Original Article

Comparison of Salivary Anti Helicobacter pylori IgG with Serum IgG and Bacteriological Tests in Detecting Helicobacter pylori Infections

H. Ghasemian safaei PhD*, A. Fazeli PhD*, H. Tamizifar PhD*, N. Rashidi MSc**

ABSTRACT

Background: This study was conducted to compare the efficacy of enzyme-linked immunosorbent assay (ELISA) for detecting anti-Helicobacter pylori (H. pylori) specific IgG antibodies in specimens of oral fluid and serum with bacteriological tests.

Methods: Antral biopsy specimens, as well as serum and oral fluid samples were collected from 97 patients who underwent upper gastrointestinal endoscopy. The presence or absence of current H. pylori infection was determined by culture, histology and urease detection. Anti-H. pylori specific IgG was detected in serum and oral fluid, using an established lab-made, and a commercial ELISA kit. The obtained data were compared with results of bacteriological tests.

Results: In all, 62 (64%) of 97 patients were positive for H. pylori by one or more of the gold standard tests (culture, histology and urease detection). Lab-made enzyme-linked immunoassay of oral fluid had a sensitivity and specificity of 92% and 83% respectively. A sensitivity and specificity of 87% and 83%, respectively, was obtained with the commercial kit. Lab-made enzyme-linked immunoassay of serum samples had a sensitivity and specificity of 90% and 88%, respectively. A sensitivity of 86% and specificity of 86% was obtained with the commercial kit.

Conclusion: Detection of anti-H. pylori specific IgG in oral fluid by ELISA is comparable in sensitivity and specificity with serum based methods. Oral fluid based ELISA could provide a reliable, non-invasive method for the diagnosis of H. pylori infection. Saliva testing may have a role in epidemiological studies.

Key words: Helicobacter pylori, ELISA, Oral fluid

Helicobacter pylori (H. pylori), a Gram negative spiral bacillus, is the major cause of chronic gastritis, peptic ulcer and probably gastric carcinoma and lymphoma[1,2,3]. The prevalence of H. pylori infection in different countries varies between 25% and 90% and is related to age[3]. Investigations have shown that H. pylori infection is acquired in childhood[4,5,6]. Therefore screening and detection of H. pylori infections are of great significance.

Diagnosis of H. pylori infection has been based on the isolation of bacteria from culture media or detection of the organism by Gram stain, urease test or gastric biopsy specimens obtained by endoscopy. These techniques are invasive, time consuming and expensive. Serological methods are also available which are non-invasive and do not require endoscopy[2,7,8,9].The urea breath test, while not invasive, may be limited by its cost or the use of radiolabeled compounds[15].

Recently, quantitative immunoassay has been used for determination of salivary H. pylori IgG antibodies[10,11,13,14]. The assay can be readily performed in the general practice setting, specimen collection is easy for the patients and health care personnel, and there is a greatly reduced risk of blood-borne infections[11,14,15]. Several studies have shown that salivary H. pylori antibody levels run at parallel levels to circulatory IgG[1,2,6,7].

Enzyme-linked Immunosorbent assay has been widely used in epidemiological studies and more recently for evaluating the results of therapeutic regimens for H. pylori infection[2,7,22].

The present study evaluated H. pylori specific IgG antibody in both saliva and serum of patients using lab-made antigens and a commercial kit[1,2,3,5,12]. We

*Assitant Professor, Department of Microbiology, Isfahan University of Medical Sciences, Isfahan, Iran.
**Postgraduate Student, Department of Microbiology, Isfahan University of Medical Sciences, Isfahan, Iran.
Correspondence to: Dr Hajieh Ghasemian safaei, Department of Microbiology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: ghasemian@med.mui.ac.ir

compared the results with those of histological methods, culture and rapid urease test from patients referring for endoscopy due to gastrointestinal symptoms.

Materials and Methods

Ninety-seven patients with gastrointestinal disorders who underwent endoscopy at Al-Zahra hospital, Isfahan, Iran, were included in this study. Oral fluid and blood samples were collected before endoscopy and two specimens were obtained from the gastric antrum during endoscopy. Demographic and clinical questionnaires were filled for all the patients.

Patients who were taking non-steroidal anti-inflammatory drugs (NSAIDs), bismuth-containing drugs, antibiotics or omeperazole were excluded, because these drugs are known to influence the diagnostic accuracy of tests for H. pylori infection.

