



## DETECTION OF HELICOBACTER PYLORI IN SALIVA AND BIOPSY SPECIMENS OF SOME IRAQI PATIENTS USING PCR TECHNIQUE

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### ABSTRACT

*Helicobacter pylori* colonize the stomachs of half of the world's population and usually persist in the gastric mucosa of human hosts for decades or life, although most *H. pylori*-positive people are asymptomatic. Objective: This study was conducted to examine the relationship between the presence of the bacteria in the oral cavity and gastric mucosa using PCR technique. Methods: The study include 74 patient 40 male and 34 female, DNA extracted from saliva and biopsy specimens, PCR was used to amplifying ure C and 16srRNA genes specific for *H. pylori*, the results clarified that all biopsy specimens presented the two genes with no significant difference between male and female, in saliva specimens the results were 6 (8.10%) for 16srRNA and 1 (1.35%) detected for ureC gene, therefore, we cannot depend on saliva in detection of *H. pylori* infection.

**KEY WORDS:** *Helicobacter pylori* PCR 16srRNA ureC gene

### INTRODUCTION

*Helicobacter pylori* is a Gram negative, spirally shaped bacterium, 0.5 – 0.9 µm wide by 2 – 4 µm long, it is micro-aerophilic and requires carbon dioxide for growth, It produces an exceptionally powerful urease which is vital for its survival in the stomach (Skirrow, 2002). This bacterium responsible for diseases such as atrophic gastritis, chronic gastritis, duodenal ulcers, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer (Kargar *et al.*, 2001). *H. pylori* is known to be acquired early in life (Kodaira *et al.*, 2002) and is likely to be transmitted from person to person, the transmission is supported by crowded living conditions, accompanied by poor hygiene and intra-familial clustering. Nevertheless, the exact manner of transmission is not completely understood (Goodwin *et al.*, 1997; Mitchell, 1999). The diagnosis of *H. pylori* infection is an important issue. Recently, there are several diagnostic assays for *H. pylori*: bacterial culture, urease test, urea breath test, histology, serology, and a stool antigen test culture. Molecular methods like polymerase chain reaction (PCR) have the potential to accurately determine both the presence of infection and the genotype of bacteria, these techniques have been used successfully to detect *H. pylori* DNA in human tissue and by amplifying 16s rRNA and ureC gene. The 16s rRNA is one of the specific targets to confirm this pathogenic bacterium and positive amplification of *H. pylori* specific DNA may be considered as a direct evidence of the presence of the pathogen (Hoshina *et al.*, 1990; Chong *et al.*, 1996; Yoshida *et al.*, 1998). The ureC gene encodes for a phosphoglucosamine mutase, this gene is not related to urease production, so it was renamed glmM. It is considered a "housekeeping" gene, which participates directly in cell wall synthesis (Bickley *et al.*, 1993; Espinoza *et al.*, 2011). It is well-established that the principal ecological niche for *H. pylori* is the gastric

mucosa. Recent studies using the polymerase chain reaction (PCR) technique for *H. pylori* diagnosis have demonstrated that *H. pylori* can be found in the human oral cavity, but it is unclear whether that cavity is a permanent or transient reservoir. This region of the body provides an excellent microaerophilic environment and is therefore a potential reservoir for *H. pylori* (Dowsett and Kowolik, 2003; Loster *et al.* 2006; Burgers *et al.* 2008). Therefore, the objectives of this paper were to determine the presence of *H. pylori* in the oral cavity and to examine the relationship between the presence of the bacteria in the oral cavity and gastric mucosa.

### PATIENTS & METHODS

Ninety seven subjects enrolled in this study, 74 patient (40 male and 34 female) suffering from Severe antrum gastropathy and other gastrointestinal tract diseases, and 23 subject (10 man and 13 female) apparently healthy (control group) with age ranged from 20 to 80 years. Samples were obtained from subjects in Gastrointestinal Endoscopy Unit at Baghdad Teaching Hospital, National Center for Early Detection of cancer medical city and the Esophago Gastroduodeno Scope Unit at Al-Kadhimiya Teaching Hospital in a period between the beginning of November 2012 and the end of May 2013. Saliva sample was taken from each patient and each control subject, they were asked to collect about 1 milliliter of saliva. The sample was collected in a sterile dry tube and preserved at -20 until analysis. Patients were advised to fast for overnight before endoscopy, the instrument used was Olympus GIF Q20 fiberoptic gastroduodenoscope, tissue biopsies were obtained from antrum, and placed in 1 ml of normal saline was preserved at -20°C for molecular analysis. Each frozen saliva and biopsy specimen was thawed; genomic DNA was then extracted directly, using the Geneaid extraction genomic DNA kit individually.

