

Case Control Study

Serum *Helicobacter pylori* KatA and AhpC antibodies as novel biomarkers for gastric cancer

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Abstract

AIM: To investigate catalase (KatA) and alkyl hydroperoxide reductase (AhpC) antibodies of *Helicobacter pylori* as biomarkers for gastric cancer (GC).

METHODS: This study included 232 cases and 264 controls. Recombinant KatA and AhpC proteins were constructed and the levels of antibodies were tested by indirect enzyme-linked immunosorbent assay (ELISA). Logistic regression was applied to analyze the relationships between KatA, AhpC and GC. The χ^2 trend test was used to evaluate the dose-response relationships between serum KatA and AhpC antibody levels and GC. Receiver operating characteristic (ROC) curve was used to evaluate the screening accuracy of KatA and AhpC as biomarkers. Combined analysis was used to observe screening accuracy of predictors for GC.

RESULTS: In all subjects, the association between KatA and AhpC and GC risk was significant ($P < 0.001$) with odds ratio (OR) = 12.84 (95%CI: 7.79-21.15)

and OR = 2.4 (95%CI: 1.55-3.73), respectively. KatA and AhpC antibody levels were strongly related to GC risk with a dose-dependent effect (P for trend < 0.001). The area under the ROC (AUC) for KatA was 0.806, providing a sensitivity of 66.81% and specificity of 86.36%; and the AUC for AhpC was 0.615, with a sensitivity of 75.65% and specificity of 45.49%. The AUC was 0.906 for KatA and flagella protein A (FlaA) combined analysis.

CONCLUSION: Serum KatA and AhpC antibodies are associated with GC risk and KatA may serve as a biomarker for GC. KatA/FlaA combined analysis improved screening accuracy.

Key words: *Helicobacter pylori*; Catalase; Serum antibody; Alkyl hydroperoxide reductase; Gastric cancer; Case-control study

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Core tip: Effective screening methods for gastric cancer (GC) have remained limited to date. The aim of this study was to explore whether serum catalase and alkyl hydroperoxide reductase antibodies of *Helicobacter pylori* could serve as novel and reliable biomarkers for GC monitoring.

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INTRODUCTION

Gastric cancer (GC) is the fifth most common malignancy and the third leading cause of cancer-related death worldwide^[1]. Although the overall incidence rate of GC continues to fall, there were still almost 1 million new cases of GC in 2012^[2]. *Helicobacter pylori* (*H. pylori*) are micro-aerophilic gram-negative bacteria that cause inflammatory reactions by selectively colonizing the gastric mucosa. The International Agency for Research on Cancer has classified *H. pylori* as a category I carcinogen since 1994^[3]. Epidemiological data also support that *H. pylori* infection is strongly associated with GC^[4-6], increasing risk by up to six-fold^[7]. In contrast, increasing data shows that *H. pylori* eradication significantly decreases the development of GC^[8,9], particularly in high-risk populations with no precancerous lesions^[10]. Eradication of *H. pylori* seems a reasonable approach for preventing GC. However, nearly 50% of the population worldwide is infected with *H. pylori*^[11]. Mass eradication therapy in the general population may bring about development of antibiotic-

resistant strains of *H. pylori* as well as over-consumption of medical resources. Therefore, there is an urgent and need to identify a reliable screening biomarker for GC.

It is reported that only a small fraction of patients infected with *H. pylori* have severe clinical outcomes, such as gastric ulcer (10%), atrophic gastritis (5%), and gastric malignancy (2%)^[3]. Research indicates that these different outcomes may be associated with the virulence factors of *H. pylori*^[12-14]. Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) virulence factors play a crucial role in protecting *H. pylori* from oxidative stress and maintaining a stable environment for the growth of bacteria^[15,16]. Huang *et al.*^[17] confirmed that KatA and AhpC were over expressed under the condition of oxidation stress (H₂O₂) in *H. pylori* strains isolated from patients with GC, gastritis, or duodenal ulcer. We previously reported that serum flagella protein A (FlaA) antibody of *H. pylori* may serve as noninvasive biomarker for early detection of GC^[18]. In this study, combined analysis was applied to explore the screening value of KatA, AhpC, and FlaA for GC. This study aims to assess the correlations between KatA and AhpC and GC and explore whether they could serve as novel and reliable biomarkers for GC.

MATERIALS AND METHODS

Study subjects

This was a hospital-based case-control study, which was approved by the Committee of Human Research of Harbin Medical University, Harbin, China. Two hundred and thirty-two cases of GC were primarily diagnosed by pathology at the Third Affiliated Hospital of Harbin Medical University between April and July 2010. The controls comprised 182 healthy people chosen from the Harbin Xiangfang Center for Disease Control and Prevention and 82 cancer-free people recruited from the neurology department at the Fourth Affiliated Hospital of Harbin Medical University between March and July 2011. All participants gave signed informed consent, and we completed a face-to-face questionnaire that included age, sex, smoking status, and alcohol consumption. Venous blood samples of 5 mL were collected from all participants, centrifuged at 3000 r/min, and stored at -80 °C.

