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*Evaluation of anti-*Helicobacter pylori *IgG2 antibody for the diagnosis of* Helicobacter pylori *infection in western and Chinese populations*

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SUMMARY

Background: The performance of commercial *Helicobacter pylori* diagnostic kits developed for particular geographic regions has often been found to be of poor diagnostic value when applied to other regions, possibly because of infections being caused by different *H. pylori* strains in different regions.

Aim: To evaluate the performance of an IgG2 anti-*H. pylori* enzyme-linked immunoassay test (Helirad Alert) for detection of *H. pylori* infection in both Australian and Hong Kong (Chinese) subjects.

Methods: Serum samples were tested for *H. pylori* specific IgG2 and IgG antibodies by enzyme-linked immunoassay kits using identical antigen preparation in 168 Australian and 160 Hong Kong (Chinese) subjects diagnosed with dyspepsia.

Results: Using a cut-off value determined by analysis of *H. pylori*-negative Australian samples, the sensitivity, specificity and accuracy of the IgG2 assay were 77.8, 97.4 and 91.1%, respectively, for the Australian samples and 96.3, 83.8 and 90% for Hong Kong samples. For the IgG assay, sensitivity, specificity and accuracy were 87.0, 99.1 and 95.2% for Australian samples and 97.5, 75 and 86.3% for Hong Kong samples respectively. Receiver-operating characteristic analysis showed better discrimination of *H. pylori* status when the IgG2 assay was applied to Hong Kong samples, while the IgG assay was better in the Australian samples.

Conclusion: These data demonstrate that the Helirad Alert enzyme-linked immunoassay could provide a reliable method for screening *H. pylori* infection in both western and Chinese populations.

INTRODUCTION

Helicobacter pylori infects half of the world population.[1, 2] Diagnosis of and screening for *H. pylori* infection in the community using a simple test is an important part of a long-term strategy to manage *H. pylori* infection. Commercial enzyme-linked immunoassay (ELISA) tests are available using different antigen preparations. However, ELISA kits developed for particular geographic regions have performed differently when they were applied to other regions.[3, 4]

Currently, defining *H. pylori* infection status in patients following eradication therapy mainly relies on the urea breath test, which is expensive and could be difficult to perform in some circumstances, as it requires special equipment. A simple non-invasive test to follow-up

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H. pylori infection status is needed. Non-invasive salivary *H. pylori* IgG has been reported as a useful tool in monitoring the efficacy of *H. pylori* eradication therapy.[5]

Our studies have shown that gastric cancer is associated with a major cytokine shift within the gastric mucosa and that this shift correlated with a selective reduction in secretion of IgG2 anti-*H. pylori* antibody.[6, 7] The question can be asked, therefore, as to whether an IgG2 antibody assay would add value to existing serology assays by identifying those at higher risk of gastric cancer, especially in regions with high prevalence of *H. pylori* infection. We have developed an IgG2 assay, which is now being evaluated for applicability to different populations.

The present study evaluates IgG2 and IgG anti-*H. pylori* antibody assays for the detection of anti-*H. pylori* antibody in serum samples of Caucasian subjects from Australia and Chinese subjects from Hong Kong. Anti-*H. pylori* IgG2 and IgG antibody levels were also measured in serum and saliva before and after eradication therapy in a subgroup to determine if there was additional value in monitoring the outcome of eradication therapy.

MATERIALS AND METHODS

Study populations

The Australian study population comprised 168 subjects with dyspepsia referred to the Centre for Digestive Disease at Sydney, Australia for the assessment of *H. pylori* status. Of 168 subjects, 54 were *H. pylori* positive (22 females and 32 males) with a mean age of 50.6 ± 14.6 years (range 29–80) and 114 *H. pylori* negative (68 females and 46 males) with a mean age of 50.2 ± 15.8 years (range 16–87). Exclusion criteria were: use of any antibiotics within 3 months prior to the study, previous therapy for *H. pylori* eradication, previous gastric surgery and current use of any acid suppressant. A subgroup of 44 subjects with *H. pylori* infection, given eradication therapy, were recruited to assess the effect of therapy on the antibody response. The mean age was 51.7 ± 12.4 years with age range 21–86 years (18 females and 26 males). Treatment was for 10 days with omeprazole (Losec, Astrazeneca Pty Ltd, North Ryde, NSW, Australia) 20 mg twice a day, amoxicillin 500 mg three times a day and clarythromycin 500 mg twice a day. A negative ^{13}C-urea breath test for post-treatment *H. pylori* status was the end point for successful eradication. Of 44 subjects with *H. pylori* infection who received treatment, 42 completed eradication therapy while two subjects withdrew from the study.