**Polymerase Chain Reaction (PCR)**

PCR amplification process was performed after extracting DNA from saliva and biopsy specimens from patients and control subjects using specific primers, the primer sequence for amplification 16s rRNA gene was as follows: 16s rRNA , F(5'- TAAGAGATCAGCCTATGTCC-3') R (5'-TCCCACGCTTTAAGCGCAAT-3') with 534bp (Kumar *et al.*, 2010), ureC gene, 300bp F (5'-GGATAAGCTTTTAGGGGTGTTAGGGG -3') R (5'-GCTTACTTTCTAACACTAACGCGC -3') (Mahsa *et al.*,

2009). The primers were supplied by the Applied Biosystems Company as a lyophilized product of different Picomole concentrations. Primers were used by making working solution, 10µl from the stock solution plus 90µl ddH<sub>2</sub>O to obtain 100µl working solution.

**PCR working solution**

Preparation of the reaction mixture on ice for amplification of *ureC* and 16srRNA gene:-For a 25µl reaction volume as in table, 1.1

**TABLE 1.1:** Component of PCR reaction mixture for *ureC* and 16srRNA gen

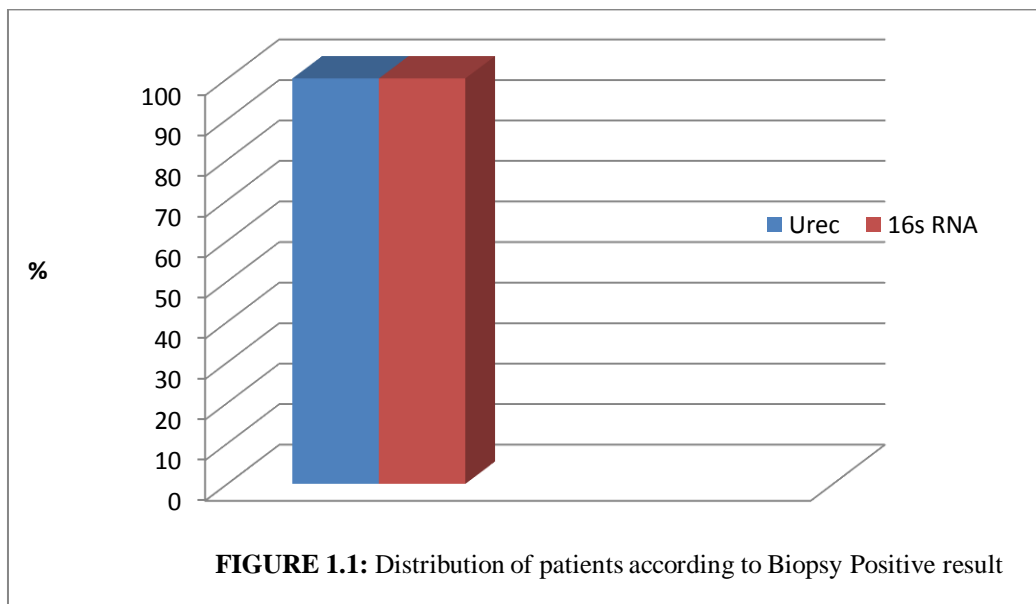
Component	Volume
GoTaq® Green Master Mix, 2X	12.5µl
Forwarded Primer, 10µM	1.5µl
Reverse primer 10 Mm	1.5µl
DNA template	3 µl
Nuclease-Free Water to	6.5 µl
Total volume : 25 µl	

The thermal cycle (Bio-Rad, Italy) was used with a thermal profile. PCR process consists of a series of thirty five cycles, each cycle consists of three steps: involving initial denaturation 5 min at 94°C, denaturation 1min.at 93 °C, annealing 1min., at 55 °C extension for 30 sec, and a final extension step at 72 for 10 min.The PCR products were identified by their size using agarose gel electrophoresis, the size of the PCR products was determined by comparing them with a DNA ladder (Promega, 1000 bp DNA, USA) which contains DNA fragments of known size.

