ISSN 1009－3095 *Journal of Zhejiang University SCIENCE* V.3，No.4，P.484－488，Sep.－Oct.，2002
http://www.periodicals.com.cn；http://www.zju.edu.cn/English
http://www.zjupress.com；http://lib.zju.edu.cn/eindex.htm；jzu_s@mail.hz.zj.cn

Study of serum *Helicobacter pylori* soluble antigen*

WU Qin-dong(吴勤动)，ZHU Yong-liang(朱永良)

(*Department of Gastroenterology，Second Affiliated Hospital，College of Medicine，Zhejiang University，Hangzhou 310009，China.*)

E-mail：wuqingdon@hzcnc.com

Received Aug. 8，2001；revision accepted Mar. 6，2002

Abstract： Objective：to explore a new serological method for detecting *Helicobacter pylori*（*H. pylori*）infection. Methods：Serum soluble antigen of *H. pylori* was detected by using avidin-biotin ELISA technique to evaluate the status of *H. pylori* infection and for comparison with rapid urease test（RUT），histologic examination and serology. Results：The sensitivity，specificity，positive predictive value and negative predictive value were 77.46%，91.07%，91.67% and 76.12 %，respectively. The prevalence rate of serum *H. pylori* soluble antigen in 138 patients undergoing endoscopy was similar to the rate obtained by ^{14}C-UBT methods（$P>0.05$）. Conclusions：The detection of serum *H. pylori* soluble antigen(HpSAg) could be used as a new serological method which is accurate，and convenient，not affected by the memorizing reaction of serum antibody；is more sensitive，more specific and suitable for clinical diagnosis，and evaluation of eradication and for follow-up of *H. pylori* as well as for detection in children and pregnant women.

Key words： *Helicobacter pylori*，Antigen，Serology
Document code： A　　**CLC number：** R573.3

INTRODUCTION

Since the first successful isolation of *H. pylori* in 1982，*H. pylori* infection has been found to be associated with various diseases including chronic gastritis，peptic ulcer diseases(Graham et al.，1992) and gastric neoplasms(Bayerdorffer et al.，1995).

Several diagnostic tests have been developed for identification of this organism. The diagnosis of *H. pylori* infections is achieved by both invasive(culture，rapid urease test，and histological staining of antral biopsy specimens) and noninvasive(urea breath test and serology) methods. All the invasive tests require upper gastrointestinal endoscopy to obtain biopsy specimens for histology，rapid urease test(RUT)，and culture. The noninvasive tests include the urea breath test(UBT) and serological detection of antibodies to *H. pylori*. Serology is a useful noninvasive test especially valuable in epidemiological survey. However，the *H. pylori* IgG(immunoglobulin G) antibody titer does not accurately reflect the current status of *H. pylori* infection(Culter et al.，1996). Serological test cannot differentiate between past or current infection of *H. pylori*. UBT has been regarded as the best noninvasive test，also has limits to widespread adoption due to problems of high cost，feasibility and production of long life radioactive waste.

In this study，a new method was developed for detecting serum *H. pylori* soluble antigen which is not affected by memorizing reaction of serum *H. pylori* antibody after eradication，and evaluated on its clinical significance.

MATERIALS AND METHODS

1. Patients

Biopsy specimens and sera were obtained from 138 adults patients who had undergone gastroduodenoscopy for upper gastrointestinal symptoms. These patients consisted of 82 males and 56 females(mean age，45.3 years；range，23－70 years). Their medical histories were

* Project supported by Zhejiang Provincial Health Bureau(No.2000A118)，China

recorded in detail. The exclusion criteria included previous surgery, gastric malignancy, upper gastrointestinal bleeding, pregnancy, breast feeding, inability to give informed consent, use of antibiotics, proton pump inhibitors, and compounds containing bismuth up to four weeks before the study. Groups of gastric diseases includes chronic superficial gastritis(CSG), chronic atrophic gastritis(CAG), and peptic ulcer(PU).

2. Rapid urease test (RUT)

The rapid urease test was performed on fresh biopsy specimens in the endoscopy suite, specimens were immersed into microtiter plates coated with test liquid and were assessed according to the *H. pylori* infection density in gastric mucosa, graded from + to + + + equating to mild, moderate, and severe intensity of *H. pylori* infection correspondingly.

