

Establishment of the possible association between the presence of *Helicobacter pylori* in the saliva and gastric biopsy by using polymerase chain reaction technique in association with oral manifestation of peptic ulcer disease

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ABSTRACT

Background: *Helicobacter pylori* are important gastrointestinal pathogen associated with gastritis, peptic ulcers, and an increased risk of gastric carcinoma. There are several popular methods for detection of *H. pylori* (invasive and non-invasive methods) each having its own advantages, disadvantages, and limitations, and by using PCR technique the ability to detect *H. pylori* in saliva samples offers a potential for an alternative test for detection of this microorganism.

Materials and methods: The study sample consists of fifty participants of both genders, who undergo Oesophageo-gastroduodenoscopy at the Gastroenterology Department of Al-Kindy Teaching Hospital Baghdad/ Iraq, during five months period from January 2014 to May 2014. They were grouped into 32 participants with PUD (case group) and 18 healthy participants (control group). A full-mouth examination was performed for every patient; saliva and gastric samples from both groups were obtained. *Helicobacter pylori* were detected in gastric biopsies by histological examination by using H & E stain, and Polymerase Chain Reaction (PCR) was carried out on the oral samples.

Results: *Helicobacter pylori* DNA was determined by PCR in oral samples in 88% patients and in gastric biopsies by histology in 86% patients, and in both samples in 84% patients. It was highly significant to find simultaneous presence for those have *H. pylori* in stomach also have such microorganism in the mouth $P < 0.05$ and there was an excellent correlation between detecting *H. pylori* simultaneously in both stomach and mouth. If we screen for stomach *H. pylori* through detecting this microorganism in the mouth; saliva samples is highly sensitive (98%) but not very specific.

Conclusion: *Helicobacter pylori* saliva test has high sensitivity, specificity, and accuracy for the diagnosis of *H. pylori* infection in Iraqi population. The test can be clinically applied as a routine diagnostic tool for *H. pylori* infection this could permit not only a target for therapeutic procedures but also a monitoring tool for the efficacy of therapy. It seems to overcome some limitations of the conventional invasive techniques.

Key words: *Helicobacter pylori* - Oral infection - *H. pylori* saliva test - PUD. (J Bagh Coll Dentistry 2015; 27(3):85-88).

INTRODUCTION

Peptic ulcer is a breach in the gastric or duodenal mucosa down to the sub mucosa ⁽¹⁾. In most cases the etiology of ulcer is unknown yet ⁽²⁾. The normal stomach mucosa maintains a balance between protective and aggressive factors. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, use of alcohol and non-steroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (*Helicobacter pylori*) ⁽³⁾. The association of *H. pylori* with peptic ulcer disease has been observed ⁽⁴⁾.

This study has been shed light on the role of (*H. pylori*) as the commonest bacterial infection worldwide, it infects more than 50% of the world's population ⁽⁵⁾, and as a main cause of PUD ⁽⁶⁾. *Helicobacter pylori* first reported in 1983 by Warren and Marshall, ⁽⁷⁾. *H. pylori* (initially termed *Campylobacter pyloridis*) is an important human pathogen ⁽⁸⁾.

H. pylori is a Gram-negative spiral bacterium that may colonize the human gastric mucosa and establish a life-long infection ⁽⁹⁾. Colonization with *H. pylori* is not a disease by itself ⁽¹⁰⁾. It has been shown that infection certainly makes the occurrence of ulcers more likely, Infection linked with the development of chronic gastritis, peptic ulcer, gastric cancer, and mucosa-associated lymphoid tissue lymphoma ^(11,12).

Because of the importance of this bacterium in the development of PUD, Rasmussen ⁽¹³⁾ evaluated the association between the presence of *H. pylori* in gastric biopsies and in the saliva of the same individuals. The relationship between gastric symptoms and *H. pylori* DNA in saliva, however, is unclear. It could be possible that the oral cavity is the initial site of infection ⁽¹⁴⁾.

Many diagnostic methods have been developed to detect *H. pylori* including the urea breath tests, rapid urease tests, and measurement of anti-*H. Pylori* antibody from serum and urine, special histologic staining and immunostaining, and stool antigen testing. Many PCR methods targeting putative *H. pylori* specific genes have also been reported ^(15,16). The goal of this study was to determine the simultaneous presence of *H.*

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pylori both in the oral cavity and gastric mucosa in patients suffering from PUD and to standardize a feasible non-invasive method for the rapid diagnosis of *H. pylori* in salivary secretion of infected patients suffering from PUD by using PCR analysis.