Cloning and expression of recombination protein

A clinical strain of *H. pylori* provisionally named H015a was isolated from a GC patient at the Second Affiliated Hospital of Harbin Medical University. Genomic DNA of H015a was extracted as a template using a DNA extraction kit (QIAGEN, Valencia, CA, United States). The *kata* and *ahpC* gene coding sequences were obtained from Genbank. Amplification of *kata* and *ahpC* gene fragments was implemented by polymerase chain reaction (PCR). The PCR primers were designed using Primer Premier 5.0 software. For *kata*, the primer sequences were 5'-CCGGAATTCATGGTTAATAAAGATGTGAACA-3'

(forward) and 5'-CCGCTCGAGTTACTTTTCTTTT-TGTGTGG-3' (reverse) that generated a 1518 bp fragment. For *ahpC*, the primer sequences were 5'-CCGGAATTCATGTTAGTTACAAAAGCTTGCCC-3' (forward) and 5'-CCGCTCGAGTTAAAGCTTAATG-GAATTTTC-3' (reverse) that generated a 597 bp fragment. *EcoRI* and *XhoI* restriction endonuclease sites were incorporated into the forward and reverse primer sequences of these two genes, respectively. Amplification was implemented under the following conditions: 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s followed by a final extension at 72 °C for 7 min. Subsequently, two PCR products were cloned into the cloning vector pMD18-T and transformed into *Escherichia coli* (*E. coli*) strain DH5 α . The positive clones were screened and cloned into the prokaryotic expression vector pET-32a. The recombinant plasmids *katA*-pET-32a and *ahpC*-pET-32a were introduced into *E. coli* BL21 (DE3) cells for expression of recombinant proteins, respectively. The target sequences of *katA* and *ahpC* gene were assayed by the dideoxy chain termination method (Biotechnology firm, BGI, Beijing, China). The recombinant *katA*-pET-32a-BL21 and *ahpC*-pET-32a-BL21 strains were cultured in lysogeny broth (LB) with 100 μ g/mL ampicillin, and induced at 30 °C by isopropylthio- β -d-galactoside with a final concentration of 1 mmol/L and 0.5 mmol/L, respectively. *E. coli* cells were harvested after 4 h and disrupted ultrasonically. The suspension and precipitate were collected and protein expression was analyzed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

H. pylori serological tests

A serological test for *H. pylori* immunoglobulin (Ig)G antibodies has already been completed and described by our group^[17].

Purification and renaturation of target recombinant proteins

The recombinant proteins were purified by Ni-NTA His Bind resin (Novagen, Darmstadt, Germany). We used stepwise dialysis to obtain the fusion protein by removing the denaturant (urea) in the purified protein. The dialysis tube was boiled 10 min in buffer (2% NaHCO₃ and 1mmol/L EDTA pH 8.0) and EDTA solution (1 mmol/L) sequentially. After cooling, the purified protein was put into the dialysis tube, and both ends were clamped with the dialysis clips. The protein was dialyzed in urea solution (pH 8.3) with a slowly decreasing concentration: 6 mol/L, 4 mol/L, 2 mol/L, 1 mol/L and 0 mol/L. Each dialysis lasted 24 h. Finally, the sample was removed from the dialysis tube and stored at -80 °C until analysis.

Detection of antibodies against recombinant proteins with enzyme-linked immunosorbent assay

An indirect enzyme-linked immunosorbent assay (ELISA) was applied to detect the serum antibodies

against *H. pylori* recombinant KatA and AhpC proteins. Recombinant KatA and AhpC proteins were diluted to 2 μ g/mL and 0.25 μ g/mL, respectively. Proteins at 100 μ L/well were incubated in a 96-well micro-plate (Costar, Washington, DC, United States) at 4 °C overnight and washed three times with phosphate buffer saline, Tween-20 (PBST), followed by blocking with 10% goat serum (AR0009; Boster, Beijing, China) and incubation for 2 h at 37 °C. Serum sample from cases and controls diluted 3200-fold with 10% bovine serum albumin (BSA) was added to the plate at 100 μ L/well and incubated for 1 h at 37 °C. Each serum sample was tested in three parallel wells. The plate was again washed three times with PBST. Peroxidase-conjugated goat anti-human IgG (H+L) (ZSGB-Bio, Beijing, China) was diluted 1:5000 with buffer, and 100 μ L was added to each well and incubated 30 min at 37 °C. Tetramethylbenzidine (TMB) substrate buffer was added to the plate at 100 μ L/well and incubated in the dark for 15 min at 37 °C. Fifty microliters stop solution was added per well to terminate the reaction. Finally, the plate was read at 450 nm absorbance using a micro-plate reader (Biotech Synergy 2, Winooski, Vermont, United States). The determination of serostatus of antibody was based on optical density (OD) value. The optimal cutoff point of OD values was used to classify samples as seropositive or seronegative.

Statistical analysis

All statistical analyses were conducted using SPSS 22.0 version software (Armonk, NY, United States). Unconditional logistic regression analysis was performed to estimate odds ratio (OR) and 95% confidence interval (CI) for the relationship between GC and antibodies. The χ^2 trend test was used to assess dose-response relationships between serum KatA and AhpC antibody levels and GC. In addition, a receiver operating characteristic (ROC) curve was plotted to identify the cutoff point of serum KatA and AhpC antibody results. Sensitivity, specificity, and area under the ROC curve (AUC) with 95%CI were calculated to evaluate the screening value of serum KatA and AhpC antibody levels for GC. Moreover, the optimal cutoff value was determined by the maximum Youden index (Youden index = sensitivity + specificity - 1). Combined analysis was used to observe screening accuracy of predictors for GC. For all tests, $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of study subjects

The characteristics of the study subjects were described in our previous study^[18].