3. Histologic examination

Biopsy specimens for histopathological analysis were fixed in 10% buffered formaldehyde and sections were stained by methylene blue (MB). Histopathologic findings regarding *H. pylori* were assessed according to Marshall's grading system. Patients with chronic gastritis were divided into two groups — chronic superficial gastritis and chronic atrophic gastritis groups, patients with peptic ulcer were divided into gastric ulcer and duodenal ulcer groups.

4. Urea breath test (UBT)

Patients took the ^{14}C-urea breath test after they underwent upper gastrointestinal endoscopy, or were in fasting conditions; specimen radioactivity was expressed by decay per-minute (dpm); detection values of 200 − 500 dpm were assumed + for the presence or mild intensity of *H. pylori* infection, values of 500 − 1000 dmp were assumed + + for moderate intensity of *H. pylori* infection, values above 1000 dpm were + + + for severe *H. pylori* infection infection, values below 200 dpm were assumed *H. pylori* negative.

5. The detection of serum *H. pylori* IgG antibody

H. pylori IgG antibody was assayed by rountine ELISA, *H. pylori* seropositive was considered when the detection values were beyound or equal to the standard value.

6. Detection of *H. pylori* soluble antigen(HpSAg)

Blood samples were collected immediately before endoscopy and sera were stored at 20°C until tested. All serum samples were tested for the presence of soluble antigen of *H. pylori* using avidin-biotin enzyme-linked immunosorbent assay (ELISA) technique to evaluate the status of *H. pylori* infection. The microwells were coated with 10 μg/ml avidin overnight, washed three times in the following day, and blocked with 10% calf serum for 30 min at 37°C. Diluted (1∶100) samples (50 μl/well) and diluted (1∶25) biotin-conjugated (biotinylated) rabbit anti-*H. pylori* soluble antibody (50 μl/well) were put into the microwells and mixed for 1 minutes. They were left there to react for 2 hours at 37℃ and then washed three times. One hundred ul diluted (1∶25) *H. pylori* antigen-horseradish peroxidase (*H. pylori*-HRP) was put into to the microwells and left there to react for 2 hours at 37℃ and then washed three times to remove any unbound material. Tetramethyl-benzidine (TMB) as substrate (100 μl/well) was put into each microwell and left there to react for 30 minutes in the dark at room temperature. The reaction was stopped by addition of 1. 8 mol/L H_2SO_4(50 μl/well). The absorbance was measured spectrophotometrically at 450 nm (the wells were read at $\lambda = 450$ nm) for each well. A standard curve was constructed and the amount of *H. pylori* soluble antigen was determined with reference to this standard curve.

7. Healthy control group

Six healthy (by examinations) volunteers comprised the controls.

8. Statiscal analysis

The chi-square test was used to analyse proportional data. Sensitivity, specificity, positive, and negative predictive values were calculated using standard methods.

RESULTS

1. Sensitivity and specificity of detecting serum *H. pylori* soluble antigen

Because no single test suffices as a criterion standard(Culter et al., 1995), *H. pylori* status was defined as positive when the concordance of

two of the four tests (histology, RUT, ^{14}C-UBT and serology) was positive. Patients with negative results on all four tests were assessed as non-infected.

The *H. pylori* soluble antigen tests were positive in 55 of 71 patients diagnosed as *H. pylori*-positive, while 51 of 56 patients with negative *H. pylori* infection showed negative in *H. pylori* soluble antigen tests. Sensitivity was 77.46% and specificity was 91.07%. The positive and negative predictive values were 91.67% and 76.12%, respectively (Table 1).

Table 1 Sensitivity and specificity of detecting serum *H. pylori* soluble antigen

H. pylori infection	*n*	Serum *H. pylori* soluble antigen test	
		Positive	Negative
H. pylori positives[a]	71	55	16
H. pylori negative[b]	56	5	51

[a] *H. pylori* positives indicate patients with positive results on two of four tests (histology, RUT, ^{14}C-UBT and serology)..
[b] *H. pylori* negative indicate patients with negative results on all four tests (histology, RUT, ^{14}C-UBT and serology).

2. Prevalence of different density *H. pylori* infection

The prevalence rate of detecting serum *H. pylori* soluble antigen in 138 patients undergoing endoscopy showed without obvious difference ($P>0.05$) in different status of *H. pylori* infection. It indicated that the method of HpSAg could be used to diagnosis different density of *H. pylori* infection (Table 2).