Blood, oral fluid and biopsy specimens were sent to laboratory under standard conditions. The oral fluid samples were frozen and stored at -20ºC until required. Sera were separated from blood specimens and stored at -20ºC until the day of the test. One biopsy sample was inoculated directly into urea broth and incubated at 37ºC. The appearance of red color was indicative of a positive result within the following 20 minutes to 4 hours. The other biopsy specimen was inoculated onto BHI agar (containing 10% fetal calf serum, 0.4% supplement and 5-8% blood) and was incubated microaerophilically at 37ºC for up to 4-5 days. Gram stain, oxidase, catalase and urease tests were performed on growing colonies of H. pylori. Positive cultures were subcultured. All of the resultant colonies were collected from culture, and a suspension equaling third of the McFarland tube was made. Suspension of the cells was sonicated in 8 cycles of 45 seconds with 45 second intervals. The protein concentration in the suspension was 4.4 mg/ml. Four serial dilutions of 1/500, 1/1000, 1/2000 and 1/4000 were obtained from this suspension (4.4 mg/ml) with concentrations of 8.8, 4.4, 2.2 and 1.1 µg/ml, respectively. Wells of ELISA plates were coated with these serial dilutions and tested with positive and negative controls. The 1/4000 dilution with a standard cut off of 0.4 was chosen, because it offered the best compromise between sensitivity and specificity. Coated plates with this concentration comprised our lab-made plates. The following method was used for lab-made antigen-based ELISA plates: A microtitration plate was coated with antigen at 4ºC overnight. On the following day the plate was emptied and 100 µl bovine serum albumin (1%) was added to each well. The plate was incubated for 1 hour at room temperature and then washed with Phosphate Buffered Solution (PBS) and 100 µl of oral fluid was added to each well. The plate was washed and 100 µl peroxidase-labeled antihuman IgG was added to it. The plate was incubated for 30 minutes at 37ºC and was then washed. 100 µl substrate solution (equal amounts of Tetramethyl Benzidine as chromogen and substrate buffer) was added to each well and incubated at room temperature in the dark for 15 minutes before adding 100 µl of 1N sulfuric acid to stop the reaction. Optical Density (OD) reading was recorded at the wavelength of 450 nm. The specificity and sensitivity of ELISA (Radim Co, and lab-made) tests were calculated, using H. pylori detection by culture, histology or urease production, or any combination of these as the gold standard.

Results

A total of 97 patients (54 female, 43 male) participated in this study. H. pylori was detected in 62 patients (64%) by either urease test, culture, or histology as follows:

19 patients were positive for H. pylori by all three tests, 31 patients by Gram stain and urease test, and 12 patients only by culture. Thirty-five patients were negative for H. pylori by all three tests (Table 1).

Table 1. Results of the bacteriologic tests of biopsy specimens

Culture	gram stain	urease detection	Number of patients
+	+	+	19
+	+	-	0
-	+	+	31
+	-	+	0
+	-	-	12
-	-	-	35

All saliva and serum samples were assayed for H.pylori IgG with commercial kit and lab-made antigen, using ELISA.

Of 97 saliva samples, 63 (65%) were positive and 34 were negative when lab-made antigen was used. Sixty saliva samples (62%) were positive while the

remaining 37 were negative (Table 2) when the commercial kit was used.

With the lab-made antigen, 60 (62%) were positive and 37 were negative. 58 samples (60%) were positive and 39 were negative with the commercial kit (Table 2).

Specificity and sensitivity of oral fluid ELISA were 83% and 92% respectively, using the lab-made kit, and 83% and 87 % respectively, using the commercial kit.

Specificity and sensitivity of serum ELISA were 88% and 90% respectively, using the lab-made kit, and 86% and 86% respectively, using the commercial kit (Table 2).

Table 2. Results of oral fluid and serum assays.

		Gold Standard			
Assay	**result**	**+**	**-**	**Sensitivity**	**Specificity**
Oral fluid ELISA (Lab-made kit)	+	57	6	92%	83%
	-	5	29		
Oral fluid ELISA (Commercial kit)	+	54	6	87%	83%
	-	8	29		
Serum ELISA (Lab-made kit)	+	56	4	90%	88%
	-	6	31		
Serum ELISA (Commercial kit)	+	53	5	86%	86%
	-	9	30		

Discussion

The close association of H. pylori with chronic gastritis, peptic ulcer and gastric cancer calls for the adoption of a new screening approach, based on its pathogenesis.

H. pylori infection is the major cause of chronic gastritis and peptic ulcer, and possibly gastric carcinoma and lymphoma[1,2,3]. Investigations have shown that in most populations, H. pylori infection is acquired in childhood[4,5,6]. Studies in Isfahan, Iran, have shown that the prevalence of the infection ranges between 50% and 70%[18,19]. H. pylori can be detected with high sensitivity and specificity by non-invasive serology techniques and urea breath test. Measurement of serum IgG antibodies to H. pylori can be used reliably for detecting current infections[13,15,16,17]. Salivary IgG can be measured; nevertheless, its diagnostic value has yet to be established[2,15,17].