MATERIALS AND METHODS

The study sample consists of fifty patients of both genders with age range (15-65) years; who undergo Oesophageogastroduodenoscopy during five months period from January 2014 to May 2014. They were grouped into 32 participants with PUD (case group) and 18 healthy participants (control group).

- **Inclusion criteria:** Symptomatic individuals were selected and these criteria were: recurrent abdominal pain, unexplained vomiting and weight loss⁽¹⁷⁾.

- **Exclusion criteria:** Gastritis patients, gastric CA, history of PUD, patients receiving antibiotics, H2 receptor antagonists or anti-acid treatment, were excluded⁽¹⁷⁾.

After informed consent was obtained from all participants, full-mouth examination was performed for each patient; saliva and gastric samples were collected from cases (whom diagnosed endoscopically by a specialist) and from healthy subjects (endoscopically no signs of PUs disease). Subjects were investigated for the presence of *H. pylori* in saliva and stomach biopsies,⁽¹⁸⁾ *Helicobacter pylori* detected in gastric biopsies by histological examination, by using Hematoxylin and Eosin stain (H & E), while Polymerase Chain Reaction (PCR) was carried out on the saliva. The lab work for the detection of *H. pylori* was done in a strictly aseptic conditions.

DNA extraction: DNA was extracted from the saliva using QIAamp® DNA Mini kit (Germany) with a bacterial DNA extraction protocol (Spin Protocol): Samples preserved in freeze thawed into room temperature then 200 µl saliva pipetted into a microcentrifuge tube, then 20 µl of proteinase K added then 200 µl lysis buffer (buffer AL) added to the mixture, then the mixture incubated at 56°C for 10 min. The mixture was then combined with 200 µl Ethanol and mixed by pulse-vortexing. The mixture was applied to the QIAamp Mini spin column which holds a silica gel membrane, and spun at 8000 r/m (round/minute) for 1 min then the spin column washed with 500 µl of buffer AW1 and then AW2 by centrifugation at 14,000 r/m for 1 and 3 minutes respectively, pure DNA bounded on a membrane eluted by 200 µl of buffer AE added and centrifuged for 1 min, then incubated at room

temperature (15-25°C) for 5 minute. Finally the resulting DNA extracts stored at -20°C until PCR assessment.

DNA amplification and gel electrophoresis:

Extracted DNA from saliva samples were amplified by using primer for urease gene ure C (136 bp) 5' – AAGCTTTTAGGGGTGTTAGGGGTTT – 3' and 5' – CGCAATGCTTCAATTCTAAATCTTG – 3' indicative of *H. pylori* infection, which have been shown previously to be highly specific and sensitive,⁽¹⁹⁾ and by using GoTaq® Green Master Mix kit (a premixed ready-to-use solution containing bacterially derived Taq DNA polymerase, dNTPs, MgCl₂ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR), the GoTaq® Green Master Mix thawed into room temperature then the reaction mixture prepared in a final volume of 50 µl. then the reactions placed in a thermal cycler. DNA amplification was carried out as follow: Denaturation at 94°C for 5 minutes in the first cycle, followed by annealing for 30 seconds at 60°C, extension for 2 minutes at 72°C, and denaturation for 30 seconds at 94°C for a total of 40 PCR cycles. The extension for the last cycle was increased to 5 minutes to ensure complete extension of the amplified fragment. The PCR products were resolved by 2% agarose gel electrophoresis and were visualized after ethidium bromide (0.5 mg/ml) staining, using an UV transilluminator and photographed by Polaroid camera.

RESULTS

The present study was carried out on 50 patients, 29 males and 21 females, their age ranged from (15-65) years. Among study groups (50) samples from oral cavity (saliva) and (50) samples from stomach (gastric biopsy) from the same individuals were collected, *H. pylori* was detected in 84% patients simultaneously in both types of samples. This study reported that there was an excellent agreement between detecting *H. pylori* simultaneously in both stomach and mouth {K > 0.70, P < 0.05, Table 1 A}. If we screen for stomach *H. pylori* through detecting this microorganism in the mouth; saliva samples is highly sensitive (98%) but not very specific (70%) (Table 1B).

Table 1: Results of detection of *H. pylori* in gastric and saliva samples:

A) Results of statistical tests

Statistical Test	Output
Fisher's exact Test	P value < 0.001
Measurement of Agreement	Kappa = 0.735
	P < 0.001

B) Results of diagnostic values if using oral sample to screen for *H. pylori* in stomach.