The study protocol was approved by the Human Ethics Committee of the Centre for Digestive Diseases, Sydney, Australia. All subjects gave written informed consent. For each subject, four biopsy specimens were obtained at endoscopy from the pyloric antrum, two for histological examination and two for a rapid urease test (CLOtest; Delta West Pty Ltd, Bentley, WA, Australia). A venous blood sample and unstimulated saliva sample were collected from each subject prior to the procedure and 6 weeks after completion of the therapy for those who received eradication treatment.

The Chinese study population comprised 160 serum samples from ethnic Chinese patients with dyspepsia (85 females and 75 males) obtained from the Department of Medicine, University of Hong Kong. Eighty serum samples were from *H. pylori*-positive subjects (mean age 45.7 ± 16.1 years, age range 18–80 years) and 80 from *H. pylori*-negative subjects (mean age 48.3 ± 12.7 years, range 22–74 years).

For both studies, endoscopic examination was performed in all the subjects. The definition (gold standard) of *H. pylori* infection for both study populations required positive CLOtest and/or positive histological examination. All the ELISA tests were performed blind without the operator's knowledge of the diagnosis or the treatment received.

Detection of Helicobacter pylori *IgG antibody.* Serum *H. pylori*-specific IgG antibody levels were measured by an ELISA kit using plates coated with an acid glycine antigen extract from *H. pylori* strain NCTC 11637, as described previously.[8]

Detection of Helicobacter pylori *IgG2 antibody.* Serum *H. pylori*-specific IgG2 antibody levels were measured using Helirad Alert ELISA kits (VRI BioMedical, Sydney, Australia) according to the instructions provided by the manufacturer. The coating antigen was exactly the same as that used in the IgG ELISA. A calibrator from known pooled *H. pylori*-positive sera was used in each plate. The same calibrator was used for the IgG and IgG2 assays.

Calculation of enzyme-linked immunoassay results. Enzyme-linked immunoassay results were expressed as ELISA Index (EI) according to the following formula:

ELISA Index

$$= \frac{\text{Mean(OD reading of unknown sample} - \text{blank OD reading)}}{\text{Mean(OD reading of calibrator serum} - \text{blank OD reading)}}$$

where, OD is the optical density. Pre- and post-treatment samples were tested on the same plate to minimize plate-to-plate variation.

Statistical analysis

Cut-off values were determined as three standard deviations above the mean antibody value in 114 *H. pylori*-negative subjects from the *H. pylori*-negative Australian study population. The sensitivity, specificity, predictive values and assay accuracy were determined for the *H. pylori* antibody tests and compared with a standardized diagnosis based on gold standard criteria of histology and results of the rapid urease test. Data were expressed as the mean ± standard error (S.E.). Antibody levels from pre- and post-treatment were analysed by the paired *t*-test. Chi-squared test was used to analyse ELISA test performance. Statistical analysis was performed by using a StatView 4.5 software program (Abacus Concepts, CA, USA). Differences were considered significant when the calculated *P*-value was <0.05.

A receiver-operating characteristic (ROC) curve was constructed to describe the effect of varying the cut-off value from which the sensitivity and specificity estimates were calculated.

