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ORIGINAL ARTICLE

Saliva or serum, which is better for the diagnosis of gastric Helicobacter pylori infection?

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ABSTRACT

Background: Helicobacter pylori is known as an agent which may involve in the occurrence of peptic ulcer, gastric cancer and also other known and unknown diseases. Treatment of the infection with antibiotics eradicates the disease and prevents its pathologic effects. A noninvasive and inexpensive method for detection of the infection is needed. In this study the diagnostic values of serum and saliva anti H. pylori IgG was evaluated.

Patients and methods: The saliva and blood samples were collected from 114 patients who underwent upper GI endoscopy and gastric biopsy. Tissue samples were examined by rapid urease test and microscopic study. Saliva and serum samples were tested by ELISA-based test for anti H. pylori IgG, using a commercial kit.

Results: From 114 cases, 61(53.5%) patients were positive for H. pylori in rapid urease test and microscopic study and 53(46.5%) were negative in both tests. Rates of positive result for H. pylori in patients with and without peptic ulcer were almost similar. Mean values of anti H. pylori IgG in saliva and serum of H. pylori positive patients were higher than H. pylori negative patients. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of tests in saliva were 83.6%, 71.7%, 77.3%, 79.1%, 78.1% and in serum were 90.2%, 86.8%, 88.7%, 88.4% and 88.6% respectively.

Conclusion: It was concluded that ELISA-based anti H. pylori IgG test in saliva could be used as an alternative diagnostic test in the absence of other invasive procedures.

Keywords: *Anti-H. pylori IgG, ELISA, Saliva*.
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INTRODUCTION

Helicobacter pylori (H. pylori) infection induces gastric inflammation in virtually all hosts, and such gastritis increases the risk for gastric and duodenal ulceration, distal gastric adenocarcinoma, and gastric mucosal lymphoproliferative disease (1-4). Marshall and Warren succeeded in culturing H. pylori in 1983 (1). Although H. pylori infection can be treated, the organism still infects approximately one half of the world's population (5). The treatment of H. pylori is complicated,

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requiring at least two different antibiotics plus gastric acid suppression for successful H. pylori eradication (6). The high prevalence and the association with peptic ulceration and gastric cancer indicate that simple, noninvasive methods should be chosen to diagnose H. pylori infection. The tests for the diagnosis of H. pylori infection fall into two categories. The invasive methods are biopsy-based including culture, rapid urease test (RUT) and histology and non-invasive testing like urea breath test (UBT) (7), serology and body materials analyzing (feces, urine and saliva). Enzyme immunoassays, which are simple, reproducible and inexpensive, can detect either antigen or antibody. Although serum-based enzyme immunoassay has been used to detect H. pylori infection (8,9), it can not distinguish between past and present infections as antibody titers decline very slowly even after successful H. pylori eradication (10).

The assay requires blood sample collection, which is not always suitable for children. Human body materials such as feces, urine and saliva, which are collected by totally non-invasive procedures, have been subjected to ELISA for the diagnosis of H. pylori infection (11,12). In this study the value of salivary test for H. pylori infection was assessed by comparing its results with those obtained by gold standard methods.

PATIENTS and METHODS

This was a cross sectional study carried out from May 2005- April 2006. The patients recruited from the Gastroenterology outpatient clinic, Imam Khomeini Hospital underwent gastrointestinal endoscopy. Patients receiving anti H. pylori drugs, non-steroid anti-inflammatory drugs and proton pump inhibitors 8 weeks before endoscopy and also those suffering from other inflammatory diseases and GI tract cancer were excluded from the study. All subjects underwent endoscopy. Chronic active gastritis was studied in gastric mucosa and also gastric biopsies were checked with rapid urease test and histological studies for presence of the bacterium. Specimens were stained with giemsa to identify H. pylori. The patients were divided into two groups, fifty three non-infected individual (46.5%) with negative rapid ureas test and negative histological studies, and sixty one new case patients (53.5%) suffering from H. pylori infection. Totally 3 ml of venous blood and 2 ml of unstimulated saliva were obtained from all subjects. Blood and saliva were sent to laboratory under standard conditions. The saliva samples were kept frozen at -20°C until analysis. Sera were separated from blood specimens and stored at -20°C until the day of test. Serum and saliva IgG against H. pylori antigens was detected by ELISA after diluting 1:100 and 1:4 by the kit diluent, respectively (Monobind, Germany). All data were expressed as the mean± SD and statistical significance was set at $p<0.05$. The data were analyzed with student t-test and chi-squire test by SPSS version 13.0 software. Specificity, sensitivity, positive and negative predictive values and precision of the saliva test were calculated.

