

Oral Fluid Antibody Detection in the Diagnosis of Gastric *Helicobacter pylori* Infection

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ABSTRACT

The aim of this study was to evaluate an enzyme - linked immunosorbent assay (ELISA) for the detection of anti - *helicobacter pylori* (*H. pylori*) specific IgG antibodies in specimens of oral fluid.

All subjects over the age 18 years undergoing endoscopy for any reason were asked to participate in the study. Two groups of 44 patients in each were selected as HP⁺ and HP⁻. At the same time, 5 milliliters of unstimulated saliva was collected from these patients, and the antibody titration against H.P. was evaluated by “ELISA” method.

In overall, the level of salivary antibody in H.P⁺ group was significantly more than those in H.P⁻ group (P<0/001). In the best cut - off, sensitivity and specificity obtained in this test were respectively 88.6% and 81.8% and positive predictive and negative predictive values were determined as 83% and 87.8%, respectively. Positive Likelihood Ratio and negative likelihood ratio were 6.8 and -0/083, respectively.

Oral fluid ELISA is relatively a comfortable, fast and noninvasive test for diagnosis of *H. pylori* infection.

Keywords: Antibodies, Enzyme-Linked Immunosorbant Assay, *Helicobacter pylori*

INTRODUCTION

Helicobacter pylori (*H. pylori*) is now accepted as the major cause of chronic active gastritis, deodenal and gastric ulceration,¹ and is associated with the development of gastric carcinoma.² *H. pylori* infection is also strongly associated with mucosal- associated tumors (MALTs) and it has been reported that eradication of *H. pylori* may lead to improvements in tumor histology.³

Oral fluid is a complex mixture of saliva secreted by parotid and other salivary glands, gingival cervical fluid from the gingival crevice and secretions from

the mucous membranes.

This mixture is sometimes called mixed saliva. The term “oral fluid” is used in this paper to describe this mixed saliva. Gingival Cervical Fluid (GCF)¹ is a transudate present in the gingival crevice in the mouth. It more closely resembles serum than salivary gland secretion. IgG, present in oral fluid, is derived from this transudate and is found at a concentration substantially lower than the serum concentration; serum levels are 500–1500 times higher than oral fluid levels.⁴ The oral fluid levels of IgG are still of sufficient concentration to be detectable by immunoassays. Immunoassays for the detection of antibodies to HIV,⁵⁻⁸ hepatitis A,⁸ hepatitis B virus core,⁸ rubella⁸ and more recently *H. pylori*⁹⁻¹² in oral fluid have been described. Oral fluid has been used in epidemiological studies of HIV infection in developing countries and

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Oral Fluid Antibody in Diagnosis of *H. pylori*

potentially has a role in seroepidemiological studies of other infectious agents.¹³ The definitive diagnosis of *H. Pylori* infection has been based primarily on the isolation of the bacterium in culture or detection of organism in histological sections of gastric biopsy specimens obtained at endoscopy. Several serological methods have also become available, and the presence of *H. pylori* IgG and IgA antibodies in serum have been found to correlate with infection. It has been recommended that salivary antibody test results can be used to screen patients prior to gastroscopy or to determine the effect of antimicrobial therapy in the eradication of *H. Pylori*. For such an assay to be clinically useful, it must accurately detect the presence or absence of *H. pylori* infection. In the present study, we compared the diagnostic accuracy of the enzyme-linked immunosorbent assay (ELISA) for the detection of anti-*H. pylori* specific IgG in oral fluid with rapid urease test and with serum-based assay.¹⁴

MATERIALS AND METHODS

Patients attending for upper gastrointestinal tract endoscopy were asked to take part in the study and an informed consent was obtained from those patients willing to participate. A sample of oral fluid (5 milliliters of unstimulated saliva) and a blood sample (5 milliliters of venous blood) were collected before endoscopy and one biopsy for Rapid Urease test was collected from the gastric antrum during endoscopy.

A total of 88 patients all over the age of 18 years participated in the study. Two groups each comprising of 44 subjects, were selected as H.P⁺ (Positive Rapid Urease test and serum – based ELISA, as the true positive subjects with 90% sensitivity and H.P⁻ (Negative Rapid Urease test and serum – based ELISA test, as the healthy subjects with 70% and 90% specificity, respectively) .

