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The frequency of *Helicobacter pylori* in dental plaque is possibly underestimated

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ABSTRACT

Objective: The commonest bacteria, causing infection across the world is *Helicobacter pylori*, which colonizes the human stomach. This bacteria has also been detected in some extra-gastric ecological niches such as the oral cavity and water. However, the results of *H. pylori* detection in extra-gastric ecological niche are controversial. The improvement of the sensitivity and the specificity of the detection methods appear to be some of the main bottleneck issues in providing compelling evidence. The aim of this study was to detect the presence of this organism in dental plaque samples using an analytically sensitive and specific Polymerase Chain Reaction (PCR) as well as a new nucleic acid detection method termed the Loop-mediated Isothermal Amplification (LAMP).

Design: In a descriptive cross-sectional study 45 participants enrolled and dental plaque samples were collected from at least two teeth surfaces (one anterior and one posterior tooth) using a sterile periodontal curette. The DNA content was extracted from the samples and the presence of *H. pylori* was determined by PCR and LAMP reactions.

Results: The frequency of detection of *H. pylori* in the dental plaque samples were 44% (20/45), 66.67% (30/45) and 77.78% (35/45) using PCR, LAMP and positivity for both tests, respectively.

Conclusion: The high frequency of *H. pylori* was detected in the dental plaque samples of the participants, which concurs with the high prevalence of this bacteria in the population. This is one of the highest reported rates around the world. The results reveal that dental plaque can be one of the main causes of re-infection and also be the cause of oral–oral transmission.

1. Introduction

Helicobacter pylori is a fastidious, slow-growing, microaerophilic, spiral or curved gram-negative bacterium.[1] This bacterium is a major etiological agent of chronic gastritis, gastric and duodenal ulcers and is a risk factor for gastric adenocarcinoma and MALT lymphoma.[2] Gastric cancer (GC) is the major public health issue and ranked as the fourth most common cancer worldwide with a high prevalence in the developing and developed countries.[3]

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Most infections are acquired before the age of 10 years, with males and females infected at approximately the same rates, with an inverse relationship between the incidence of infection and socioeconomic status.[4] *H. pylori* gets transformed into a non-culturable but viable coccoid form in certain environmental and suboptimal conditions. Person-to-person transmission via the gastric–oral and/or faecal–oral route is the most important means by which the organism is spread.[4,5]

As *H. pylori* DNA has been detected from gastric juices, vomitus, saliva and dental plaque it is inferred that the oral–oral route is one of the most likely transmission pathway.[6] *H. pylori* needs a special condition for colonization, that is provided in the alveolar cavity, dental plaque under the gum and periodontal pockets. This colonization can also play an important role in the recurrence of infection and treatment failure.[5,7]

PCR targeted a variety of *H. pylori* genes which have been used in the detection of *H. pylori* in clinical and environmental samples including gastric biopsy, gastric juice, saliva, dental plaque, stool, and water.[8]

As PCR has recognized the highest level of *H. pylori* in dental plaque this approach is accepted as the best method of detection of the bacteria in the alveolar cavity samples.[9,10]

Although PCR is a rapid and sensitive technique, it involves some constraints such as time consuming than LAMP, sensitivity to inhibitors, and requires some special devises for thermal cycling and electrophoresis.

The Loop-Mediated Isothermal Amplification, a new method of highly specific and sensitive DNA amplification, has been developed by Notomi et al., in 2000,[11] which has the potential to overcome the PCR limitations.

The purpose of this research was to assess *H. pylori* prevalence in the dental plaques of patients who are suffering from chronic periodontal diseases using Loop-Mediated Isothermal Amplification.

2. Materials and methods

The population under this cross-sectional study consisted of 45 participants referred to Department of Periodontology, School of Dentistry, Kermanshah University of Medical Sciences, in 2013. All the participants gave signed consent and were fully informed regarding the work. Patients with natural tooth in oral cavity and periodontal plaque were included. Diabetics, pregnant women, HIV-positive patients, smokers, and patients who used antacids and anti *H. pylori* antibiotics like amoxicillin, tetracycline, metronidazole and clarithromycin during the prior week were excluded from the research. All participants were examined by a dentist and dental plaque was collected from the at least two teeth surfaces (one anterior and one posterior tooth) using a sterile periodontal curette. All participants brushed once or twice daily. The dental plaque samples were transferred to the laboratory in 2 h.

The dental plaque samples were homogenized with a glass rod and centrifuged at 8000 rpm for 5 min. The pellet was subjected to DNA extraction using the DNA extraction kit according to the manufacturer's instructions (Accuprep Genomic DNA Extraction Kit, Bioneer, South Korea). The DNA extracted was maintained at −20 °C before it was used in the PCR and LAMP reactions.

The PCR reaction was done using the specific primers of the *H. pylori ureC* gene which were designed and described in an previously published article[12] (Table 1).

DNA amplification was performed in a BioRad thermocycler (BioRad, Singapore) using the following programme: an initial denaturation step at 95 °C for 5 min was followed by 37 cycles of amplification each consisting of 95 °C for 30 s, and annealing at 56 °C for 30 s, with extension at 72 °C for 60 s, and a final extension step at 72 °C for 5 min. Negative and positive control were included in each run. After amplification, the PCR products were subjected to 1% agarose gel electrophoresis and staining using ethidium bromide. The sizes of the amplicons were estimated in comparison with the 100 bp molecular weight marker. All the reactions were performed with a negative control containing all of the reagents without the DNA template and also positive control. A single 214 bp band indicated as a positive reaction.

The specific primers for *H. pylori* were designed based on the *ureC* (*glmM*) gene as a target. The *glmM* gene, the most promising target, is relatively well conserved, and present in all the *H. pylori* isolates.[13–18] In addition, the *glmM* gene has a high degree of sensitivity and specificity in the diagnosis of *H. pylori*.[16,19] The LAMP method requires a set of four specific primers: a forward inner primer (HP-*ureC* FIP), a backward inner primer (HP-*ureC* BIP), and two outer primers (HP-*ureC* F3 and HP-*ureC* B3), and also two loop primers (HP-*ureC* LF and HP-*ureC* LB) which accelerate the reaction, by recognizing six distinct regions of the *ureC* gene sequences. All the primers were designed based on the *ureC* gene sequence of the *H. pylori* 26695 strain (Accession No. AE000511) using Primer Explorer V4. The loci and sequences of the primers are shown in Table 1.

The LAMP reactions were performed as described earlier with some modifications.[20,21] The Optimal LAMP reaction was

Table 1 – PCR and LAMP primers that used in this study.

Primer name		Sequence 5′–3′	Size of product (bp)	Reference
PCR	HP-*ureC*F	CAT CGC CAT CAA AAG CAA AG	214	[12]
	HP-*ureC*R	CAG AGT TTA AGG ATC GTG TTA G		
LAMP	HP-*ureC* BIP	CCATCGCCTGTTTTAGCGTAACATGCAATTGAATAAAGCCAAT	119	This study
	HP-*ureC* FIP	GTGTGCGCTTTGCAAGTGAGGGTTTAACGCAACAGAGCT		
	HP-*ureC* B3	GGATAAGTTTGTGAGCGAATG		
	HP-*ureC* F3	ACTAGGCTTTGGGGGTAT		
	HP-*ureC* LB	TCGCTAAAAATGATATGCCCGC		
	HP-*ureC* LF	CGTTAGTGTTAGAAAGCAAGCAGG		