

Comparison of the serum and salivary antibodies to detect gastric *Helicobacter pylori* infection in Kashan (Iran)Ahmad Piroozmand¹, Babak Soltani^{2,3}, Mohsen Razavizadeh⁴, Amir Hasan Matini⁵, Hamid Reza Gilasi⁶, Abbas Nassaji Zavareh⁷, Siamak Soltani⁸

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Abstract

Background and aim: *Helicobacter pylori* (*H. pylori*) is an important and common contagious human pathogen which may cause peptic ulcer and also gastric cancer. The definite diagnosis of it is made through invasive tests. Recently, non-invasive tests including serologic tests of serum and saliva have been conducted for diagnosis of *H. pylori* infection. In this research, the diagnostic values of serum and salivary serology were compared together to use salivary anti-*H. pylori* test as an alternative method in the future.

Methods: During this prospective case-control study on patients who were candidates for endoscopy and gastric biopsy from March 2015 to April 2016 in Shahid Beheshti hospital, Kashan, Iran, serum and salivary samples were obtained for measurement of Immunoglobulin G (IgG) antibody levels against *H. pylori* by enzyme-linked immunosorbent assay (ELISA). Histopathology was the gold standard test. Statistical analysis was performed by SPSS software version 16. Statistical tests included Kolmogorov-Smirnov, independent-samples t-test, Chi-square, Mann-Whitney U, Kruskal-Wallis, McNemar and correlation.

Results: Of 123 patients, sixty-one patients (49.6%) were *H. pylori*-positive according to histology. The median levels of anti-*H. pylori* antibodies in serum ($p < 0.001$) and saliva ($p < 0.001$) of *H. pylori*-positive cases were significantly higher than *H. pylori*-negative cases. Sensitivity, specificity, positive likelihood ratio, negative likelihood ratio and accuracy of serologic tests in serum were 75%, 79%, 3.5, 0.3, 77% and for saliva were 85%, 82%, 4.7, 0.18, 84% respectively.

Conclusion: Diagnostic values of salivary ELISA are comparable to serum ELISA and can be used as an alternative modality for diagnosis of *H. pylori* infection.

Keywords: Salivary, Serum, *Helicobacter pylori*, ELISA, IgG

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1. Introduction

Helicobacter pylori (*H. pylori*), one of the most common chronic infections, can lead to chronic gastritis, gastric and duodenal ulcers and sometimes gastric lymphoma and adenocarcinoma (1-3). Colonization is usually lifelong without treatment, and its prevalence varies from 20% to 50% in developed countries to more than 80% in resource limited countries (4-6). The definitive diagnosis of *H. pylori* is isolation of the microbe or its detection in histology of gastric biopsy by endoscopy, which are expensive and invasive tests (7). Some serologic tests are inexpensive, rapid and acceptable with high accuracy rates and seem to be appropriate for epidemiologic studies especially in untreated cases and children (8-12). Saliva is more advantageous over serum due to its easier collection, non-invasiveness and lesser risk of blood infection (7). Anti-*H. pylori* antibody measurement in serum, urine and saliva among children, is not reliable due to wide variety of sensitivity and specificity of tests (13). In a study, the sensitivity of salivary IgG against *H. pylori* was low (5) and in another investigation, the low salivary IgG specificity was reported (1). In another survey, the sensitivity and specificity of salivary anti-*H. pylori* IgG were acceptable (14). Due to contradiction between the studies concerning reliability of salivary ELISA for detection of *H. pylori* infection and furthermore, in our country, only serum antibody test is routine, we decided to compare the diagnostic values of serum with salivary antibody tests for detection of *H. pylori* infection to recommend salivary ELISA as a non-invasive method instead of serum antibody test.