The following patient groups were excluded from the study: Those <18 years; pregnant women; patients with bleeding diatheses and those on anticoagulants; patients who had taken antibiotics, or proton pump inhibitors or bismuth preparations during the previous 4 weeks.

The urease test (CP test: yamanouchi Pharma) was carried out according to the manufacturer's instruct-

tions, and samples were examined for the presence of positive red color at 30 minutes and 24 hours, serum and saliva antibodies against *H. pylori* antigen were detected by ELISA, using a commercially available kit (Italain Radim Kit). In order to achieve optimal accuracy saliva samples were diluted to 1:2 and serum samples were diluted to 1:300.

In brief, a whole *H. pylori* strain isolated from samples was used as a source of antigen, diluted with coating buffer, added to each well, and incubated for 2h at 37°C, the plates were washed with washing buffer, and binding sites were blocked by addition of 2% serum albumin in washing buffer and incubation for 18h at 4°C, diluted saliva samples were added separately to each well and incubated for 90 minutes at 37°C. Anti-human antibody conjugated with horseradish peroxidase was added and incubated for 1h at 37°C, and mixed with tetra methylbenzidine as a chromogen. The colorimetric reaction was then prolonged for 15min at room temperature in the dark and terminated with the addition of 50µ lit of 4 N H₂SO₄ per well. The absorbance value (optical Density) at 450 nm was recorded with an automated plate reader.

The sensitivity, specificity and productivity values (positive and negative) and likelihood Ratios (positive and negative) values of the oral fluid ELISA were calculated by using the detection *H. pylori* in antral biopsy specimens by Rapid Urease test and serum – based test, as the “true positive”.

RESULTS

A total of 88 patients including 44 males and 44 females with a mean age of 44 ± 17.8 participated in this study and were divided into two groups (H.P⁺, H.P⁻).

In overall, the level of salivary IgG antibody in H.P⁺ group was found significantly more than those in the H.P⁻ group (Figure 1). Antibody titration in serum and oral fluids in Urease positive patients and Urease negative subjects is shown in tables 1 and 2. The P value of less than 0.001 addressing the difference between antibody titer of the two groups is provided in table 3.

Table 1. Antibody titration in serum and oral fluids of Urease positive patients.

Samples	No.	Results in Antibody Titration			
		Range of Titers	Mean Titers	Standard Deviation	Standard Error
Serum	44	2.3-110	40.9	29.3	4.9
Oral Fluid	44	16.2-50	42.48	28.11	4.23

Table 2. Antibody titration in serum and oral fluids of Urease negative subjects.

Samples	No.	Results in Antibody Titration			
		Range of Titers	Mean Titers	Standard Deviation	Standard Error
Serum	44	0.2-48	10.4	11.9	2.2
Oral Fluid	44	0.22-14.2	12.71	6.49	0.97

Table 3. Mean of oral fluid antibody titers in two groups.

Groups	No.	Mean Titers	Standard Deviation	Standard Error
Positive	44	42.4818	28.1181	4.2390
Negative	44	12.7173	6.4912	0.9786

P-value : 0.000

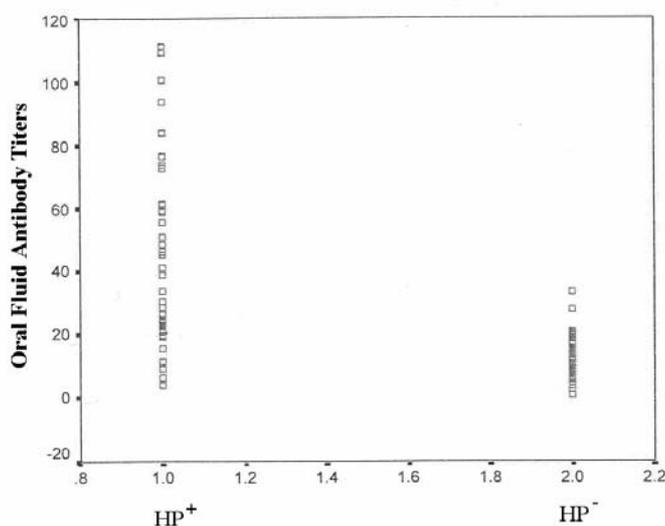


Figure 1. Comparison of antibody titer in two groups.

The best cut – off for determining sensitivity and specificity of the test was also determined and found 18.6 as shown in figure 1.