Cloning and expression of the recombinant proteins

Nucleotide homology of the cloned *katA* gene compared to *H. pylori* 26695 was 95.52% The homology of

Table 1 Association between gastric cancer and seropositivity of catalase and alkyl hydroperoxide reductase antibodies in study subjects *n* (%)

Virulence factors serostatus	All subjects				<i>H. pylori</i> positive subjects				<i>H. pylori</i> negative subjects			
	Case	Control	OR (95%CI)	<i>P</i> value [†]	Case	Control	OR (95%CI)	<i>P</i> value [†]	Case	Control	OR (95%CI)	<i>P</i> value [†]
KatA												
Negative	78 (33.62)	228 (86.36)	1.0 (Reference)	< 0.001	47 (35.61)	104 (88.14)	1.0 (Reference)	< 0.001	26 (29.21)	109 (83.85)	1.0 (Reference)	< 0.001
Positive	154 (66.38)	36 (13.64)	12.84 (7.80-21.15)		85 (64.39)	14 (11.86)	14.59 (6.84-31.13)		63 (70.79)	21 (16.15)	12.15 (5.79-25.51)	
AhpC												
Negative	56 (24.14)	121 (45.83)	1.0 (Reference)	< 0.001	33 (25.00)	57 (48.31)	1.0 (Reference)	< 0.001	54 (54.00)	103 (70.55)	1.0 (Reference)	< 0.001
Positive	176 (75.86)	143 (54.17)	2.40 (1.55-3.73)		99 (75.00)	61 (51.69)	2.30 (1.25-4.23)		46 (46.00)	43 (29.45)	2.04 (1.10-3.78)	
Combination of KatA and AhpC												
Negative	78 (33.62)	226 (85.61)	1.0 (Reference)	< 0.001	49 (37.12)	104 (88.14)	1.0 (Reference)	< 0.001	33 (33.00)	127 (86.99)	1.0 (Reference)	< 0.001
Positive	154 (66.38)	38 (14.39)	11.64 (7.12-19.01)		83 (62.88)	14 (11.86)	13.40 (6.29-28.53)		67 (67.00)	19 (13.01)	13.91 (6.74-28.74)	

[†]The *P* value was obtained from logistic regression analysis adjusted for age, sex, family history of gastric cancer, smoking, and alcohol consumption. KatA: Catalase; AhpC: Alkyl hydroperoxide reductase.

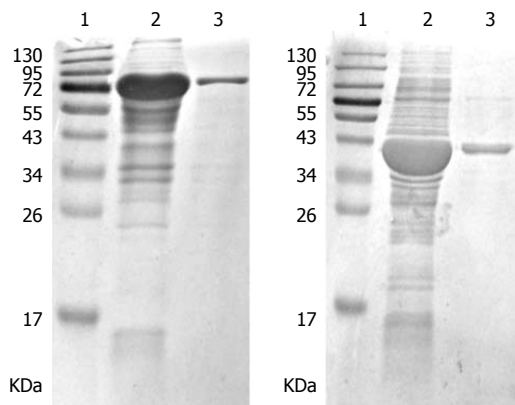


Figure 1 SDS-PAGE analysis of purified recombinant proteins. A: KatA; B: AhpC. 1: Marker; 2: Unpurified protein; 3: Purified protein. KatA: Catalase; AhpC: Alkyl hydroperoxide reductase.

ahpC nucleotide was 96.48% compared with *H. pylori* J99.

A prokaryotic expression system was constructed. After induction by isopropyl beta D thiogalactoside (IPTG), proteins with the expected size were clearly present as inclusion bodies in the ultrasonic precipitation by SDS-PAGE. Finally, the purified fusion proteins were obtained (Figure 1).

Association between serum positivity of antibodies and GC

As shown in Table 1, an association between KatA and GC risk was observed, with OR = 12.84 (95%CI: 7.79-21.15), 14.59 (6.84-31.13), and 12.15 (5.79-25.51) in all, *H. pylori*-positive and *H. pylori*-negative subjects, respectively (*P* < 0.001). Dose-dependent effects showed that KatA antibody levels were

strongly related to GC risk in the three populations mentioned above (*P* for trend < 0.001) (Table 2). Similarly, a significant association between GC risk and serum positivity of AhpC was observed with OR = 2.40 (95%CI: 1.55-3.73) in all subjects, 2.30 (1.25-4.23) in *H. pylori*-positive subjects, and 2.04 (1.10-3.78) in *H. pylori*-negative subjects (*P* < 0.001) (Table 1). Correspondingly, AhpC antibody level was significantly related to GC risk in a dose-dependent manner (*P* for trend < 0.001) (Table 2). Moreover, an evident association between GC risk and serum positivity of combination of KatA and AhpC was present, with OR = 11.64 (95%CI: 7.12-19.01), 13.39 (6.29-28.53), and 13.91 (6.74-28.74) in all, *H. pylori*-positive and *H. pylori*-negative subjects, respectively (*P* < 0.001) (Table 1).