Of 97 patients studied with bacteriological methods (direct smear, urea test and culture), 62 (63.9%) were H. pylori positive. Navabakbar and Nafisei reported that 50% - 70% of patients evaluated with bacteriological methods were H. pylori positive in Isfahan[18,19]. Simoor et al, Marshal et al, and Christie showed that 50%, 42% and 40% of their patients were H. pylori positive with bacteriological methods[1,2,4].

In this study, we measured salivary and serum H. pylori IgG with commercial kit (Radim Co., Italy) and lab-made kit, with ELISA. We attempted to assess the value of measuring salivary H. pylori antibodies in establishing the presence of infection in patients. Collecting and testing salivary specimens is non-invasive, painless, convenient, and fast and carries no risk of needle stick injury. Whole-cell antigen for lab-made kit was prepared via sonication of cultured bacteria, consistent with the protocol used by Marshal and Luzza[1,3]. Iranian strains of H. pylori were used in lab-made antigens, thus offering advantages such as higher specificity, availability and low cost.

Testing saliva, specificity and sensitivity of ELISA were 83% and 92%, respectively, with lab-made antigen, and 83% and 87% respectively, with the commercial kit. Chi-square test showed no significant difference between the specificity and sensitivity of the two kits ($P>0.05$).

Testing serum specimens specificity and sensitivity of ELISA were 88% and 90% respectively with the lab-made antigen, and 86% and 86% respectively, with the commercial kit. Chi-square test

showed no significant difference between the specificity and sensitivity of the two kits (P>0.05).

There was a good correlation between levels of salivary and serum IgG antibodies, and there was no significant difference between them regarding specificity and sensitivity (P>0.05). Marshal[1] reported that specificity and sensitivity of ELISA were 85% and 94% respectively, with lab-made antigen used for testing saliva, and 91% for both, with the lab-made kit (made as in our study) used for testing serum specimens.

Fransisco Luzza showed that specificity and sensitivity of ELISA were 82% and 93% respectively, with lab-made antigen used for testing saliva, and 3% - 6% higher when testing serum; which were similar to our data[3]. Simoor reported specificity and sensitivity of 75% and 81%, respectively for ELISA with lab-made antigen used for testing saliva[2], which were less than our results.

In this study immunoglobulin degradation by salivary protease possibly contributed to false negative Helisal (Helicobacter salivary) assay results.

In addition, some patients may have a low inoculum of organisms. False negative results may also occur in recently infected patients, before an antibody response has developed. False positive test results may be due to the presence of cross-reacting bacterial antigens. Also the biopsy specimens may have failed to detect H. pylori owing to patchy infection. False positive results may also be seen in patients who have undergone eradication therapy for H. pylori but may remain antibody-positive for at least six months after treatment, while biopsy is negative for H. pylori[1,2,3,4,5].

In this study the prevalence of H. pylori in patients with gastritis was 48% which was higher than in other patients; this was similar to Marshal's results[1]. In our study, one patient, positive for H. pylori proved to have gastric carcinoma, a likely hint to the presumed correlation between H. pylori infection and carcinoma. Chronic gastritis may advance towards gastric carcinoma, but further investigations are warranted[20]. Hence, early diagnosis and treatment of H. pylori infections, as well as an effective screening policy are of great importance. Seventy percent of patients with gastric ulcer were H. pylori positive. Five individuals with normal saliva had H. pylori, which may be due to new infection, undetectable antibodies or non-pathogenic strain contamination. Based on our findings, there is no significant difference in the rate of infection between male and female individuals. Incidence of infection increases with age. The lowest rate of infection (48%) was seen in the 16-25 years age range, and individuals aged between 66 and 75 years had the highest rate of infection (83%). Our results are consistent with similar investigations[21].

We believe that salivary anti H.pylori IgG test prior to endoscopy is a useful diagnostic screening test for seroepidemiological studies. Negative results may help reducing the number of unnecessary endoscopies, and for determining the outcome of antimicrobial therapy for eradication of H. pylori infection.