RESULTS

*Performance of the anti-*Helicobacter pylori *serum IgG antibody enzyme-linked immunoassay test*

The results for sensitivity, specificity, positive and negative predictive values and accuracy of the assays were determined by comparing *H. pylori* antibody status with the 'gold standard'. For the Australian samples, the sensitivity and specificity of the IgG assay were 87.0 and 99.1%, respectively, with an assay accuracy of 95.2% based on a cut-off value (1.88 EI) determined as three standard deviations above the mean antibody value for 114 *H. pylori*-negative subjects (Table 1). Using the Australian determined cut-off value for the Hong Kong samples, the test had a 97.5% sensitivity and a 75% specificity with an assay accuracy of 86.3% (Table 1). However, the performance of the test based on the ROC curve was better using Australian samples in terms of a better discrimination of *H. pylori* status (Figure 1). Twenty of 80 *H. pylori*-negative Hong Kong samples had values above the cut-off compared with one of 114 *H. pylori*-negative Australian samples ($P < 0.0001$).

Table 1. Data analysis of *Helicobacter pylori* IgG and IgG2 cut-off value

Cut-off value	% Sensitivity	% Specificity	% PPV	% NPV	% Accuracy
Mean IgG + 3s.d. (1.88)					
Australia	87.0	99.1	97.9	94.2	95.2
Hong Kong	97.5	75.0	79.6	96.8	86.3
Mean IgG2 + 3s.d. (0.93)					
Australia	77.8	97.4	93.3	90.2	91.1
Hong Kong	96.3	83.8	85.6	95.7	90.0

*Performance of the anti-*Helicobacter pylori *serum IgG2 antibody enzyme-linked immunoassay test*

Based on the cut-off value of 0.93 EI, the IgG2 test had a 77.8% sensitivity and a 97.4% specificity with an assay accuracy of 91.1% for Australian samples (Table 1). For Hong Kong samples, the test had a sensitivity of 96.3% and a specificity of 83.8% with an assay accuracy of 90% (Table 1). ROC analysis showed a better discrimination of *H. pylori* status for Hong Kong samples with

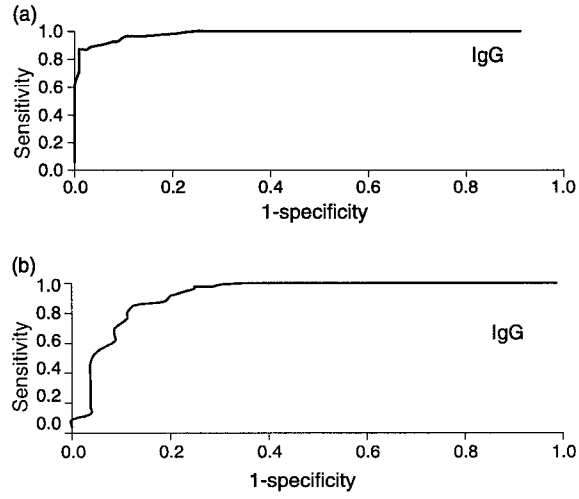

Figure 1. Receiver-operating characteristic curves for *Helicobacter pylori* IgG in (a) Australian samples and (b) Hong Kong samples.

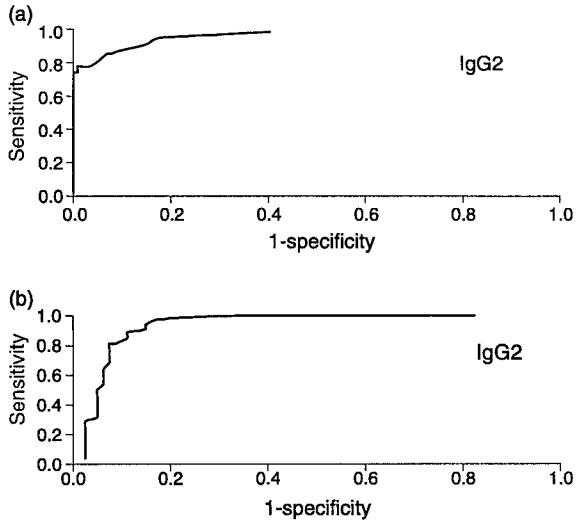

Figure 2. Receiver-operating characteristic curves for *Helicobacter pylori* IgG2 in (a) Australian samples and (b) Hong Kong samples.

12 of 54 *H. pylori*-positive samples from Australia having an EI lower than 0.93 compared with only three of 80 *H. pylori*-positive samples from Hong Kong ($P < 0.001$) (Figure 2).

