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Original Article

Detection of *Helicobacter pylori* urease antigen in saliva in patients with different gastric *H. pylori* status

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Abstract

Background: Finding a simple, accurate, and noninvasive diagnosis method is a substantial challenge for the detection of *Helicobacter pylori*. The aim of the present study was to compare the presence of *H. pylori* urease antigen in saliva with the presence of this bacterium in gastric mucosa.

Methods: Saliva samples and gastric biopsies were taken from 153 consenting Moroccan patients. Saliva samples were analyzed using an immunochromatographic test for urease antigen *H. pylori* detection. Thereafter, the gastric biopsies were analyzed by histology and polymerase chain reaction (PCR) to detect this bacterium.

Results: From a total of 153 recruited Moroccan patients, *H. pylori* was detected in 28 (18.30%), 87 (57.24%), and 69 (45.10%) cases by saliva test, histology, and PCR, respectively. A significant association was observed between the presence of *H. pylori* antigen in saliva and age. However, no association was found with sex, *H. pylori* virulence factors, gastric disease outcome, and density of the bacterium on the gastric mucosa. Considering that only 90 patients presented concordant results on *H. pylori* diagnosis (positive or negative) by both histology and PCR, the immunochromatographic test showed very low sensitivity (29.79%) and high specificity (90.70%). Of these two tests, the positive and negative predictive values were 77.78% and 54.17%, respectively. The accuracy of the test for salivary detection of urease antigen *H. pylori* was 58.89%.

Conclusion: This study demonstrated a low detection rate of *H. pylori* antigens in saliva compared with the presence of this bacterium in gastric mucosa, suggesting that saliva cannot be used as a suitable sample for the diagnosis of *H. pylori* in our study population.

Keywords: *H. pylori* diagnosis; *H. pylori* urease antigen; PCR−histology; saliva

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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1. Introduction

Helicobacter pylori infection is a public health problem that is becoming an increasingly troublesome economic and healthcare burden in many countries around the world. This follows from its involvement in varied gastric pathologies such as peptic ulcer, gastric adenocarcinoma, and MALT (mucosa-associated lymphoid tissue) lymphoma. Therefore, the accurate and timely diagnosis of this bacterium remains the first step to address these burdensome problems. After the successful culture of *H. pylori* in 1982, efforts have been directed to improve the methods of detection of this bacterium. At present, there are many techniques used for the detection of *H. pylori* bacterium, and these tests fall into two categories: invasive and noninvasive. The invasive methods, which are biopsy-based, include: culture, rapid urease test, polymerase chain reaction (PCR), and histology. All of these tests require an endoscopic procedure, are quite expensive, and can miss *H. pylori* infection if a biopsy sample from one part of the stomach does not contain the bacteria. These methods also require the use of specialized equipment, experienced personnel, and substantial time to obtain the results. Noninvasive tests or indirect techniques do not require an endoscopy, and include: (1) blood tests (which detect antibodies to *H. pylori*); and (2) a urea breath test that requires very expensive equipment.[1]

It has long been speculated that the presence of *H. pylori* has not been limited only to gastric mucus but can also be present in the oral cavity, and for this reason, may affect the outcome of eradication therapy. Therefore, failure to eliminate the organism from the mouth can lead to gastrointestinal reinfection.[2] Therefore, it will be of manifest interest if we can better identify the oral *H. pylori* infection. The aims of this study were to compare the presence of *H. pylori* urease antigen in saliva, with the presence of bacterium in gastric mucosa, and to determine the possible association between the saliva test results and age, sex, gastric disease outcome, *H. pylori* virulence factors, and density of the bacterium on the gastric mucosa.

2. Methods

2.1. Biopsy sample collection

A total of 153 consenting Moroccan patients between 16 years and 90 years of age, who were attending the gastroenterology department of Hospital University (CHU) Hassan II of Fez, Morocco, and who had undergone endoscopy for the diagnosis of abdominal pain or discomfort were included in this study. Three gastric biopsies were taken from each patient, and two biopsies (one from the antrum and one from the corpus) were fixed in 10% buffer formalin and used for histological examination and *H. pylori* detection. The presence of the bacterium was scored in a semiquantitative approach as 1, 2, and 3 (the grades denoting small, moderate, and large numbers of *H. pylori*, respectively).