Screening utility of serum antibody for GC

An ROC curve was plotted to explore the screening value of KatA and AhpC for GC. The AUC for KatA was 0.806 (95%CI: 0.768-0.845), 0.805 (0.751-0.853), and 0.801 (0.741-0.861) in all, *H. pylori*-positive and *H. pylori*-negative subjects, respectively (Figure 2). The AUC for AhpC was 0.615 (95%CI: 0.566-0.665) in all subjects, 0.629 (0.560-0.699) in *H. pylori*-positive subjects, and 0.605 (0.530-0.680) in *H. pylori*-negative subjects (Figure 3). As shown in Table 3, the optimal cutoff value of KatA and AhpC for GC was 0.3583 and 0.3647 in all subjects, providing a sensitivity of 66.81% and 75.65% and a specificity of 86.36% and 45.49%, respectively. AUC for the combination of KatA and FlaA was 0.906 (95%CI: 0.879-0.932), and the optimal cutoff value was 0.4305 with a sensitivity of 78.88% and a specificity of 89.02% (Figure 4A). For combination of KatA, FlaA, and AhpC, the AUC was 0.910

Table 2 Dose-dependent association between gastric cancer risk and serum catalase and alkyl hydroperoxide antibodies levels in study subjects *n* (%)

All subjects					<i>H. pylori</i> positive subjects					<i>H. pylori</i> negative subjects				
Antibody level (OD) ¹	Case	Control	OR (95%CI) ²	<i>P</i> value for trend	Antibody level (OD)	Case	Control	OR (95%CI) ²	<i>P</i> value for trend	Antibody level (OD)	Case	Control	OR (95%CI) ²	<i>P</i> value for trend
KatA														
≤ 0.4187	167 (71.98)	66 (25.0)	1.0 (Reference)	< 0.001	≤ 0.4152	92 (69.70)	29 (24.58)	1.0 (Reference)	< 0.001	≤ 0.4167	66 (74.16)	32 (24.58)	1.0 (Reference)	< 0.001
0.4187-0.5313	36 (15.52)	66 (25.0)	4.25 (2.49-7.27)		0.4152-0.5133	23 (17.42)	30 (25.42)	3.79 (1.78-8.06)		0.4167-0.5568	9 (10.11)	33 (25.42)	6.67 (2.70-16.51)	
0.5313-0.6799	18 (7.76)	66 (25.0)	9.95 (5.05-19.62)		0.5133-0.6692	9 (6.82)	30 (25.42)	9.69 (3.81-24.70)		0.5568-0.6824	11 (21.36)	33 (25.42)	7.00 (2.68-18.30)	
> 0.6799	11 (4.74)	66 (25.0)	15.85 (6.97-36.06)		> 0.6692	8 (6.06)	29 (24.58)	16.55 (5.51-49.76)		> 0.6824	3 (3.39)	32 (24.58)	19.89 (4.32-91.70)	
AhpC														
≤ 0.2168	88 (37.93)	66 (25.00)	1.0 (Reference)	< 0.001	≤ 0.2182	51 (38.64)	29 (24.58)	1.0 (Reference)	< 0.001	≤ 0.2110	31 (34.83)	32 (24.58)	1.0 (Reference)	< 0.001
0.2168-0.3265	69 (29.74)	66 (25.00)	1.26 (0.76-2.11)		0.2182-0.3433	41 (31.06)	30 (25.42)	1.10 (0.54-2.25)		0.2110-0.3310	29 (32.58)	33 (25.42)	1.44 (0.69-3.00)	
0.3265-0.4888	49 (21.12)	66 (25.00)	1.41 (0.82-2.43)		0.3433-0.4908	28 (21.21)	30 (25.42)	1.54 (0.70-3.38)		0.3310-0.4948	16 (17.98)	33 (25.42)	1.83 (0.80-4.23)	
> 0.4888	26 (11.21)	66 (25.00)	3.54 (1.84-6.82)		> 0.4908	12 (9.09)	29 (24.58)	3.40 (1.32-8.73)		> 0.4948	13 (14.61)	32 (24.58)	3.33 (1.31-8.46)	

¹Serum positivity for the antibodies to KatA and AhpC was categorized by quartiles of antibody levels in controls; ²Adjusted for age, sex, family history of gastric cancer, smoking, and alcohol consumption. *H. pylori*: *Helicobacter pylori*; KatA: Catalase; AhpC: Alkyl hydroperoxide reductase.

(95%CI: 0.885-0.935), offering a sensitivity of 80.17% and a specificity of 88.64%, while the optimal cutoff value was 0.4354 (Figure 4B).

DISCUSSION

Gastric carcinogenesis is a multifactorial process, and *H. pylori* infection plays an important role in the initial stage^[19]. Patients with malignant tumors are often diagnosed at an advanced stage, and 5-year survival rate is < 10%^[20]. Therefore, early detection is a crucial factor for GC prevention. However, it is difficult to diagnose GC any earlier because the symptoms of gastric pre-cancerous and malignant diseases are non-specific and vague. At present, endoscopy is the gold standard for screening GC and is commonly used in the clinic. A large case-control study from Japan indicated that GC mortality was reduced 30% by endoscopic screening compared with no screening^[21]. In spite of this finding, limitations of endoscopy, such as the existence of over diagnosis and unwillingness of asymptomatic patients because of pain as well as cost make endoscopy unsuitable for population-based screening. Serological testing is widely available and is a low-cost noninvasive diagnostic method. In the present study, we explored whether serum *H. pylori* antibody could serve as a biomarker for GC monitoring.