RESULTS

A total of 114 patients [59 male (51.8%), 55 female (48.2%)] with the mean age of 44.68 years (15-85 years old) were participated in this study. Fifty three cases (46.5%) who were negative for H. pylori by either urease rapid test or histological study. H. pylori was detected in 61 patients (53.5%) by the two tests. H. pylori positive patients showed significantly higher titers of anti H. pylori IgG (1.77 ± 0.950) in serum samples than H. pylori negative subjects (0.547 ± 0.443) ($p<0.001$). H. pylori-positive patients also showed significantly higher titers of anti H. pylori IgG (0.55 ± 0.238) in saliva samples than H. pylori negative subjects (0.279 ± 0.274) ($p<0.001$) (figure 1).

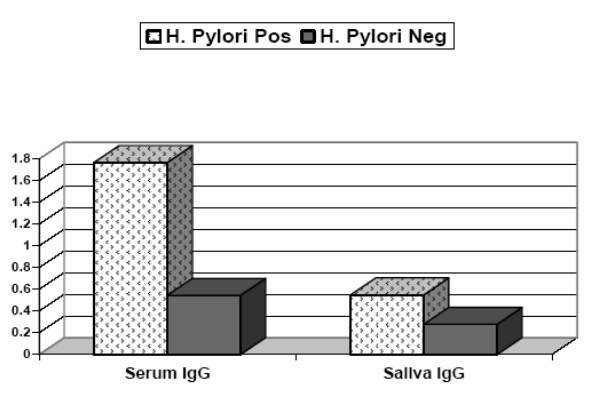

Figure 1. Optic density (Index of anti-H.pylori IgG titer) in serum and saliva in the H.pylori positive and negative subject

True-positive rates (sensitivity) and false-positive rates (1-specificity) were calculated at different cut-off values and plotted to obtain a receiver operating characteristic (ROC) curve (figures 2 and 3). Commercially kit cut off was 20 U/ml with OD near 0.8 for sera and 8 U/ml with OD near 0.33 for saliva.

In this analysis, the point that enclosed the largest area, represented the best compromise between sensitivity and specificity and was chosen for our initial analysis. At this cut off rate, the salivary IgG test was considered positive for 51 of 61 H. pylori positive patients (sensitivity 83.6%) and 15 of 53 H. pylori negative patients (specificity 71.7%) (table1).