The third antral biopsy was directly used for the molecular detection of *H. pylori*, *cagA* status *vacA s*, *vacA m*, and *vacA i* as described in a previous study.[3,4]

2.2. Saliva samples

A minimum of 1 mL of saliva was collected in a sterile disposal testing container prior to endoscopy, and the test was performed within 5 minutes in patients who either did not eat or drink within the hour preceding the test. These specimens were used to detect *H. pylori* antigens with the noninvasive "one-step *H. pylori* saliva antigen" (HPS) test (Ameritek, Everett, WA, USA). After the saliva specimens were collected, the test disk was opened and laid flat on a dry work surface. Then, four drops of saliva and two drops of buffer were added into the test tube, and four drops of mixture (saliva and buffer) were added into the sample well. If the purple color did not move across the "result window" in the center of the disk in approximately 30 seconds, two additional drops of mixture were added into the sample well. Finally, the test results were interpreted at 20−30 minutes. The presence of two color bands ("T" band and "C" band) within the result window, regardless of which band appears first, indicates a positive result, and the presence of only one purple color band indicates a negative result, where the result is considered invalid if no band is visible.

In this study, only samples showing concordant results on histology and PCR were considered. Therefore, a sample was considered positive for *H. pylori* infection when both of these tests were positive, and negative when both histology and PCR results were negative.

To compare the presence of *H. pylori* in saliva and gastric mucosa, two age groups were defined and correlated; Group 1 included patients aged $\leq$ 50 years of age, whereas Group 2 included the elderly patients.

Subsequently, we tested the association of *H. pylori* antigen presence in saliva with age, sex, *H. pylori* virulence factors, gastric disease outcome, and density of the bacterium on the gastric mucosa.

2.3. Statistical methods

The statistical analysis for our study was done using SPSS, version 20 (SPSS Inc., Chicago, IL, USA) software. Chi-square or Fisher's exact tests were applied to establish all statistical associations, and $p < 0.05$ was considered to be statistically significant. To determine the accuracy of the salivary test, only samples that showed concordant results in PCR and histology were considered for comparison and considered as a reference test, and some qualitative parameters were statistically calculated: sensitivity (ability to detect positive cases) = true positive/(true positive + false negative); specificity (ability to exclude negative cases) = true negative/(true negative + false positive); positive predictive value (PPV) = percent of true positives to all positive cases; negative predictive value (NPV) = percent of true negative extent to all negative cases; and accuracy (E) = (true positive + true negative)/(true positive + true negative + false positive + false negative).

2.4. Informed consent

All participants were informed about the study objectives, methods, confidentiality, and potential outcomes, and they

provided written consent for their participation. This study was approved by the Institutional Review Board of the Hassan II University Hospital of Fez, Fez Morocco.

3. Results

Saliva and biopsy specimens were obtained from 153 consenting patients for the purposes of this study. There were 85 (55.56%) men and 68 (44.44%) women, with an average age of 49 years (range, 16–90 years). Of these 153 patients, 101 (67.79%) had chronic gastritis, 36 (24.16%) had gastroduodenal ulcer, and 12 (8.05%) were diagnosed with gastric cancer. For each patient, *H. pylori* detection was done using saliva test, histopathology, and PCR. *H. pylori* was detected in 28 (18.30%), 87 (57.24%), and 69 (45.10%) tested patients by the saliva test, its histology, and PCR, respectively. The saliva test results were correlated to age and sex. This analysis indicated a higher rate of *H. pylori* antigen detected in Group 1 (≤50 years) compared with Group 2 ($p = 0.007$). No association was found between the rate of *H. pylori* detection in saliva and sex (Table 1).