Sensitivity and specificity obtained in this test were 88.6% and 81.8%, respectively. Positive predictive value and negative predictive value were found 83% and 87.8%, respectively. The positive and negative likelihood Ratios were determined as 6.8 and –0.083, respectively.

DISCUSSION

The administration of antimicrobial agents along with standard anti-ulcer medications for patients with peptic ulcer disease and *H. pylori* infection has been recommended in order to eradicate the organism and thereby to prevent ulcer relapse. Therefore, the ability to reliably detect infection caused by *H. pylori* has become an important determinant in the management of these patients. Endoscopy with gastric biopsy has been the standard diagnostic procedure. This allows visualization of the gastroduodenal mucosa, confirmation of the presence of ulcers, and provision of tissue for microbiology and histologic examinations. However, it is a costly and invasive procedure with potential risks and discomfort for the patients. Radiolabelled urea breath tests have been found to be accurate noninvasive means of detecting *H. pylori* infection,^{15,16} but these tests are not widely available .

In the past few years, commercial enzyme immunoassays have been developed for the detection of serum *H. pylori* antibodies. Although several studies have found an excellent correlation between *H. pylori* serology and the presence of infection,¹⁷ other studies have failed to reproduce these results, the reported sensitivities and specificities of commercially available serologic kits have ranged from 68 to 97% and 53 to 83%, respectively.¹⁸

Preliminary results suggested that serology might play a useful role in screening patients prior to endoscopy¹⁸ or in monitoring the effect of antimicrobial therapy directed at the eradication of *H.pylor*,¹⁸ although these findings need to be confirmed in larger clinical trials.

Detection of antibodies in saliva has been used increasingly in the past few years for the diagnosis of a variety of infectious disease.¹⁹ The use of this type of specimen offers definite advantages including ease of sample collection, lack of patient discomfort, and no risk of needle stick injury. Although studies have examined the use of saliva antibody tests in epidemio-

Oral Fluid Antibody in Diagnosis of *H. pylori*

logic screening or surveillance,²⁰ there have been fewer evaluations of these tests for the diagnosis of disease in individual patients. An accurate assay for the detection of *H. pylori* antibodies in saliva would be a useful and noninvasive way to identify infection, permit selective use of endoscopy, and monitor the response to antimicrobial therapy.

The sensitivity (88.6%) and specificity (81.8%) found in the present study are comparable to those reported previously for salivary antibody tests used to diagnose *H. pylori* infection.^{15,17,21}

Patel et al. assessed a modification of a serum ELISA for the measurement of salivary *H. pylori* IgG in 119 patients referred for endoscopy. The sensitivity and specificity of the test was 85% and a good correlation between levels of salivary and serum IgG antibodies was found.^{11,17} Clancy and co-workers compared the salivary - based assay with serology and found a good correlation of salivary antibody results with serum antibody titers. There appeared to be a more rapid decline in salivary antibody levels than in serum antibody levels following antimicrobial treatment.²¹

Several factors might have contributed to the discrepant results obtained in the present evaluation. Salivary samples were stored at room temperature prior to testing, as recommended by the manufacturer.²¹

It is possible that antibody degradation by salivary protease might have contributed to False-Negative results. False-Negative results may also possibly occur in patients recently infected, before an antibody response has developed. False-Positive test results may be due to the presence of cross-reacting bacterial antibodies. Apparently False-Positive results may also, occur because of sampling error in obtaining gastric biopsy specimen: infection of the gastric mucosa may be patchy, so that examination of biopsy specimens may occasionally fail to identify truly infected patients.

While endoscopy and tissue biopsies remain irreplaceable for the definitive confirmation of the *H. pylori* status, the present study supports a role for the salivary IgG antibody response in screening patients. Although certain ulcers and gastritis occur independently of *H. pylori* infection, a negative anti-*H. pylori* salivary IgG status may help in reducing the number of unnecessary endoscopies, specially in low-

risk patients, such as subjects under 45 years of age.^{22,23}

These results indicate that the "ELISA" test in detection of salivary IgG antibody against *H. pylori* is moderately accurate enough and its sensitivity and specificity is the same as the "ELISA" test which is used to detect the IgG level in serum. Therefore, this test is moderately accurate method with a high sensitivity and a high negative predictive value and a high positive likelihood ratio for detecting *H. pylori* infection in adults.

ACKNOWLEDGEMENT

This work was supported by a grant from office of research of Hamadan University of Medical Sciences.

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