2. Material and Methods

2.1. Study design and population

This prospective case-controlled study was conducted on cases who were candidates for upper endoscopy from March 2015 to April 2016 in Kashan Shahid Beheshti hospital. A total of 123 patients with age range of 19 to 89 years participated in the study. Indications for endoscopy included abdominal discomfort or pain, nausea and vomiting with epigastric pain and dyspepsia. Inclusion criteria included the patients greater than or equal to 18 years of age and those who were referred for endoscopy according to the aforementioned symptoms. Exclusion criteria included use of any antibiotics, bismuth, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids or proton pump inhibitors (PPIs) during the previous 2 weeks, recent upper gastrointestinal (GI) bleeding, pregnancy, acute or chronic renal failure, chronic liver disease, age less than 18 years, gastric carcinoma and diabetes mellitus. Twenty cases were excluded before entering the study (10 patients used PPIs, 5 patients used NSAIDs and 5 cases refused to undergo endoscopy). Following enrollment, no individuals were excluded from the study. The patients were chosen according to convenient sampling strategy. The patients were divided regarding biopsy samples into two groups (*H. pylori*-negative and *H. pylori*-positive groups). Serum and salivary samples were taken for measurement of Immunoglobulin G (IgG) antibody levels against *H. pylori* by enzyme-linked immunosorbent assay (ELISA) and their diagnostic values were compared based on histopathology (gold standard test). Endoscopy was performed by one physician. Endoscopic findings included normal endoscopy, gastric ulcer (GU), duodenal ulcer (DU) and gastritis.

2.2. Sample size

Sample size was estimated according to $\alpha = 0.05$, sensitivity = 89%, specificity = 82%, and $d = 0.05$ (14). The total sample size according to sensitivity was calculated as 151 and regarding specificity, was estimated as 228.

2.3. Research ethics

The study was approved by the Ethics Committee of Kashan University of Medical Sciences (No. 29/5/1/5021). Informed written consent was obtained from all participants and their information was written in questionnaires. All cases enrolled in the study voluntarily. Harms and benefits of the procedures were explained to the patients. For lessening of complications, an expert endoscopist and anesthesiologist were present. Furthermore, a complete resuscitation set was prepared. Any patient was free to exclude themselves from the study at any time. All information was kept confidential. No expenses were burdened to any patient.

2.4. Laboratory methods

About 2 ml of un-stimulated saliva and 3ml of serum samples were obtained from cases and stored at -20°C . Laboratory tests were performed by a member of staff. Following thawing saliva and serum samples, the saliva samples were centrifuged for twenty minutes at 2000 r.p.m and the supernatants were extracted and anti-*H. pylori* IgG was measured in both samples by ELISA kit (Radim Co. Italy). Serum and saliva samples were diluted to 1:300 and 1:2 respectively. Antigen of *H. pylori* was diluted by coating buffer and added to wells, incubated at 37°C for 2 hours, then washed with buffer containing 2% serum albumin and incubated at 4°C for 18 hours. Thereafter, diluted samples were added and incubated for 90 minutes at 37°C . Then, anti-human antibody supplemented with

horseradish peroxidase was added and incubated for 1 hour at 37 °C, and tetra methylbenzidine was mixed to it. Prolongation of colorimetric reaction was done for 15 minutes at room temperature and finally, 50 μ liters of 4 NH₂SO₄ was mixed to wells. The optical density (OD) at 450 nm was recorded by an automated reader.

2.5. Histopathology

Two biopsy samples were taken from gastric corpus and antrum, and were fixed in 10% formalin and were stained by Giemsa and hematoxylin-eosin to determine the chronic gastritis and other histopathologic changes. Immunohistochemistry (gold standard test) was performed to detect *H. pylori* infection with 1/100 dilution (Biocare Med., California, USA). Histopathologic evaluations were conducted by one pathologist.

2.6. Statistical analysis

Data were entered into SPSS software version 16 (Chicago, Illinois, USA). Normal distribution of continuous data was detected by Kolmogorov-Smirnov test. Continuous data with normal distribution were described by mean \pm SD, and continuous data without normal distribution were reported as median (range) or mean \pm SE. Anti-*H. pylori* antibody levels in serum and saliva were indicated by median (range) because they had no normal distributions. Categorical values were described by frequency and percent. Comparison of antibody concentrations between two groups (*H. pylori* positive and negative groups) and sexes was made by Mann-Whitney U test. The difference of antibody titers among endoscopic findings was detected by Kruskal-Wallis test. The relation between age and antibody levels was estimated by Pearson correlation. Association between age and *H. pylori* infection was determined by independent-samples t-test. Chi-square test determined the association of sex and endoscopic findings with *H. pylori* infection. Cut-off value was chosen as 10 U/ml according to recommended ELISA kit for serum and salivary samples and according to it, true positive, false positive, false negative and true negative rates were estimated and used for calculation of diagnostic values (sensitivity, specificity and others) of two sample tests. Comparison of the sensitivity and specificity of serum and salivary ELISA tests was made by McNemar test. $P < 0.05$ was considered statistically significant.