KatA is a ubiquitous enzyme that protects *H. pylori* cells from extracellular H₂O₂ attack^[22,23] and plays an important role in colonization of gastric mucosa^[15]. AhpC is the most abundant and essential antioxidant protein of *H. pylori*^[16], and it protects bacteria from lipid peroxidation and DNA damage^[24,25]. We used a commercial ELISA method to detect *H. pylori* infection status. However, this method may fail to detect prior *H. pylori* infection in GC patients, and patients positive for anti-CagA (cytotoxin-associated gene A) antibody may have negative results for *H. pylori* serological testing^[26,27]. In order to eliminate these possible influences on our results, *H. pylori*-negative and overall subjects were also analyzed to observe the associations between GC and the KatA and AhpC antibodies. The results indicated that we should be more vigilant regarding antibody titer and seropositivity. Meanwhile, we found that the median of KatA and AhpC antibody levels were lower in cases group than in the controls (data not shown). This finding implied that the high antibody titer of *H. pylori* KatA and AhpC may protect against the occurrence of GC.

A Latin American study showed that seropositivity of KatA in a population within a high risk of GC area was higher than that in a low-risk population^[28]. Our results confirmed that KatA was associated with GC, and seropositivity of KatA antibody showed a 14.59-fold increased risk of GC. Yan *et al*^[29] found that AhpC antibody of *H. pylori* may be related to the development of gastric diseases using the gerbil model to simulate human *H. pylori* infection. In addition, Huang *et al*^[30] indicated that AhpC was expressed in greater amounts in GC than gastritis strains. In our study, there was a significant association between AhpC antibody and GC, based on epidemiology data. Further analysis found that KatA and AhpC antibody levels were strongly related to GC risk in a dose-dependent manner. In order to explore whether KatA and

Table 3 Sensitivity and specificity of different catalase and alkyl hydroperoxide reductase critical values

Percentile ¹	All subjects			<i>H. pylori</i> positive subjects			<i>H. pylori</i> negative subjects		
	Critical value (OD) ²	Sensitivity	Specificity	Critical value (OD) ²	Sensitivity	Specificity	Critical value (OD) ²	Sensitivity	Specificity
KatA									
Optimal cutoff point ²	0.3583	66.81%	86.36%	0.3557	64.39%	88.14%	0.3730	70.79%	83.85%
25%	0.2800	46.55%	93.18%	0.4152	69.70%	74.58%	0.2773	50.56%	90.00%
50%	0.4305	75.00%	71.59%	0.5133	87.12%	49.15%	0.4447	78.65%	69.23%
75%	0.5958	90.95%	36.36%	0.6692	93.94%	24.58%	0.6107	92.13%	36.15%
90%	0.7418	97.84%	16.29%	0.9042	100.00%	8.47%	0.7873	97.75%	14.62%
AhpC									
Optimal cutoff point ²	0.3647	75.65%	45.49%	0.3613	75.76%	48.31%	0.2330	43.82%	70.77%
25%	0.1953	30.43%	78.95%	0.1953	30.30%	80.51%	0.1917	30.34%	77.69%
50%	0.2830	59.57%	57.14%	0.2865	59.85%	60.17%	0.2913	62.92%	57.69%
75%	0.4267	84.35%	32.71%	0.4325	83.33%	33.05%	0.4313	85.39%	23.85%
90%	0.5747	95.65%	14.29%	0.5302	93.94%	13.56%	0.6410	96.63%	13.85%

¹Percentiles of serum KatA and AhpC antibody levels in controls; ²Optimal cutoff point in the different parameters was identified according to the maximum Youden's index (sensitivity + specificity - 1). *H. pylori*: *Helicobacter pylori*; KatA: Catalase; AhpC: Alkyl hydroperoxide reductase.

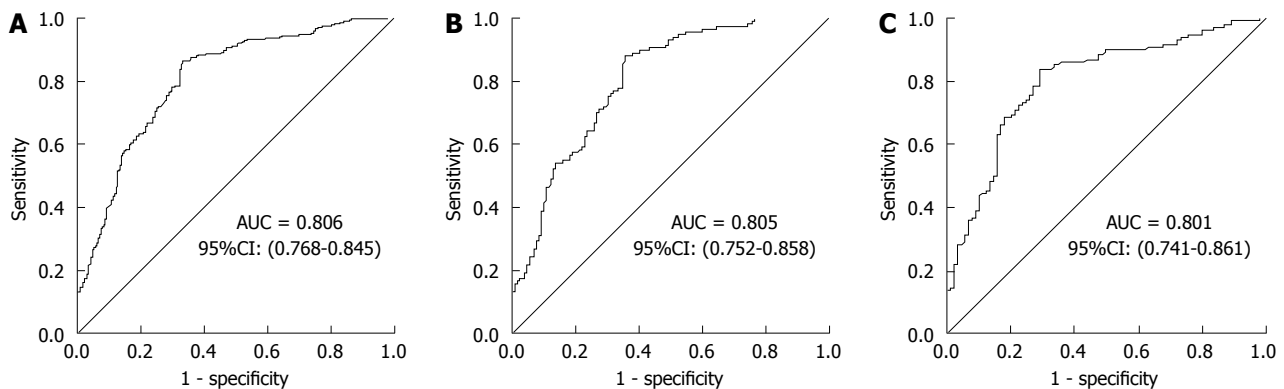


Figure 2 Receiver operating characteristic curve for serum catalase antibody. A: All subjects; B: *H. pylori*-positive subjects; C: *H. pylori*-negative subjects. *H. pylori*: *Helicobacter pylori*.