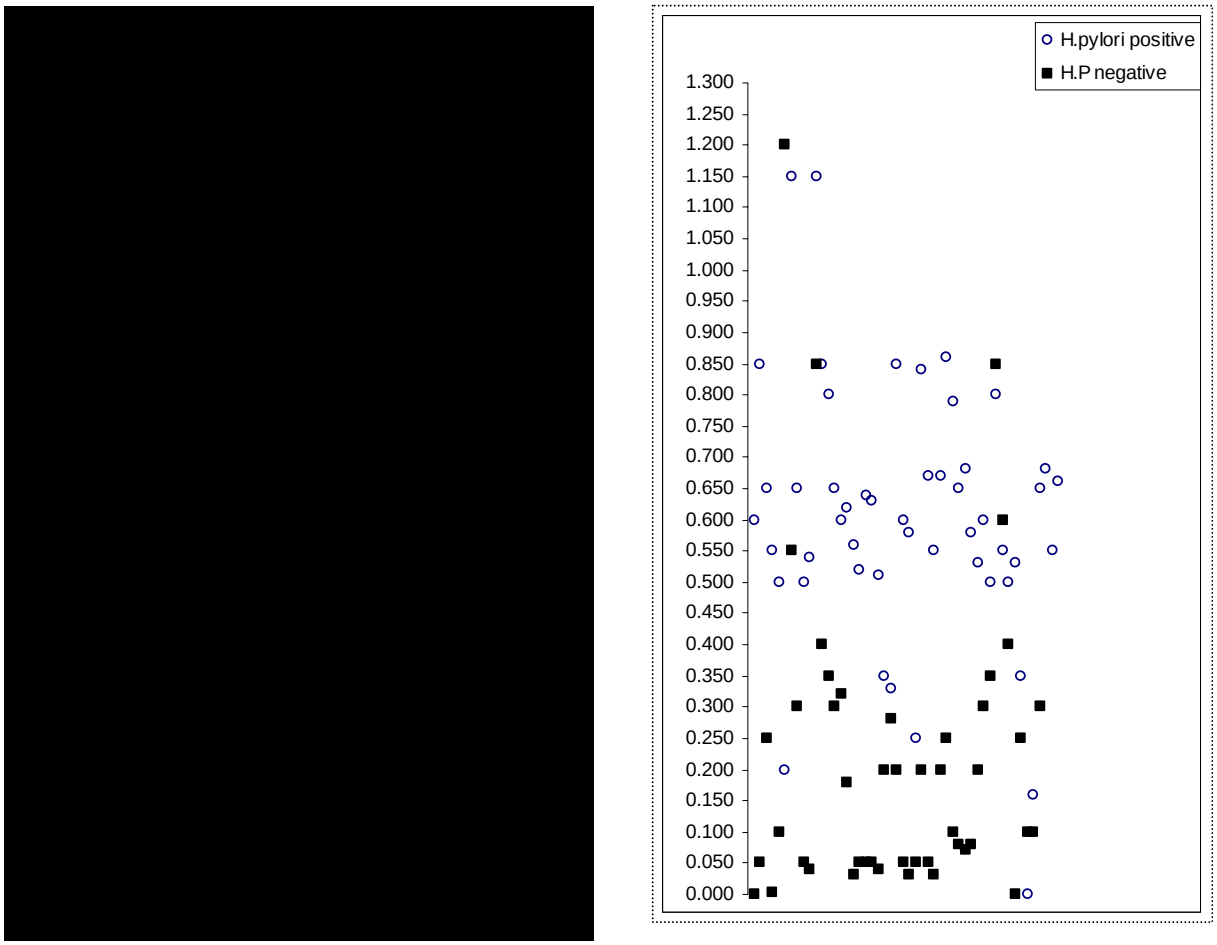

Figure 2. The values of Anti-H.pylori IgG (optical density) in serum (Panel A) and saliva (Panel B) in 114 patients. H.pylori positive and negative patients are shown by red circles and blue squares. Dotted lines represent the cut off points.

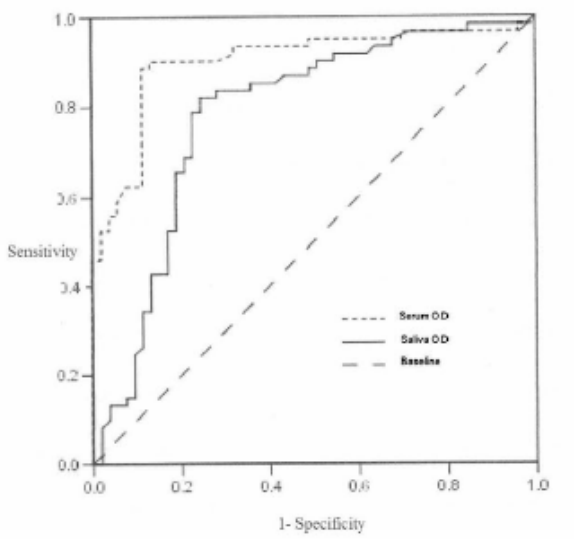

Figure 3. The ROC curve of ELISA test for serum and saliva anti-H. pylori IgG

Table 1: The sensitivity, specificity, positive and negative predictive values and precision (95% CI) of anti-H.pylori IgG tests in serum and saliva

	Saliva	Serum
Sensitivity	83.6(76.7-90.4)	90.2(84.6-95.7)
Specificity	71.7(63.3-80)	86.8(80.5-93)
Positive Predictive Value	77.3(69.5-85)	88.7(82.8-94.6)
Negative Predictive Value	79.1(71.5-86.6)	88.4(82.4-94.3)
Precision of Diagnosis	78.1(70.4-85.4)	88.6(82.7-94.5)

DISCUSSION

The serologic tests are based on the detection of specific anti H. pylori IgG antibodies in the patient's serum. Serology was the first non-invasive technique, even though it has some limitations (13, 14). The most important point is that we are not able to distinguish between active infection and a pervious contact. Some studies have reported that saliva is a non-invasive sample for detection of antibodies to H. pylori. Since saliva can be obtained easily, it has been analyzed by enzyme immunoassay to detect antibodies to H. pylori. Saliva contains IgA and low levels of IgG, the former being produced locally by salivary gland (15).

The salivary IgG is mainly derived by transudation from blood to gingival fluid (12). In this study, we measured salivary and serum H. pylori IgG with commercially-ELISA kit. We attempted to assess the value of measuring salivary H. pylori antibodies in confirming the presence of infection in patients. Collection and testing salivary specimens is non-invasive, painless, convenient, and fast and carries no risk of needle stick injury. Specificity and sensitivity of ELISA sera were 83.6% and 71.7% for saliva and 86.8% and 90% for sera, respectively. There was a good correlation between levels of salivary and serum IgG antibodies, and there was no significant different between them regarding specificity and sensitivity ($p > 0.05$).

Results of this study are comparable with majority of other similar studies. The specificity and sensitivity of ELISA in detection of H. pylori in saliva samples were reported 71% and 82% respectively (7), which were similar to our results. On the other hand, our results are also in agreement with those reported by Simor et al in the case of detection of H. pylori infection by analyzing saliva (16).

It was concluded that ELISA for detection of salivary anti H. pylori IgG is a rapid, non-invasive, inexpensive test that may be considered as an alternative to the serum IgG test when blood samples are not available or in pediatric population (17,18). While endoscopy and tissue biopsies remain irreplaceable for the definitive confirmation of the H. pylori status, present study supports a role for the salivary IgG antibody response in screening patients with dyspepsia.

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, especially in low-risk patients (13).

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