/m2*	-	*s1c/m2*
16	*s2/m1a*	-	*s2/m2*

PCR tests for dental samples looking for *H. pylori* gene clues were negative. The *H. pylori* detection rate was statistically associated with the type of sample (P = 0.01). All patients with positive *H. pylori* in their saliva had a positive PCR reaction for gastric biopsy samples simultaneously.

Upon analysis of the results, in some cases we found different genotypes of *H. pylori* from the saliva, gastric biopsies and stool of the same patient. As presented in Table 3, in 6 (24.00%) patients, isolated *H. pylori* strains from gastric biopsies and the saliva of every patient showed a different genotype. In 11 (6.58%) patients, the genotypes of stool strains differed from genotypes of gastric isolates, and in one (4.00%) patient there were three different genotypes in his gastric biopsy, saliva and stool specimen (Table 3). However, variation of *H. pylori* genotypes in different studied sites were statistically non-significant (P > 0.05).

DISCUSSION

Infection by *H. pylori* remains one of the most important scientific phenomena in the biomedical literature worldwide and represents the most prevalent chronic bacterial disease because it affects more than half of the world's population, with a distribution related to the degree of economic development in each country[3]. The prevalence of *H. pylori* differs significantly both between and within countries, with high rates of infection being associated with low socioeconomic status and high densities of living[15]. For instance, in Japan, South America, Turkey and Pakistan, the prevalence is more than 80%, while in Scandinavia and England, the prevalence is between 20% and 40%[11]. The prevalence of this bacterium in Iran is 60%-90%, indicating Iran is a high risk region for *H. pylori* infection. The prevalence of this bacterium was 77.66% in our study and it was therefore compatible with other reports in Iran[2,11]. In our study, the rate of *H. pylori* in different sites of the gastric tract (0% dental plaques, 10.72% saliva, 77.66% gastric biopsy and 71.67% stool) varied, which is inconsistent with other studies[16,17]. There are several hypotheses which can explain the low rate of *H. pylori* in oral cavity compare to gastric biopsy and stool samples. First may be due to the fact that eradication therapy usually removes the gastric infection while it does not necessarily affect oral and intestinal colonization[16]. The second reason for such decreasing level of the rate of bacterium can be related to the presence of oral normal flora, which is able to affect the *H. pylori* growth by producing bacteriocin-like inhibitory proteins against *H. pylori* strains[1]. The third reason is based on the hypothesis that the *H. pylori* may persists in yeast while is in mouth. The *Candida spp.* could be the reservoir for *H. pylori* and play an important role in the bacterial re-inoculation in gastric tissue or transmission to a new host[18], so may yeast protects *H. pylori* from the stressful conditions in the mouth and carries it to the gastrointestinal tract of human[19]. According to Gatti *et al*[20] from Brazil and Bindayna *et al*[21] from India in 2006, there was a significant relationship between *cagA* gene and the inflammation of gastric tissue. The prevalence of *cagA*$^{+}$ gene in their samples was 79% and 59% respectively. However, Kangsadalampai *et al*[22] from Thailand in 2005 and Cirak *et al*[23] from Turkey in 2003, and Gutiérrez *et al*[24] from Cuba in 2005 failed to confirm such relationship between *cagA* status and gastric disorders. The prevalence of *cagA* gene was 31% in Thailand, 71% in Turkey and 88.5% in Cuba. In our survey, the prevalence of *cagA* gene was 94.42% in gastric biopsy samples and due to high prevalence of *cagA* in our studied isolates, we did not find any significant relationship between this gene and gastric disorders. The prevalence of *cagA* gene in our study was in accordance with our previous report[9] and similar to East Asian countries where the most of isolates are positive for *cagA* gene. Also this finding was different with major of previous

Table 4 Summary of studies which analysed *Helicobacter pylori* status in different oral cavity, stool and gastric sample