Saliva and serum-specific antibody in subjects successfully eradicating H. pylori

Six subjects were confirmed to have failed to eradicate *H. pylori*. The eradication rate by intention-to-treat analysis was 85.7% (36/42, 95% CI: 75–96) based on ^{13}C-urease breath test. As shown in Table 2, subjects who successfully eradicated the bacterium following triple therapy had a significant drop in both salivary IgG2 ($P < 0.002$) and IgG ($P < 0.001$) antibody levels. The average decline was 31% for IgG2 and 15% for IgG ($P = 0.07$). In 15 subjects, the salivary IgG2 antibody levels had declined by at least 50%, whereas there was no change in eight subjects. As shown in Table 3, the specific serum IgG and IgG2 antibody levels dropped significantly in subjects with successful eradication of *H. pylori* at 6 weeks. However, 11 subjects did not show any change of serum antibody levels. Stability of results was shown over 10 months of sample storage at −20 °C (data not shown).

For IgG, a similar decline was noted in both serum and saliva samples (15%). For IgG2, saliva antibody levels declined more (31% decrease) than levels in the serum samples (6.3% decrease). In contrast, in six subjects who failed to eradicate *H. pylori* infection after triple

Table 2. Decline of salivary specific antibody at 6 weeks after eradication therapy

	Saliva *H. pylori* IgG (mean ± s.e.)		Saliva *H. pylori* IgG2 (mean ± s.e.)	
	Eradication ($n = 36$)	Failed ($n = 6$)	Eradication ($n = 36$)	Failed ($n = 6$)
Pre-treatment	2.72 ± 0.22	3.06 ± 0.62	1.5 ± 0.26	1.39 ± 0.37
Post-treatment	2.06 ± 0.2	2.48 ± 0.49	0.81 ± 0.11	1.24 ± 0.25
P-value	$P < 0.001$	N.S.	$P < 0.002$	N.S.

Levels of specific anti-*H. pylori* IgG and IgG2 were measured by ELISA as described in 'Materials and methods'.

Table 3. Decline of serum-specific antibody at 6 weeks after eradication therapy

	Serum *H. pylori* IgG (mean ± s.e.)		Serum *H. pylori* IgG2 (mean ± s.e.)	
	Eradication ($n = 36$)	Failed ($n = 6$)	Eradication ($n = 36$)	Failed ($n = 6$)
Pre-treatment	3.62 ± 0.3	3.45 ± 1.07	2.88 ± 0.34	2.9 ± 1.2
Post-treatment	2.95 ± 0.22	2.9 ± 1.02	2.43 ± 0.31	2.6 ± 0.92
P-value	<0.005	N.S.	<0.002	N.S.

Serum samples were collected from individual subjects at 6 weeks after completion of eradication therapy.

therapy, no significant change in either IgG or IgG2 levels was observed.

DISCUSSION

This is the first study to assess the potential clinical value of an IgG2 anti-*H. pylori* antibody assay for the diagnosis of *H. pylori* infection in dyspeptic subjects in communities with a low (Sydney, Australia) and high (Hong Kong) prevalence of *H. pylori*-related incidence of gastric cancer. In the current study, both IgG and IgG2 assays gave acceptable diagnostic accuracy when used on serum samples from Australia and Hong Kong, although minor differences in performance were observed. The potential value of using the IgG2 antibody assay to monitor successful eradication therapy using saliva was also investigated.

Commercial *H. pylori* IgG ELISA assays have been extensively used for the detection of *H. pylori* infection. The parameters of these assays vary with sensitivity ranging between 81 and 100% and specificity between 79 and 98%.[3, 9, 10] Previous studies have shown that the performance of diagnostic kits is often poor in geographically different regions, possibly because of the use of less relevant antigens.[4, 11] In the present study, the whole-cell antigen extract from the *H. pylori* strain NCTC 11637 gave reasonable accuracy in an Australian population. For the IgG assay using a cut-off determined as the mean + 3s.d. of *H. pylori*-negative Australian samples, a specificity of 99.1%, a sensitivity of 87.0% and an assay accuracy of 95.2% was recorded. These results are similar to those reported by others for assays using acid-glycine extracts of *H. pylori*.[12]