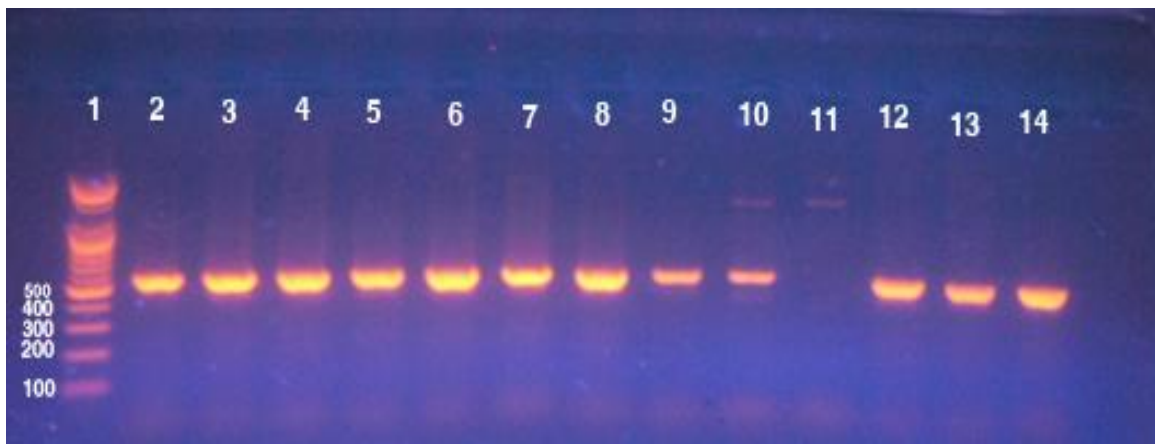
**RESULTS & DISCUSSION**

**Detection of *H. pylori* DNA using biopsy specimens**

Detection of some genes of *H. pylori* by conventional PCR was confirmed the occurrence of this bacterium conducted through amplification of DNA extra from tissue biopsy of all patients and control individuals using a *Helicobacter* species-specific primers for two genes 16srRNA and ure C PCR amplification.DNA extracted from seventy four specimen human tissue taken from patient clarified the presenting of 16srRNA and *ure C* gen using conventional PCR, the 23 control group were negative for the *ure C* and 16srRNA genes. The present of DNA band with 534bp in comparison with DNA ladder, evidence of the detection of 16srRNA gene specific for *H.pylori*, this gene was detected in all 74 patients that they infected with different gastropathy, while it was negative in control biopsy (Figure, 1.1 and 1.2).



**FIGURE 1.1:** Distribution of patients according to Biopsy Positive result

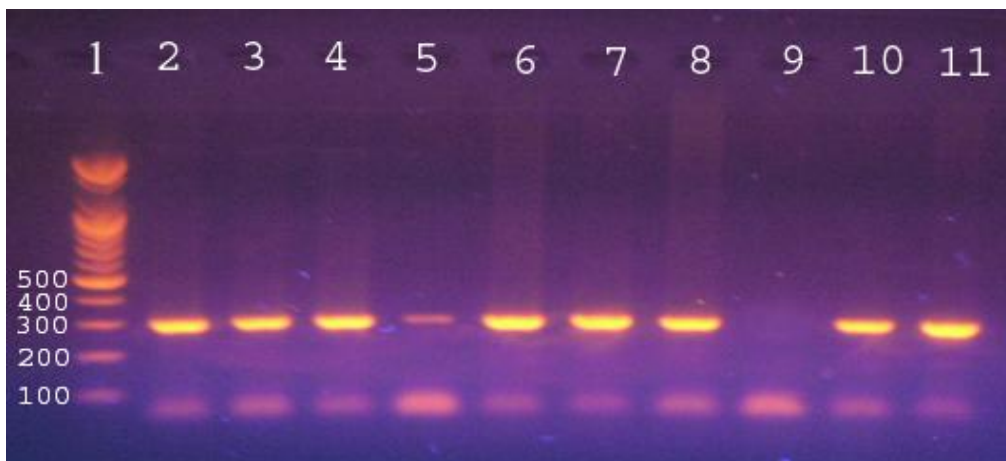


**FIGURE 1.2:** Detection of the PCR product DNA bands of *Helicobacter pylori* 16s rRNA gene (534bp). The amplified fragments were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide at 70 volts/cm for 1 hour. photographed under UV light.

Lane 1       $\longrightarrow$       DNA ladder 100bp  
 Lane 2       $\longrightarrow$       10 534bp for 16srRNA  
 Lane 11      $\longrightarrow$       Control negative  
 12, 13, 14  $\longrightarrow$       534bp for 16srRNA (positive)

The molecular detection of this study, also involved detection of ure C gene in all patients and control biopsy. The results clarified that showed the presenting DNA band

of 300bp, which referred to this gene, whereas the biopsy of control didn't appeared this band (Figure, 1.3).