References

1. *Sunnerstam B, Kjerstadiust, Jansson L, Giesecke J, Bergstrom M, and Ejderhamn J. Detection of H. pylori antibodies in a pediatric population: comparison of three commercially avilable serological tests and one in house enzyme immunoassay. J. clin. Micro. , 1999; 37(10): 3328-3337.*
2. *Brade B, Posselt HG, Ahvens P, Kitz R, Dietrich CF, Caspary WF. New immunoassay in stool provides an accurate noninvasive diagnostic method for Helicobacter pylori screening in children. Pediatrics, 2000 Jul; 106 (1 pt 1): 45-7.*
3. *Malaty M, Logan D,Graham D, Ramchatesingh J, and Reddy G. Helicobacter pylori infection in asymptomatic children: comparison of diagnostic tests. Helicobacter, 2000 ; 5: 155.*
4. *Marshal B, Howat A and wright P. Oral fluid antibody detection in the diagnosis of Helicobacter pylori infection. J.med. Microbiol, 1999; 48: 1043- 1046.*
5. *Simor A, Lin E, Saibil F, Conen L, Louiempearen S, Donhoffer H. Evaluation of enzyme immunoassay for detection of salivary antibody to Helicobacter pylori. J. clin. Microb. , 1996 ; 550- 553.*
6. *Luzza F, Oderd A, Maletta M, Imeneo M, Mesuraca L,Chioboli E, et al. Salivary immunoglobulin G assay to diagnose Helicobacter Pylori infection in children. J. clin, Microb., 1997 Dec; 3358- 3360.*
7. *Christic J, Mcnulty C, Shephered N, Valori R. Is saliva serology useful for the diagnosis of Helicobacter pylori? Gut, 1996; 39: 27-30.*

8. Herbrink P, Doorn L. Serological methods for diagnosis of Helicobacter pylori infection and monitoring of eradication therapy . Euro. J. Clin. Microb & Infec. Dis., 2000 April; 19,3: 164-173.
9. Odera G, Rapa N, Ronchi B, Lerro P, Pastore M, Staiano A, et al. Detection of H. pylori in stool specimens by non- invasive antigen enzyme immunoassay in children: multicenter Italian study. BMJ, 2000; 320:347-348.
10. Gilger MA, Tolia V, Johnson A, Rabinowitz S, Jibaly R, Elitsur Y, et al. The use of an oral fluid immunoglobulin G Elisa for the detection of Helicobacter pylori infection in children. Helicobacter, 2002 Apr; 7(2): 105-10.
11. Cockburn M, Collett J, Cox B. Validation of the saliva- based H. pylori test, helisal, and its in prevalence surveys. Epid miol Infect, 2001 Apr; 126(2): 191-6 .
12. Luzza F, Imeneo M, Marasco A, Crotta S, Ierardi E, Usai P, et al. Evaluation of a commercial serological kit for detection of salivary immuno globulin G to Helicobacter pylori: a multicenter study. Eur J Gastroenterol Hepatol, 2000 Oct; 12(10): 1117-20.
13. Garsia R, Gisbert J, Hermida C, Cabrera M, Diaz J, Pajares J, et al. Antibodies against Helicobacter pylori in saliva . Study of their validity versus breath test and its agreement with serology . Aten primaria, 2000 ; 25(6): 390-4.
14. Bode G, Marchildon P, Peacock J, Brenner H, Rothenbacher D. Diagnosis of Helicobacter pylori infection in children: comparison of a salivary immunoglobulin G antibady test with the [C] urea breath test. Clin. Diag.Lab. Immun., 2002 Mar; 9 , 2: 493-95.
15. De Pascalis R, Del pezzo M, Nardone G, Budillon G, and Lavitola A. Performance characteristics of an Enzyme- Linked Immunosorbent Assay for determining salivary immunoglobulin G Response to Helicobacter pylori. J. clin. Microb, 1999 Feb; 37(2): 430- 432.
16. Goeln, Sherwal B, Patwari A, Bajaj P, Choudhury M. Evaluation of invasive and non- invasive diagnostic modalitie, Helicobacter pylori infection in children. Indian Pediatr, 2003 Feb; 40(2): 141-6.
17. Reilly TG, Poxon V, Sanders D, Elliott T and Walt R. Comparison of serum, salivary, and rapid whole blood diagnostic tests for H. pylori and their validation against endoscopy based tests. Gut, 1997; 40: 454- 458.
18. Navab Akbar F. Diagnosis strains of H. pylori with PCR- RFLP method. Medical University of Isfahan. 2002.
19. Nafisi M. Study membrane Hg of H. pylori (urease) for detection antibody against it. Medical University of Isfahan. 1998.
20. Ohshima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors. Mutat. Res, 1994; 305:253-64.
21. Forman SF. H. pylori infection rates in relation to age social class in population of welsh men. Gut, 1991; 32: 25-8.
22. Stone M, Mayberry J. Non – invasive testing for Helicobacter pylori . Postgrad Med J, 1999; 75:74-77.