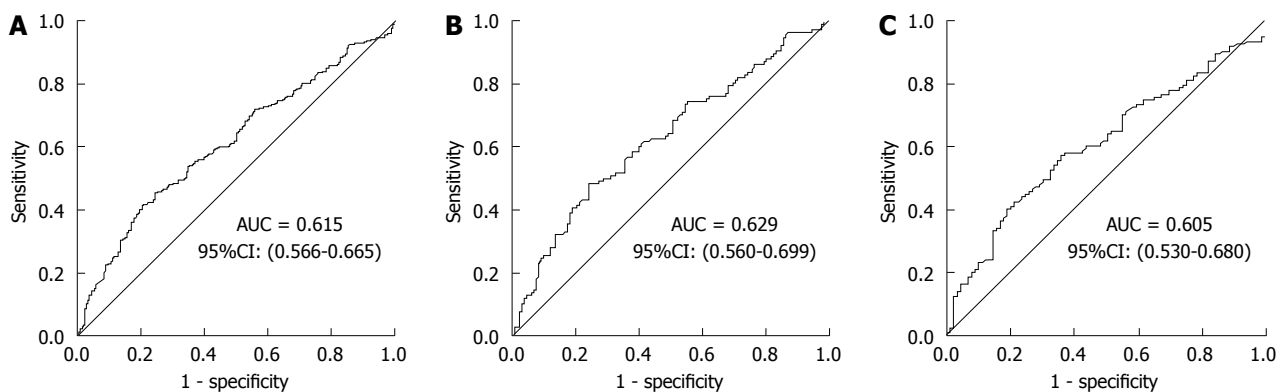


Figure 3 Receiver operating characteristic curve for serum alkyl hydroperoxide reductase antibody. A: All subjects; B: *H. pylori*-positive subjects; C: *H. pylori*-negative subjects. *H. pylori*: *Helicobacter pylori*.

AhpC could serve as biomarkers for GC, ROC curves were plotted to evaluate the screening value of the antibodies. The results showed that the AUC for KatA was 0.806, which was higher than the general standard for diagnosis ($AUC \geq 0.7$)^[31,32]. Unfortunately, the AUC for AhpC was lower. Generally, a single indicator for screening has a lower screening yield. At this point, we

attempted to develop a combined analysis to assess the value of screening. Our previous study found that the sensitivity was 74.1%, and the specificity was 64.4%, while FlaA served as a screening biomarker for GC alone^[17]. The combined results for KatA, FlaA, and AhpC showed that the AUC for combination of KatA and FlaA was elevated by 0.10, and sensitivity and specificity

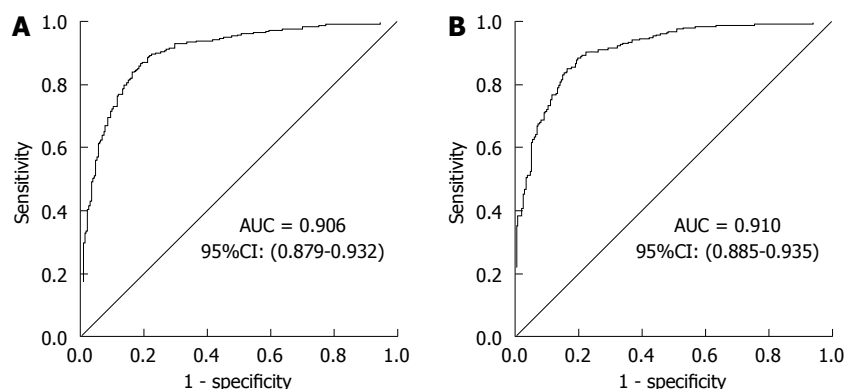


Figure 4 Receiver operating characteristic curve for combined analysis in all subjects. A: KatA + FlaA; B: KatA + FlaA + AhpC. KatA: Catalase; AhpC: Alkyl hydroperoxide reductase; FlaA: Flagella protein A.

were increased by 12.07% and 2.66%, respectively, in all subjects compared to KatA alone. Yet, combination of KatA, FlaA, and AhpC did not improve screening power in the identification of patients with GC compared to combination of KatA and FlaA.

Indirect ELISA method was adopted to detect serum KatA and AhpC antibodies in this study, and this method might be accompanied by the non-specific signal caused by cross-reactivity. In other words, KatA and AhpC will not only react with the corresponding specific antibody but also with the non-specific antibodies in the present study. Because of this non-specific signal, some *H. pylori*-negative subjects were classified as KatA or AhpC positive.