Author name	Country	Target population	Number of sample	Type of specimens	Method	Positive rate %
Cześnikiewicz-Guzik *et al*[16]	Poland	Gastrointensinal patients	100	Gastic biopsy, saliva and gingival plaques	ELISA	51 biopsy 54 saliva and 48.3 gingival pockets
Medina *et al*[3]	Argentina	Gastroduodenal patients	98	Saliva, dental plaque and gastric biopsy	PCR	88.4 biopsy and 18.98 oral samples
Iamaroon *et al*[7]	Thailand	Recurrent auphtus ulcer patients and healthy volunteers	22 patients/15 normal people	Mocusa	Nested PCR	4.5 auphtus patients and 4.5 normal patients
Tanahashi *et al*[37]	Northern California	Gastric patients	16 infected 10 uninfected	Stool, saliva and vomits	PCR and culture	18.8 saliva, 21.8 stool and 37.5 vomits
Silva *et al*[6]	Brazil	Gastric patients	30	Gastric biopsy, saliva and dental plaque	Single step and nested PCR	80 gastric biopsy, 30 saliva and 20 dental plaque
Fernández-Tilapa *et al*[5]	Mexico	Adults without dyspepsia	200	Gastric biopsy, saliva and dental plaque	Nested and semi nested PCR, ELISA	62 biopsy and 17 oral samples
Wang *et al*[8]	Tennessee	Gastric patients	31	Gastric biopsy and saliva	PCR and DNA sequencing	100 gastric biopsy and 71 saliva
Current study	Iran	Gastroduodenal patients	300	Gastric biopsy, saliva, dental plaque and stool	PCR	77.66 biopsy, 10.72 saliva, 0 dental plaque and 71.67 stool

ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase chain reaction.

reports from Iran, which the *cagA* positive rate was 44% to 91% and similar to European isolates[11,25,26]. This phenomenon may be because of changes in Iranian isolates status or targeting different part of *cagA* gene for amplification. According to López-Vidal *et al*[27] from Mexico in 2008 *vacA s1b/m1*, Linpisarn *et al*[28] from Thailand in 2007, *vacA s1a/m1* and *vacA s1c/m1*, Ahmad *et al*[29] from Pakistan in 2009, *vacA s1b/m2* and *vacA s1a/m1a*, Rudi *et al*[30] from Germany in 1998, Miciuleviciene *et al*[31] from Lithuania in 2008 and Saribasak *et al*[32] from Turkey in 2004, Hussein *et al*[33] from Iraq in 2008 and Momenah *et al*[34] from Saudi Arabia in 2006, *vacA s1a/m2* were the prominent strains in their country. We have found *vacA s1a/m2* as a predominant genotype in gastric specimens of Iranian patients with gastroduodenal diseases which was similar to Germany, Lithuania, Turkey, Iraq and Saudi Arabia but far different with Mexico, Thailand and Pakistan. There was statically significant correlation between *vacA s2/m2* genotype and NUD (P = 0.05) and *vacA s1a/m1a* genotype with PUD (P = 0.04). This finding is in accordance with the major of studies which believe *s1/m1* isolate are more virulent than *s2/m2*[27,29]. Similar to the previous reports from Iran, from statistical point of view no relationship was found between gastric cancer and *vacA* status (P = 0.1)[2,11]. Gastric epithelial cells seem to be the main niche of the *H. pylori*, however there are limited studies considering *H. pylori* status in oral cavity. Some of studies have detected *H. pylori* from different sites of the oral cavity[7,9] and some other groups failed to detect *H. pylori* from saliva, subgingival plaques and gingival pockets[35,36].

Medina *et al*[3] from Argentina in 2010 found *H. pylori* in 18.4% of saliva and Fernández-Tilapa *et al*[5] from Mexico in 17% of dental plaque during 2011. Cześnikiewicz-Guzik *et al*[16] from Poland in 2004 find this bacterium in 54% of saliva and 48.3% of gingival packets while Iamaroon *et al*[7] from Thailand in 2003 did not find *H. pylori* in oral aphtous ulceration patients (Table 4). In this study we found *H. pylori* in 10.72% of saliva and none of dental plaques. That's may be because of the high level of hygiene in our studied population[1]. Some authors have suggested that *H. pylori* may belong to the normal oral flora of the human oral cavity, maintaining a commensal relation with the host, but sometimes present in very low numbers which is difficult for identification. Others have suggested that *H. pylori* are not consistently present in dental plaque and saliva so when present, may be the result of occasional gastroesophageal reflux[1]. Some researchers suggest that *H. pylori* in oral cavity may serve as a source of gastric reinfection by this bacterium[7]. According to Tanahashi *et al*[37] from Austria, 93.7% of stool samples were *H. pylori* positive and Parsonnet *et al*[17] found this bacterium in 88% of the specimens. Both of them applied PCR assay for detection of *H. pylori*. In our study, 71.67% of stool samples of infected patients were *H. pylori* positive which is somehow accordance with other studies[17,38]. The lower prevalence of *H. pylori* in feces rather than stomach may is due to the effect of the intestinal tract normal flora. Our results showed high homology (58%) in *vacA* genotype in saliva and gastric samples from the same patients. This result was consistent with the findings of study by Wang *et al*[8] which showed 64% homology between saliva and gastric samples from the same patients. These findings support the hypothesis that saliva is a possible source of *H. pylori* infection. The major difference between gastric biopsy, stool and saliva is that saliva represents the entire oral cavity, but punch biopsy and stool sample serve only as a fraction of the total gastric mucosal surface. Interestingly the *H. pylori* isolated from gastric samples showed high diversity compare to those isolated from saliva and stool which may indicate that gastric biological nature support survive of all different genotype of *H. pylori*. Saliva is more likely to contain the