Using the 90 *H. pylori*-positive and *H. pylori*-negative (by concordance of histology and PCR) results, *H. pylori* urease antigen was detected only in 14 (29.79%) cases in the saliva of gastric *H. pylori*-positive patients and negative in 39 (90.70%) cases with no gastric *H. pylori* infection (Table 2). The *H. pylori* detection results obtained via saliva test and PCR–histology according to age groups showed that the antigen was higher in younger (46.2%) than in older (8.3%) gastric *H. pylori*-positive patients (Table 3).

The *H. pylori* genotypes were determined using PCR. When the *cag A* status of *H. pylori* was determined, it was positive in 19 (40.43%) of 47 cases. However, the *vacA* gene was detected and characterized in 43 (91.49%) of 47 cases. The most dominant genotype in this series was *vacA s2m2i2*, with a rate of 31.91% ($n = 15$). No association was detected between saliva test detection and *cagA* or *vacA H. pylori* genotypes.

The rate of *H. pylori* detection by use of the saliva test did not show any correlation, nor was there any correlation with the pathological profile or with the density of *H. pylori* on the gastric mucosa.

To assess the accuracy of the salivary test, some qualitative parameters were first statistically calculated by taking into consideration the entire population, and then according to age groups, such as sensitivity, specificity, positive and negative predictive values, and accuracy. The concordant results of PCR and histology were used as reference. The values obtained for each parameter are reported in Table 4 and shows very low sensitivity in the studied population, especially in older patients compared with younger patients.

Table 1
Correlation of *Helicobacter pylori* with age and sex.

	Saliva test			
	Positive (%)	Negative (%)	*n*	*p*
Age group 1 (≤ 50 y)	20 (26.7)	55 (73.3)	75	0.007
Age group 2 (51–90 y)	8 (10.3)	70 (89.7)	78	
Male	16 (18.82)	69 (81.18)	85	0.85
Female	12 (17.65)	56 (82.35)	68	

Table 2
Comparison of saliva test with polymerase chain reaction (PCR)–histology results on *Helicobacter pylori* detection.

		Saliva test		
		Positive (%)	Negative (%)	Total
Histology and PCR	Positive	14 (29.79)	33 (70.21)	47 (52.22)
	Negative	4 (9.30)	39 (90.70)	43 (47.78)
	Total	18 (20)	72 (80)	90

Table 3
Comparison of saliva test and polymerase chain reaction (PCR)–histology results for *Helicobacter pylori* detection according to the age.

		Histology and PCR	
		Positive (%)	Negative (%)
Age group 1	Positive	12 (46.2)	2 (11.8)
	Negative	14 (53.8)	15 (88.2)
Age group 2	Positive	2 (8.3)	2 (8.7)
	Negative	22 (91.7)	21 (91.3)

4. Discussion

Different methods were developed for the noninvasive detection of *H. pylori*; some of them were based on immunological techniques and used whole blood, serum, urine, stool, or saliva as specimens.[5–8] *H. pylori* has been detected in dental plaques and saliva by PCR, culture, and rapid urease test.

The presence of the bacterium in the oral cavity was reported in 1989.[9] Nevertheless, it is unclear whether the oral cavity is a permanent or transient reservoir.[1,10,11] A number of recent publications have demonstrated that the human oral cavity is an excellent microaerophilic environment, and thus a potential reservoir for *H. pylori*.[12–15] However, other studies have shown that the oral cavity is most likely a transitory reservoir for *H. pylori* via regurgitation or vomiting.[16–18] The use of saliva or dental plaque as samples makes the diagnosis noninvasive, and several authors consider that it can provide a global picture of *H. pylori* in the stomach.[19] This is in contrast with results involving invasive tests that explore only a small portion of the gastric mucosal surface[20] and are subject to sampling error.[21] Nevertheless, the use of these specimens for the diagnosis is still extensively discussed.[9,15,17,22,23]

Table 4
Saliva test performance characteristics.

	Histology and PCR (%)				
	Sensitivity	Specificity	PPV	NPV	*E*
Saliva test (all population)	29.79	90.7	77.78	54.17	58.89
Age group 1	46.15	88.24	85.71	51.72	62.79
Age group 2	8.33	91.3	50	48.84	48.93

E = accuracy; PCR = polymerase chain reaction; NPV = Negative predictive value; PPV = Positive predictive value.