Some evidence indicates that *H. pylori* infection increases the risk of non-cardia GC^[7,33]. Nine (3.88%) cardia GC cases were included in our study. However, their involvement did not affect the overall results and conclusion.

In conclusion, the data indicate that serum KatA and AhpC antibodies are associated with GC risk and that KatA may serve as a novel biomarker for GC screening. Combined analysis of KatA and FlaA could improve screening accuracy. However, serum AhpC antibody performed poorly as a marker for GC. Our study offers a basis for early diagnosis of GC, and further prospective studies are needed to verify our findings.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) infection is a crucial cause of gastric cancer (GC). Eradication of *H. pylori* seems a reasonable approach for preventing GC, but it is not feasible in large populations due to financial limitations. Therefore, a sensitive and low-cost screening biomarker for GC is urgently needed.

Research frontiers

Invasive endoscopy is the gold standard for GC detection, but it is not suitable for population-based screening. Serological testing is a widely available and noninvasive diagnostic method. In this study, the authors explored the value of serum catalase (KatA) and alkyl hydroperoxide reductase (AhpC) antibodies of *H. pylori* as biomarkers for GC monitoring.

Innovations and breakthroughs

This study indicated that KatA and AhpC antibodies are associated with GC risk and that KatA may serve as a novel biomarker for GC screening. Besides, combining for KatA and flagella protein A could improve screening accuracy.

Applications

These finding offers a basis for early diagnosis of GC.

Peer-review

This is a well-designed study showing that KatA and AhpC antibodies are associated with GC. The methodology is well described. Exploration of KatA and AhpC as biomarkers has important value for GC prevention.

REFERENCES

- 1 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- 2 Ang TL, Fock KM. Clinical epidemiology of gastric cancer. *Singapore Med J* 2014; **55**: 621-628 [PMID: 25630323 DOI: 10.11622/smedj.2014174]
- 3 Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. *H. pylori* infection and gastric cancer: state of the art (review). *Int J Oncol* 2013; **42**: 5-18 [PMID: 23165522 DOI: 10.3892/ijo.2012.1701]
- 4 Limburg P, Qiao Y, Mark S, Wang G, Perez-Perez G, Blaser M, Wu Y, Zou X, Dong Z, Taylor P, Dawsey S. Helicobacter pylori seropositivity and subsite-specific gastric cancer risks in Linxian, China. *J Natl Cancer Inst* 2001; **93**: 226-233 [PMID: 11158192]
- 5 El-Omar EM, Oien K, Murray LS, El-Nujumi A, Wirz A, Gillen D, Williams C, Fullarton G, McColl KE. Increased prevalence of precancerous changes in relatives of gastric cancer patients: critical role of *H. pylori*. *Gastroenterology* 2000; **118**: 22-30 [PMID: 10611150]
- 6 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789 [PMID: 11556297]
- 7 Helicobacter and Cancer Collaborative Group. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001; **49**: 347-353 [PMID: 11511555]
- 8 Uemura N, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, Sasaki N, Haruma K, Sumii K, Kajiyama G. Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. *Cancer Epidemiol*