A higher sensitivity for the IgG assay was obtained in the Hong Kong population when the same cut-off value was applied, although the specificity was lower. This is reflected in a higher level of false-positive results in the Hong Kong samples by ROC analysis. Reduced specificity has been noted in other population groups with a high prevalence of *H. pylori* infection and in older populations.[13] These observations may reflect 'cryptic' infection not detected by the traditional 'gold standard' parameters of a positive urease test and detection of spiral organisms on microscopy.[14] Changing the cut-off level would enhance performance against 'gold standard' criteria, although this reflects the probability of cryptic infection by urease-negative organisms. The IgG2 antibody assay had a better sensitivity, with an accuracy of 90% when a cut-off value of 0.93 EI (mean ± 3s.d.) was used for the Hong Kong samples. This cut-off value was too high for the Australian population with an increase in the number of false-negative results. These differences may in part be the result of variable strains of *H. pylori* and the variable host immune response noted for IgG subclasses,[12, 15] because of variations in lipopolysaccharide (LPS) content.[16] The induction of an IgG antibody response to *H. pylori* infection is determined largely by polysaccharide antigens on the LPS moiety of *H. pylori*. Thus, a high serum IgG antibody response is caused by the presence of an immunodominant epitope, whereas low reactivity is because of strains showing low antigenicity.[17] Antigenic determinants on highly immunogenic LPS are predominantly recognized by IgG2 subclass antibody.[18] A second explanation for the differences is a variation in host production of interferon (IFN)-γ, a cytokine which promotes IgG2 secretion.[19]

Detection of antibodies in saliva has been used for the diagnosis of infectious disease, including monitoring response to eradication therapy in *H. pylori* infection.[5] Tests performed with saliva have shown an overall sensitivity ranging from 81 to 94% and specificity from 70 to 90%.[20, 21] Similar sensitivity and specificity parameters were found for saliva testing as were observed with the serum test.[22] The present study demonstrated a similar decline in *H. pylori*-specific IgG in saliva and serum after successful eradication of *H. pylori* infection. Salivary IgG2 antibody levels, however, had a fourfold decrease than the serum levels. This could be because of a local source of IgG antibody in saliva more reflective of mucosal antigen or to different kinetics of a downregulated mucosal environment.[5] In animal models, it has been shown that secretory IgG antibody correlates with the kinetics of local IgA secretion rather than with those of serum IgG antibody.[23] Saliva IgG antibody does increase following mucosal stimulation with microbial antigens, with a poor correlation noted between IgG antibody levels in serum and saliva,[5] while others have noted that the distribution of saliva IgG subclass antibody differs from that of serum IgG.[24] The difference noted in IgG2 subclass antibodies in the present study is consistent with these observations providing strong additional support to the concept that saliva IgG antibody is secreted from a mucosal compartment separate from systemic immunity. The IgG2 saliva antibody levels may also reflect IFN-γ within the mucosa, which has been shown to reflect a dominant T-helper (Th)1 response in *H. pylori*-infected mucosa.[25]

Rapid decline in specific IgG2 would logically follow a reduction in Th1 cytokines (including IFN-γ) because of a reduction in bacterial load consequent upon effective eradication of *H. pylori*. The relatively minor fall in saliva antibody level in subjects who failed to eradicate *H. pylori*, while not statistically significant, encourages more extensive study of the kinetics of saliva IgG2 antibody as a potentially valuable parameter to monitor eradication therapy.

These results demonstrate that the Helirad Alert IgG2 anti-*H. pylori* ELISA assay performs as well as currently used commercial assays for the diagnosis of *H. pylori* infection with respect to sensitivity, specificity, predictive values and accuracy in both Australian and Chinese populations. However, a minor adjustment of cut-off values may improve diagnostic ability in different geographic areas. The assay may also have particular value in detecting risk of gastric cancers in high-prevalence populations. We have previously shown a link between a low level of anti-*H. pylori* IgG2 and gastric cancer (Z. Ren, T. Borody, G. Pang, L. C. Li, M. Dunkley, R. Clancy, unpublished data). Further study of this application is directed at confirming a cut-off of positivity, above which the risk of gastric cancer would be low, where 'test and treat' is considered an initial treatment option.

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