**FIGURE 1.3:** Detection of PCR product DNA bands of *Helicobacter pylori* ure C gene (300bp). The amplified fragments were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide at 70 volts/cm for 1 hour. photographed under UV light.

Lane 1       $\longrightarrow$       DNA ladder 100bp  
 Lane 2       $\longrightarrow$       8 300bp for ure C gene  
 Lane 9       $\longrightarrow$       Control negative  
 Lane 10 , 11  $\longrightarrow$       300bp for ure C gene (positive)

16S rRNA gene primers were used for the detection of *H.pylori* in many researchs. These primers were demonstrated to be highly sensitive and specific biomarker for detection of *H.pylori*. It is one of the specific targets to confirm *H. pylori* infection, and positive amplification of *H. pylori* specific DNA may be considered as a direct evidence of the presence of the pathogen (Hoyle *et al.*, 1991; Chong *et al.*, 1996; Riggio *et al.*, 2000). Amplification of this gene segments has a theoretical advantage, as the high copy number of rRNA per bacterial cell increases the target DNA copies (templates) by several thousand fold. Therefore, this amplification was

suggested to give more reliable results (Engstrand *et al.*, 1992; Germani *et al.*, 1997). In similar study performed by Sushil *et al.* (2010) study reported that *H. pylori* was successfully isolated from biopsy samples of 168 patients in Varanasi (North) and Hyderabad (South India). Isolates of *H.pylori* were identified on the basis of amplification of the *H. pylori* specific 16srRNA gene, all the strains showed amplification of desired fragment of 16srRNA (534bp) with *H. pylori* species- specific primer .

*Helicobacter* DNA in the present study was investigated using a *Helicobacter* species-specific 16s rRNA and ureC gene PCR amplification. PCR-based detection of the ureC

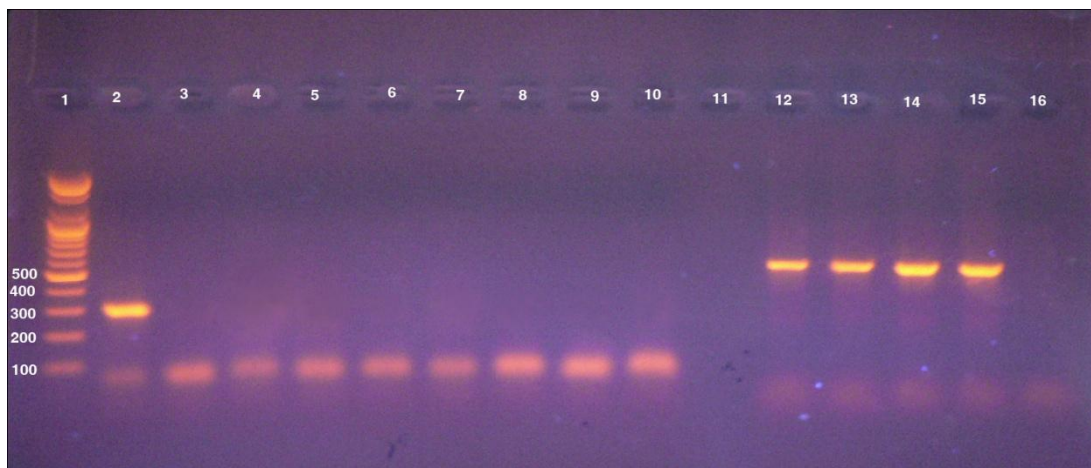
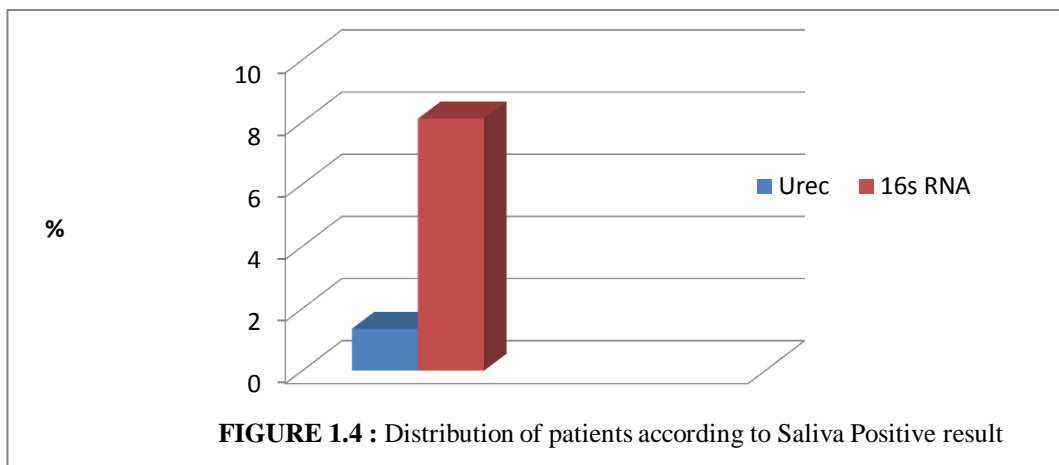
gene appears to be the most promising for detection of *H. pylori*. The ureC gene, renamed glmM by De Reuse *et al.*, in 1997 encodes phosphoglucosamine mutase, which is unrelated to urease production; it is an enzyme catalyzing the inter-conversion of glucosamine-6- phosphate into glucosamine-1-phosphate, which is subsequently transformed into N-acetylglucosamine. Therefore, *H. pylori* glmM is essential for cell growth. glmM gene is a housekeeping gene that is present in all *H. pylori* strains. Its sequence appears to be relatively well conserved between strains (Lu *et al.*, 1999). Salehi *et al.* (2009) reported that among patients with gastric ulcer, gastritis, and duodenal ulcer, 91.7% glmM PCR positive biopsy specimens were detected, which is similar to the present study.