Table 2 Prevalence of *H. pylori* infection in different density

Degree of *H. pylori* infection	*n*	HpSAg		χ^2	*P* *
		Positive	Prevalence(%)		
+	7	6	85.7	0.603	0.74
+ +	22	16	72.7		
+ + +	42	33	78.6		

* Likelihood Ratio Chi-Square Test

3. Comparison of the prevalence of *H. pylori* infection in HpSAg with ^{14}C-UBT in patients with different chronic gastric diseases

Comparison of the prevalence test for detecting *H. pylori* soluble antigen with that of ^{14}C-UBT tests in different chronic gastric diseases revealed that there was no significant difference in different groups of patients with chronic gastritis, peptic ulcer and control group ($P>0.05$) (Table 3).

Table 3 Comparison of prevalence of *H. pylori* infection in HpSAg with ^{14}C-UBT in different chronic gastric diseases

Group	*n*	*H. pylori* soluble antigen		^{14}C-UBT		χ^{2*}	*P*	r_s^{**}
		positive	prevalence(%)	positive	prevalence(%)			
CSG	87	44	50.57*	45	51.72*	0	1	0.701
CAG	31	18	58.06**	17	54.84**	0	1	0.542
PU	20	14	70.0	14	70.0	0	1	0.813
CONTROL	6	1	16.67	1	16.67	0	1	

* McNemar Chi-Square Test, ** Spearman Correlation coefficient ($P<0.01$)

4. Prevalence of *H. pylori* infection detected by 5 tests

The prevalence rate detecting serum *H. pylori* soluble antigen in 138 patients undergoing endoscopy was similar to non-serological methods ($P>0.05$), but the prevalence rate of detecting serum *H. pylori* antibody is 72.60%, significantly higher than that of other methods ($P<0.05$). Because patients with positive serum *H. pylori* antibody may be consist of those with current and past *H. pylori* infection. The patients with false positive of *H. pylori* antibody must be excluded to evaluate the accuracy of diagnosis (Table 4).

Table 4 Prevalence of *H. pylori* Infection Detected by 5 tests

Test	No. of patiens	No. of *H. pylori* positive	Prevalence (%)
RUT	138	75	54.34
MB stain	138	68	49.28
^{14}C-UBT	138	71	51.44
HpSAg	138	69	50.00
HpAb	138	100	72.26*

* $P<0.05$

DISCUSSION

Since the discovery of *H. pylori* in 1982 (Graham et al., 1992; Bayerdorffer et al., 1995), many tests have been proposed for the detection of *H. pylori* infection. These included tests performed on biopsy samples (RUT, histological and smear examinations, culture and polymerase chain reaction), noninvasive tests (^{13}C-urea and ^{14}C-urea breath tests, and serological tests for specific antibody detection). Tissue-based tests are highly specific but less sensitive because of patchy distribution of *H. pylori* in the stomach (Graham et al., 1992; Culter et al., 1995). Bacterial culture is rarely performed because of the fastidious nature of the bacterial, leading to inadequate sensitivity (Goodwin et al., 1985). Commercially available nonendoscopic tests for *H. pylori* fall into two categories: antibody tests, and urea breath tests. Although serological testing for special *H. pylori* antibodies is also a sampling method (Reilly et al., 1997) which, to a certain extent, reflects the extent of *H. pylori* infection in the stomach; the serum antibody levels are maintained for a long time (>5 or 6 months) and patients should not be expected to become seronegative after *H. pylori* is successfilly eradicated (Culter et al., 1996). Serological test cannot distinguish between past and current *H. pylori* infection. ^{13}C-UBT is more expensive and usually requires specialized, costly equipment- isotope ratio mass spectrometer to measure ^{13}C in the exhaled breath. ^{14}C-UBT exposes the patient to a small dose of radiation by ^{14}C, whose half-life is up to 5730 years, and so, cannot be performed on women who may be pregnant and children (Marshall et al., 1988).

However, all these have limits in clinical application. Ideally, the test for *H. pylori* should be safe, accurate, noninvasive, easy to perform, and inexpensive. The detection of serum *H. pylori* soluble antigen fulfill these requirements. It is minimally invasive, requires only a small blood sample obtained by venipuncture; and is relatively easy to perform. Unlike tests that rely on bacterial urease activity (UBT and RUT), which may yield false-negative results in patients taking antibiotics, proton pump inhibitors, and compounds containing bismuth (Laine et al., 1998), the detection of serum *H. pylori* soluble antigen infection is not affected by these medications. Gastric epithelial cells are tightly joined to each other, forming a continuous barrier that selectively restricts the movement of substances between the external and internal compartments. Any disruption or modification of the epithelial architecture can lead to an alteration of barrier function. When the gastric or duodenal mucosa is colonized with these organisms, *H. pylori* could alter in vitro the barriers properties of the epithelium by increasing the permeability of the tight junctions and dramatically decreasing the transepithelial electrical resistance (TER). The increased permeability may provide a potential mechanism by which *H. pylori* soluble antigens can cross the mucosa barrier and be released into the blood (Terrés et al., 1998). Thesesoluble antigens mostly derived from outer membrane protein, including urease, proteinase, lipopolysaccharide, cytotoxin, neutrophil-activation protein, and heat shock protein, etc, with molecular weight of 80 000 to 550 000. Most of these antigens mentioned above exist in the blood circulatory system