3. Results

Of 123 cases, 63 (51.2%) males and 60 (48.8%) females with mean age of 41.3 \pm 16.3 years (range of 19-89 years) were enrolled in the present study. Sixty-one patients (49.6%) were *H. pylori* positive according to histology. Endoscopic findings were included: 11 (8.9%) normal, 33 (26.8%) gastric ulcers, 55 (44.7%) duodenal ulcers and 24 (19.5%) gastritis. Positive *H. pylori* was reported in 3 (27.3%) of normal endoscopies, 18 (54.5%) of gastric ulcers, 27 (49.1%) of duodenal ulcers and 13 (54.2%) of cases with gastritis ($p=0.4$). Antibody levels [median (range)] in serum were significantly higher in *H. pylori*-positive patients [19.3 (10.1-38)] rather than *H. pylori*-negative patients [4.5 (2.6-9.4)] ($p<0.001$). Also, antibody levels [median (range)] in saliva were significantly higher in *H. pylori*-positive subjects [16.5 (12.6-32.9)] rather than *H. pylori*-negative subjects [4.5 (1.8-7.7)] ($p<0.001$). Positive serum and salivary *H. pylori* IgG were 48% and 50.4% respectively. There was no sex preponderance for *H. pylori* infection ($p=0.4$) or serum and salivary antibody levels ($p=0.73$ and $p=0.83$ respectively). Also, there was no significant association between sex and serum and salivary IgG positivity ($p=0.24$ and $p=0.17$). Table 1 shows the diagnostic values of the ELISA tests in serum and saliva. According to Table 2, there was no significant association between endoscopic findings and antibody titers of serum and saliva ($p=0.72$ and $p=0.95$ respectively). The relationship between age and antibody concentrations of serum and saliva was not significant ($r = -0.08$, $p=0.4$ and $r=0.15$, $p=0.09$ respectively). No significant association was detected between age and *H. pylori* infection ($p=0.8$). By the McNemar test, the sensitivity and specificity of serum and salivary antibody titers were not different statistically, between *H. pylori* positive and negative groups ($p=0.27$ and $p=0.82$ respectively).

Table 1. Diagnostic values of anti-*H. pylori* IgG in saliva and serum

| Variables | Saliva | Serum |
|---------------------------|--------|-------|
| Sensitivity | 85% | 75% |
| Specificity | 82% | 79% |
| Positive Predictive Value | 83% | 78% |
| Negative Predictive Value | 85% | 77% |
| LR- | 0.18 | 0.3 |
| LR+ | 4.7 | 3.5 |
| Accuracy | 84% | 77% |

H. pylori, *Helicobacter pylori*; LR-, negative likelihood ratio; LR+, positive likelihood ratio

Table 2. Association of endoscopic findings with serum and salivary antibody levels

| Endoscopic findings | Sample | Antibody levels (mean ± SE) | p-value ¹ | p-value ² |
|---------------------|--------|-----------------------------|----------------------|----------------------|
| Normal | Serum | 13.8±6.8 | 0.72 | 0.95 |
| | Saliva | 16.8±9.2 | | |
| Gastric ulcer | Serum | 20.7±6.4 | | |
| | Saliva | 16.3±3 | | |
| Duodenal ulcer | Serum | 21.2±5.1 | | |
| | Saliva | 21.3±5.03 | | |
| Gastritis | Serum | 27.4±8.7 | | |
| | Saliva | 19.08±5.9 | | |

SE, standard error; p-value¹, for serum antibody level; p-value², for salivary antibody level.