Indicator of Test Performance	P value	95%CI
		[Upper; Lower]
Sensitivity	0.98	[0.86; 1.00]
Specificity	0.71	[0.30; 0.95]
Accuracy	0.94	[0.82; 0.98]
PPV	0.95	[0.83; 0.99]
NPV	0.83	[0.36; 0.99]

Also the current study found that presence of *H. pylori* in saliva was significantly associated with gingival inflammation and halitosis $P < 0.05$. But no significant associations with teeth erosion, burning mouth syndrome (BMS), oral aphthous $P > 0.05$. Also there was highly significant correlation between presence of *H. pylori* in saliva and PUD group $P < 0.001$ (Figure 1).

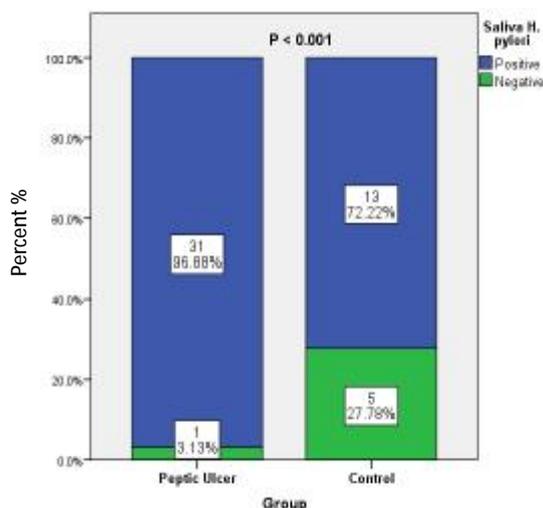


Figure 1: Distribution of study sample according to study group and to presence of *H. pylori* in saliva.

DISCUSSION

In this study, *H. pylori* was identified simultaneously in both kinds of samples (gastric biopsy and in saliva) for 84% participants. The present findings showed statistical significance of the positive relation of *H. pylori* in gastric biopsies and oral samples, these results coincide

with that reported by Miyabayashi⁽²⁰⁾ and Rasmussen⁽¹⁵⁾, indicating that in many cases, patients with positive results of gastric biopsies were also positive in oral samples. In this study, the *H. pylori* saliva test had a high sensitivity 98%, which enabled it to detect a low titer of *H. pylori*, but it's not very specific 71%, this finding agree with a study reported by Song⁽²¹⁾.

Also the finding showed particular attention is paid to the association of the *H. pylori* reservoir in the oral cavity with gingivitis, this finding agrees with Cellini⁽²²⁾. It is also suspected that the presence of this bacteria in PUD patients may lead to oral inflammation and the formation of gingival/periodontal pockets, which develop favorable conditions for bacterial growth⁽²³⁾. The findings of this study support the concept of a potential association between halitosis and the presence of *H. pylori* in the oral cavity, this findings is highly similar to that reported by Moshkowitz⁽²⁴⁾ and Suzuki⁽²⁵⁾.

The finding show no significant association between *H. pylori* presence in saliva and dental erosion, this may be explained by the fact that dental erosion doesn't occur until gastric acid had acted on the dental hard tissue regularly over a period of several years. In agreement with studies of other researchers^(26,27).

The present study showed no significant relation between *H. pylori* and burning mouth syndrome. At variance to other trial, reported by Idan and Abdul-Razaq⁽²⁸⁾, found highly statistical significant relationship between *H. pylori* and burning mouth.

The findings suggest that salivary *H. pylori* does not play a role in the pathogenesis of RAU, in agreement with Ghanaei⁽²⁹⁾ found that these bacteria not involved as a cause of recurrent oral aphthous ulcers because *H. Pylori* DNA could not be found in the aphthous ulcers of these patients.

In conclusion there is strong relation between presence of *H. pylori* in stomach and oral cavity, it was identified simultaneously in both kinds of samples, in gastric biopsy histologically and in saliva by PCR. The successful detection of *H. pylori* DNA directly from saliva samples indicates that this approach is feasible and saliva could serve as an effective and valuable noninvasive specimen to diagnose and monitoring the efficacy of therapy in patients with active *H. pylori* infection. This method not only reduces the economic burden of treatment, but also lowers the risk of increasing the resistance of *H. pylori* to antibiotics.

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