- Biomarkers Prev* 1997; **6**: 639-642 [PMID: 9264278]
- 9 **Fukase K**, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Amagai K, Hayashi S, Asaka M. Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. *Lancet* 2008; **372**: 392-397 [PMID: 18675689 DOI: 10.1016/S0140-6736(08)61159-9]
 - 10 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194 [PMID: 14722144]
 - 11 **Correa P**, Piazuelo MB. Natural history of *Helicobacter pylori* infection. *Dig Liver Dis* 2008; **40**: 490-496 [PMID: 18396115 DOI: 10.1016/j.dld.2008.02.035]
 - 12 **Bagnoli F**, Buti L, Tompkins L, Covacci A, Amieva MR. *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proc Natl Acad Sci USA* 2005; **102**: 16339-16344 [PMID: 16258069 DOI: 10.1073/pnas.0502598102]
 - 13 **Lahner E**, Bernardini G, Santucci A, Annibale B. *Helicobacter pylori* immunoproteomics in gastric cancer and gastritis of the carcinoma phenotype. *Expert Rev Proteomics* 2010; **7**: 239-248 [PMID: 20377390]
 - 14 **Yan J**, Mao YF, Shao ZX. Frequencies of the expression of main protein antigens from *Helicobacter pylori* isolates and production of specific serum antibodies in infected patients. *World J Gastroenterol* 2005; **11**: 421-425 [PMID: 15637759]
 - 15 **Hazell SL**, Evans DJ, Graham DY. *Helicobacter pylori* catalase. *J Gen Microbiol* 1991; **137**: 57-61 [PMID: 2045782]
 - 16 **O'Riordan AA**, Morales VA, Mulligan L, Faheem N, Windle HJ, Kelleher DP. Alkyl hydroperoxide reductase: a candidate *Helicobacter pylori* vaccine. *Vaccine* 2012; **30**: 3876-3884 [PMID: 22512976 DOI: 10.1016/j.vaccine.2012.04.002]
 - 17 **Huang CH**, Chiou SH. Proteomic analysis of upregulated proteins in *Helicobacter pylori* under oxidative stress induced by hydrogen peroxide. *Kaohsiung J Med Sci* 2011; **27**: 544-553 [PMID: 22208537 DOI: 10.1016/j.kjms.2011.06.019]
 - 18 **Tian W**, Jia Y, Yuan K, Huang L, Nadolny C, Dong X, Ren X, Liu J. Serum antibody against *Helicobacter pylori* FlaA and risk of gastric cancer. *Helicobacter* 2014; **19**: 9-16 [PMID: 24118166 DOI: 10.1111/hel.12095]
 - 19 **Graham DY**. *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology* 2015; **148**: 719-31. e3 [PMID: 25655557 DOI: 10.1053/j.gastro.2015.01.040]
 - 20 **Msika S**, Benhamiche AM, Jouve JL, Rat P, Faivre J. Prognostic factors after curative resection for gastric cancer. A population-based study. *Eur J Cancer* 2000; **36**: 390-396 [PMID: 10708942]
 - 21 **Hamashima C**, Ogoshi K, Okamoto M, Shabana M, Kishimoto T, Fukao A. A community-based, case-control study evaluating mortality reduction from gastric cancer by endoscopic screening in Japan. *PLoS One* 2013; **8**: e79088 [PMID: 24236091 DOI: 10.1371/journal.pone.0079088]
 - 22 **Free C**, Lee RM, Ogden J. Young women's accounts of factors influencing their use and non-use of emergency contraception: in-depth interview study. *BMJ* 2002; **325**: 1393 [PMID: 12480855]
 - 23 **Wang G**, Alamuri P, Maier RJ. The diverse antioxidant systems of *Helicobacter pylori*. *Mol Microbiol* 2006; **61**: 847-860 [PMID: 16879643 DOI: 10.1111/j.1365-2958.2006.05302.x]
 - 24 **Wang G**, Hong Y, Johnson MK, Maier RJ. Lipid peroxidation as a source of oxidative damage in *Helicobacter pylori*: protective roles of peroxiredoxins. *Biochim Biophys Acta* 2006; **1760**: 1596-1603 [PMID: 17069977 DOI: 10.1016/j.bbagen.2006.05.005]
 - 25 **Wang G**, Conover RC, Olczak AA, Alamuri P, Johnson MK, Maier RJ. Oxidative stress defense mechanisms to counter iron-promoted DNA damage in *Helicobacter pylori*. *Free Radic Res* 2005; **39**: 1183-1191 [PMID: 16298744 DOI: 10.1080/10715760500194018]
 - 26 **Ekström AM**, Held M, Hansson LE, Engstrand L, Nyrén O. *Helicobacter pylori* in gastric cancer established by CagA immunoblot as a marker of past infection. *Gastroenterology* 2001; **121**: 784-791 [PMID: 11606491 DOI: 10.1053/gast.2001.27999]
 - 27 **Annibale B**, Lahner E, Santucci A, Vaira D, Pasquali A, Severi C, Mini R, Figura N, Delle Fave G. CagA and VacA are immunoblot markers of past *Helicobacter pylori* infection in atrophic body gastritis. *Helicobacter* 2007; **12**: 23-30 [PMID: 17241297]
 - 28 **Camargo MC**, Beltran M, Conde-Glez CJ, Harris PR, Michel A, Waterboer T, Carolina Flórez A, Torres J, Ferreccio C, Sampson JN, Pawlita M, Rabkin CS. Serological response to *Helicobacter pylori* infection among Latin American populations with contrasting risks of gastric cancer. *Int J Cancer* 2015; **137**: 3000-3005 [PMID: 26178251 DOI: 10.1002/ijc.29678]
 - 29 **Yan J**, Kumagai T, Ohnishi M, Ueno I, Ota H. Immune response to a 26-kDa protein, alkyl hydroperoxide reductase, in *Helicobacter pylori*-infected Mongolian gerbil model. *Helicobacter* 2001; **6**: 274-282 [PMID: 11843959]
 - 30 **Huang CH**, Chuang MH, Lo WL, Wu MS, Wu YH, Wu DC, Chiou SH. Alkylhydroperoxide reductase of *Helicobacter pylori* as a biomarker for gastric patients with different pathological manifestations. *Biochimie* 2011; **93**: 1115-1123 [PMID: 21440595 DOI: 10.1016/j.biochi.2011.03.008]
 - 31 **Zheng J**, Ding X, Tian X, Jin Z, Pan X, Yan H, Feng X, Hou J, Xiang H, Ren L, Tian P, Xue W. Assessment of different biomarkers provides valuable diagnostic standards in the evaluation of the risk of acute rejection. *Acta Biochim Biophys Sin (Shanghai)* 2012; **44**: 730-736 [PMID: 22759804 DOI: 10.1093/abbs/gms056]
 - 32 **Hanley JA**, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982; **143**: 29-36 [PMID: 7063747]
 - 33 **Kamangar F**, Dawsey SM, Blaser MJ, Perez-Perez GI, Pietinen P, Newschaffer CJ, Abnet CC, Albanes D, Virtamo J, Taylor PR. Opposing risks of gastric cardia and noncardia gastric adenocarcinomas associated with *Helicobacter pylori* seropositivity. *J Natl Cancer Inst* 2006; **98**: 1445-1452 [PMID: 17047193 DOI: 10.1093/jnci/djj393]

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