4. Discussion

In the present study, the salivary sensitivity and specificity for detection of *H. pylori* infection were 85% and 82% respectively, and serum sensitivity and specificity were 75% and 79% respectively, that all of them were acceptable. Our results showed 49.6% prevalence of *H. pylori* infection, which was less than 68.9% reported in Egypt (5). Hooshmand et al. reported 50% prevalence of *H. pylori* infection which was in line with ours (14). During another study, 52% of patients were *H. pylori* positive which was compatible with the current investigation (3). In an Australian survey, prevalence rate was 21.5% which was less than present results (15). The cause of these varieties may be due to different socioeconomic levels between communities. In the present study, there was no sex predominance in prevalence of *H. pylori* infection, whilst Yucel et al. (16) indicated a higher prevalence rate among females but Abdul Rahim et al. reported male predominance (15). Our results indicated no association between sex and positivity of serum and salivary IgG against *H. pylori*. In an investigation, salivary IgG positivity was associated with female gender, which was not in line with ours (17). The current results showed no relationship between age and serum and salivary antibody levels. We also found no association between age and *H. pylori* infection. Shu et al. indicated a significant association between *H. pylori* infection with age, male gender and family members (18). This study showed 48% and 50.4% positive serum and salivary *H. pylori* IgG respectively. She et al. reported 35.6% positive serum *H. pylori* IgG, which was less than ours (19). In an investigation in India, the positivity rate of *H. pylori* IgG in saliva was reported as 67.5%, which was more than our results (1). The current results regarding serum *H. pylori* IgG, revealed a sensitivity of 75% and specificity of 79%, but Khalilpour et al. found the sensitivity and specificity of serum IgG up to 100%, which was substantially more than us (20). An investigation in Iran, showed high sensitivity (90.2%) and low specificity (61.1%) of serum *H. pylori* IgG, which were incompatible with our results (21). Rahman et al. reported sensitivity and specificity of serum IgG against *H. pylori* about 96.7% and 42.8% respectively, that the sensitivity was more than our findings and the specificity was lesser than our results (22). In current work, the salivary IgG revealed a sensitivity and specificity of 85% and 82% but El-Mekki et al. indicated a high sensitivity (95%) and low specificity (70%) of ELISA test (23). In a survey of Krishnaswamy et al., sensitivity (79.31%) of salivary IgG was acceptable, but specificity (63.64%) was low (1). Hooshmand et al. reported sensitivity and specificity of salivary anti- *H. pylori* IgG as about 88.6% and 81.1% respectively that the sensitivity was higher than ours (14). During a survey, the sensitivity and specificity of salivary antibody for detection of *H. pylori* were 80.9% and 95.3% respectively, that the specificity was significantly higher than ours (24). El-Mekki et al. found no significant difference between serum and salivary antibody levels regarding endoscopic findings, which was concordant with our study (23). In this research, serum and salivary antibodies for diagnosis of *H. pylori* were acceptable with no statistical difference, while El-Fakhfakh et al. found that the salivary antibodies were more sensitive and specific than serum antibodies (5). Leal et al. revealed no significant difference between sensitivity and specificity of serum and saliva which was consistent with this study (10). Luzzza et al. performed a multicenter investigation and found that salivary ELISA had significantly lower sensitivity, specificity and accuracy compared with serum ELISA, which was incompatible to our results (25). The causes of various sensitivities and specificities in the aforementioned studies, may be due to different antigens contained in commercial kits, diversity of ELISA kit efficacy in different geographic areas, variant choices of cut-off values, contamination of samples with other bacteria, concomitant infections and diversity of technical methods. Prevalence of *H. pylori* can affect negative and positive predictive values of the serologic tests. Some factors which may increase false negative results include recent infection before rising of antibody and probable degradation of antibody by salivary protease. Cross-reaction of oral normal flora antigens, recent antibiotic use and inappropriate gastric biopsy may lead to false-positive results. The strong point of our survey includes detecting associations between serologic tests and some factors such as age, sex and endoscopic findings. The limitations of the present

study include: 1) Use of histology as the only gold standard test and, because of probable false positive and negative results, some other tests such as culture, PCR, rapid urea test (RUT) or urea breath test (UBT) would be better to use accompanied by histology. 2) Limited sample size. 3) We did not evaluate some risk factors of *H. pylori* infection and positivity of serologic tests such as family members and parental education. 4) We, and most of the other cited studies evaluated the prevalence of *H. pylori* infection in suspected cases, not the general population. So, more comprehensive multicenter investigations with larger sample sizes that represent the prevalence of *H. pylori* infection in the general population, and evaluation of risk factors for *H. pylori* infection and its serologic positivity are recommended in the future.

5. Conclusions

Diagnostic values of salivary IgG tests for detection of *H. pylori* infection are comparable with serum IgG ELISA, and may be used as an appropriate alternative to serum serology. It is an inexpensive, rapid, non-invasive and easy modality. Although invasive methods have higher sensitivities and specificities, salivary IgG tests may be a valuable aid for epidemiologic studies, screening of dyspeptic patients and finally, reducing the undue endoscopies. Furthermore, it is more acceptable for children rather than a serum serology test.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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