by free status while some of these antigens could be neutralized by special antibodies to product antigen-antibody complex and then are cleared from the system. It is known that low-molecular weight, soluble non-polymer *H. pylori* antigens can induce immunologic tolerance. So it is possible that these low-molecular weight and low-dose soluble *H. pylori* antigens may result in deficiency or suppression of the immune response. Therefore these soluble *H. pylori* antigens can exist in peripheral blood because of absence of neutralization by special antibodies. The maintenance of the blood concentration of soluble antigens coming from *H. pylori* outer membrane protein depends on *H. pylori* infection in the gastric muosa and continuous reproduction of *H. pylori* there . The soluble antigen released by *H. pylori* could disappear or decrease rapidly after *H. pylori* in the gastric mucosa is successfully eradicated. Therefore the detection of serum *H. pylori* soluble antigen can be used to distinguish between the current and past *H. pylori* infection in the gastric mucosa, observe whether *H. pylori* was been successfully eradicated after treatment. Our results showed that the sensitivity and specificity of detecting serum *H. pylori* soluble antigen is more than 70% and 90% respectively; and that the prevalence of *H. pylori* in different groups of patients with chronic gastritis and peptic ulcer ranges from 51.72% – 70% similar to that of ^{14}C-UBT.

In conclusion, the detection of serum *H. pylori* soluble antigen is a new serological method which is sensitive, specific, accurate, convenient, inexpensive, is not affected by the memorizing reaction of serum antibody, and suitable for clinical diagnosis, evaluation of eradication and follow-up of *H. pylori* as well as for detection of *H. pylori* infection in children and pregnant women.

References

Bayerdorffer, E., Neubauer, A., Rudolph, B., Thiede, C., Lehn, N., Eidt, S., Stolte, M. 1995; Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue after cure of *Helicobacter pylori* infection. *Lancet*, **345**: 1591 – 1594.

Cutler, A. F., Havstad, S., Ma, C. K., Blaser, M. J., Perez-Perez, G. I., Schubert, T. T. 1995; Accuracy of invasive and noninvasive tests to diagnose *H. pylori* infection. *Gastroenterol*, **109**: 136 – 141.

Cutler, A. F., Prasad, V. M. 1996; Long-term follow-up of *Helicobacter pylori* serology after successful eradication. *Am J Gastroenterol*, **91**: 85 – 88.

Goodwin, C. S., Blincow, E. D., Warren, J. R., Waters, T. E., Sanderson, C. R., Easton, L. 1985; Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J Clin Pathol*, **38**: 1127 – 1131.

Graham, D. Y., Lew, G. M., Klein, P. D., Evans, D. G., Evans, D. J. Jr., Saeed, Z. A., Malaty, H. M. 1992. Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer: a randomized, controlled study. *Ann Intern Med*, **116**: 705 – 708.

Laine, L., Estrada, R., Trujillo, M., Knigge, K., Fennerty, M. B. 1998; Effect of proton-pump inhibitor therapy on diagnostic testing for *Helicobacter pylori*. *Ann Intern Med*, **129**: 547 – 550.

Marshall, B. J., Surveyor, I. 1988; Carbon-14 urea breath test for the diagnosis of *Campylobacter pylori* associated gastritis. *Nucl Med*, **29**: 11 – 16.

Reilly, T. G., Poxon, V., Sanders, D. S. A., Elliott, T. S., Walt, R. P. 1997; Comparison of serum, salivary, and rapid whole blood diagnostic tests for *Helicobacter pylori* and their validation against endoscopy based tests. *Gut*, **40**: 454 – 458.

Terrés, A. M., Pajares. J. M., Hopkins, A. M., Murphy, A., Moran, A., Baird, A. W., Kelleher, D. 1998; *Helicobacter pylori* disrupts epithelial barrier function in a process inhibited by protein kinase C activators. *Infect Immun*, **66**: 2943 – 2950.