entire DNA from every strain colonizing the oral cavity but at concentrations that may be close to or below the detection level of our PCR assay. In current study we found several genotypic diversities between *H. pylori* strains isolated from saliva, stool and stomach of the same patient. Our data indicated that isolates from different sites of a single individual tend to be more a like than strains isolated from the same site of different individuals (P = 0.001). This is in agreement with our previous report which there was 61% homology between *H. pylori vacA* genotypes in saliva and gastric biopsy of same individuals[9]. The heterogeneity of *H. pylori* may be due to genotypic variation among strains and/or variations in *H. pylori* populations within an individual host, as proposed by Blaser[13]. Genotypic variation of *H. pylori* has been documented in point mutations and variation in the gene order[31,32]. Although high rate of similarity was seen among *H. pylori* isolates from different anatomical sites, but 16% of patients were infected with 3 different strains. This finding supports the idea that humans can be simultaneously infected with two or more *H. pylori* genotypes[39]. Variation might be because of co-existence of these bacteria together or occurring mutations[1].

In conclusion, there is high similarity between *H. pylori* strains isolated from saliva, stool and gastric specimens so it indicates that the possibly role of saliva and stool as *H. pylori* infection sources. However, the diversity of *H. pylori* genotypes between stomach, stool and saliva in the same patient suggest that more than one *H. pylori* strains may exist in the saliva and stomach of the same patient due to co-infection or genetic variation.

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COMMENTS

Background

Helicobacter pylori (*H. pylori*) infection is widespread throughout the world, and it is estimated that more than half of people are infected with this bacterium, but the exact route of transmission has not yet been fully clarified and remains poorly understood.

Research frontiers

Overall there are limited studies considering *H. pylori* status in oral cavity or feces. Some limited studies suggest that dental plaques, oral cavity and feces have important role in infection transmission and may serve as a reservoir for *H. pylori*, however some other studies did not find such correlation.

Innovations and breakthroughs

To date there has been a very limited study considering genotyping of *H. pylori* in oral cavity and feces. In this study, the authors employed genotyping in more detail using well-known virulence marker genes such as cytotoxin associated gene A (*cagA*) and voculating cytotoxin gene A (*vacA*). Furthermore, more anatomical sites of each patient including dental plaques, saliva, gastric and stool were analyzed for *H. pylori* genotyping by authors. Current study confirmed the significant role of saliva and feces but not dental plaques as a possible mean of *H. pylori* transmission and reservoir.

Applications

By finding correlation between *H. pylori* genotypes isolated from saliva and stool with gastric biopsy, the authors concluded that control of *H. pylori* in saliva and stool is crucial for managing of *H. pylori* infection in gastric tissue.

Terminology

Genotype: The genotype is the genes makeup and characteristic of an organism, a cell or an individual which reflect genetic profile of the cell. Genotyping is the process of determining and classification of organisms or cell based on differences in the genetic makeup (genotype) using biological techniques. Compare to observable characteristics (phenotype) of organisms, genotyping can provide a more accurate view of the biological and genetical status and be expected to be more useful for evaluating, for example, the source of infection, the mode of infection transmission and genetic variation.

Peer review

In the current cross-sectional study on high number of patients, the authors analyzed *H. pylori* genotype status in digestive system from mouth to rectum by targeting 8 regions of two important virulence marker genes, *cagA* and *vacA* alleles. The result indicate that although saliva and stool seems to be major source of *H. pylori* which infects gastric, however remarkable number of patients carry different genotypes in their gastrointestinal tract.

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