To our knowledge, the immunochromatographic test for the detection of urease antigen was performed on children's stool and urine, and showed a good performance with a high sensitivity and specificity.[24,25] However, there are several studies that used it on saliva.

The aim of the present study was to compare the presence of *H. pylori* in the stomach and in the saliva of Moroccan patients.

The use of concordant results of PCR and histology to determine the *H. pylori* status in gastric biopsy was chosen to increase the reliability and the accuracy of diagnosis. However, discordant results were obtained when comparing salivary *H. pylori* antigen and the invasive tests (histology and PCR). The results of this study showed a low *H. pylori* urease antigen detection rate in saliva (20% *H. pylori* positives) compared with the reference examination in gastric mucosa (52.2% *H. pylori* positives). This can be attributed to

(1) The low sensitivity (29.79%) of the used test, which may be related to the possible low affinities of the used monoclonal antibodies of *H. pylori* antigen
(2) The low inoculums concentration of *H. pylori* in the mouth, which may be explained by the hypotheses reported in other studies:
    (a) Ability of oral normal flora to affect the *H. pylori* growth by producing bacteriocin-like inhibitory proteins against *H. pylori* strains
    (b) Effect of yeast that protects *H. pylori* from the stressful conditions in the mouth and carries it to the human gastrointestinal tract
    (c) The short life of *H. pylori* in the oral cavity following the high oxygen concentration in this location[15,26]

However, evaluation of the saliva test is impractical because the presence of *H. pylori* in saliva is not confirmed and remains unclear, because studies evaluating the presence of this bacterium in saliva or oral cavity showed conflicting data. Also, there is no gold standard applicable in these milieu, and despite the use of PCR or nested PCR in some studies the detection of this bacterium is rare and ranges from 0% to 1.9%.[27−29]

Nevertheless, a high rate of positive HPS was found in the studies of Yee et al,[1] Song and Li,[30] and Yang et al.[31] These studies were conducted on Asian populations (Taiwanese, Chinese). This population focus helped to underscore the fact that: (1) the high genetic diversity of the bacterium observed between different geographical areas can account for the different behavior of the bacteria toward the environment in which it is located; and (2) the detection of *H. pylori* urease antigen can be age-dependent because all of these studies have considered the entire population. Overall, a precise comparison with other studies remains difficult, because the studied population and the reference tests used were different.

The detection of *H. pylori* using the saliva test in cases with negative results by both histological examination and PCR can be explained by: (1) the presence of the *H. pylori* antigen in the mouth even if the bacterium is not present[1,17]; and (2) the detection of urease antigen of other species of the *Helicobacter* genus (*Helicobacter felis* or *Helicobacter heilmannii*). Effectively, *H. felis* and *H. pylori ure* gene products showed a high degree of conservation,[32] and some epitopes were conserved among the urease of various gastric *Helicobacter* spp.[33]

In this study, considering that the saliva flow decreases at age 50 years and older,[34,35] and this may influence the *H. pylori* growth and thus the detection rate in this milieu, we determined the rate of bacterium detection in saliva according to patient age. The results showed a significant association between the rate of *H. pylori* antigen detection in saliva and age. A high rate was obtained in younger patients ($\leq$50 years old; $p = 0.007$). This finding was confirmed when comparing the saliva test and the concordant results of PCR−histology according to age. We conclude that the antigen is more detectable in younger (46.2%) compared with older (8.3%) gastric *H. pylori*-positive patients. In spite of the small size of the studied samples, our data do not support the idea of Sreebny,[35] who hypothesized that the low salivary secretion rates, related to advanced age, create favorable conditions for the growth of bacteria, including *H. pylori*.

In conclusion, this study demonstrated a low detection of *H. pylori* in saliva compared with the gastric mucosa, suggesting that saliva cannot be considered as a reservoir for *H. pylori*. Therefore, measuring the extent of *H. pylori* in saliva will not facilitate an accurate diagnosis of this bacterium in our study population.

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