Different primers used by many researchers for detection this gene, as in the study of Zivar *et al.* (2011) who confirmed the present of *ureC* gene by using primers glmF

and glmR for amplification, the expected PCR product of 294 bp was obtained in 126 (75%) biopsy specimens . Recently Reza *et al.* (2013) using primers HP-F and HP-R to amplify the *ureC* gene, the expected PCR product of 294-bp was obtained in all strain of *H.pylori*.

**Detection of *H. pylori* DNA using saliva specimens**

Using the saliva by some researchers in previous studies to detection the presence of *H.pylori* encourage us to understand the role of saliva in diagnosis of *H.pylori* infection as easy and comfortable method for patient instead of endoscopy, which is painful for them. The results revealed that only 6 (8.10%) of saliva specimens of patients that complaining from sever gastritis detected positive for 16srRNA and 1 (1.35%) detected for ure C gene, also three (3/23) of the apparently healthy group gave positive results for the 16srRNA gene with band 534bp, no one of them presented the ure C gene (300bp) (Figure, 1.4 and 1.5) .



**FIGURE 1.5:** Detection of PCR product DNA bands of *Helicobacter pylori* ure C gen (300bp) and 16srRNA(534bp) from saliva specimens, the amplified fragments were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide at 70 volts/cm for 1 hour. photographed under UV light.

- Lane 1                      ⇨                      100bp DNA ladder
- Lane 2                      ⇨                      300bp for ure C gene
- Lane 3                      ⇨                      Negative
- Lane 12,13,14,15       ⇨                      534bp for 16srRNA
- Lane 16                    ⇨                      Negative

Several authors reported the low prevalence of *H.pylori* in saliva, in Iran Hassan *et al.* (2012) found that 271

(90.33%) patients from 300 patient were positive for *H. pylori*, but *H. pylori* was detected in 25 (10.72%) of saliva



and they are not able to detect this bacterium in the dental plaques of studied patients. In study of Myriam *et al.* (2013), they analyzed the samples from 30 patients (17 males (56.7%) and 13 females (43.3%)), 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, the researchers attributed this result to PCR technique variations, lower concentration of the bacteria in salivary samples, suggested that other oral bacteria may cause false positive results. The patients in the current work, however, were not ideal for the collection of oral specimens, all were due to undergo endoscopy, so many had cleaned their teeth immediately before arrival, collection of dental plaque particularly difficult. All had been fasting so very little saliva could be aspirated without citric acid stimulation. In this study the three apparently healthy who have *H. pylori* in the saliva, and did not have active infection in the stomach, the possible explanation is that these subjects had only a low absolute number of *H. pylori* in their mouths, not enough to colonize in the stomach. If a favorable environment was provided (*e.g.*, there were some oral diseases), the bacteria may increase to some level sufficient to cause gastric infection or reinfection. In saliva the result of PCR detection was 1.035%, 8.10 for ure C, 16srRNA respectively, there is high significant different in compared with other methods refer that; we cannot depend on saliva for detection of *H. pylori*.

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