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***Case Control Study***

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

Zhang B *et al*.KatA/AhpC and gastric cancer

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**AIM:** To investigate catalase (KatA), 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 ELISA. Logistic regression was applied to analyze the relationships between KatA, AhpC and GC. The *x*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; Alkyl hydroperoxide reductase; Serum antibody; 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 reductaseantibodies 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 Cancerhas classified *H. pylori* as a category I carcinogen since 1994[3]. Epidemiological data also supports 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 indentify 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 gastric (5%) and gastric malignancy (2%)[3]. Research indicates that these different outcomes may be associated with the virulence factorsof *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 andGC 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 including 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'-CCGCTCGAGTTACTTTTTCTTTTTTGTGTGG-3' (reverse) that generated a 1518-bp fragment. For *ahpC*, the primer sequences were 5'-CCGGAATTCATGTTAGTTACAAAACTTGCCC-3' (forward) and 5'-CCGCTCGAGTTAAAGCTTAATGGAATTTTC-3' (reverse) that generated a 597-bp fragment. *Eco*RI and *Xho*Irestriction 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 DH5α. 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 LB medium with 100 µg/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% SDS-PAGE.

***H. pylori serological tests***

A serological test for *H. pylori* IgG 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 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 ELISA***

An indirect 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 µg/mL and 0.25 µg/mL, respectively. Proteins at 100 µL/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% BSA was added to the plate at 100 µL/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 µL was added to each well, and incubated 30 min at 37 °C. TMB substrate buffer was added to the plate at 100 uL/well and incubated in the dark place for 15 min at 37 °C, Fifty microliters per well stop solution was added 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 OD value. The optimal cutoff point of OD values was used to classify samples as seropositive or seronegative.

***Statistical analysis***

All the statistical analyses were conducted using SPSS 22.0 version software. Unconditional logistic regression analysis was performed to estimate odds ratio (OR) and 95% confidence interval (CI) for the relationship between GC and antibodies. The *x*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 *ahpC* nucleotide was 96.48% compared with *H. pylori* *J99.*

A prokaryotic expression system was constructed. After induction by 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 these three populations mentioned above (*P* for trend < 0.001) (Table 2). Similarly, a significant association between GC risk and serum positivity of AhpC was also 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 also 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 (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 crucial factor for GC prevention. However, it is difficult to diagnose GC earlier because the symptoms of gastric pre-cancerous and malignant diseases are non-specific and vague. At present, endoscopy as 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, limitations of endoscopy, such as the existence of over diagnosis and unwillingness of the asymptomatic patients because of pain, as well as costs 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 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 patient 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 showed that we should pay more attention to the antibody titer and seropositivity. Meanwhile, we found that the median of KatA and AhpC antibody levels were lower than in the controls (data not shown). This 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 with high risk of GC area was higher than in a low-risk population[28]. Our results verified 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. Also, Huang *et al*[30] indicated that AhpCwas 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 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 ≥ 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 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 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 combined KatA and FlaA.

Indirect ELISA method was adopted to detect serum KatA and AhpC antibodies in this study, which might be accompanied by the non-specific signal caused by cross-reactivity. This means that KatA and AhpC will not only react with the corresponding specific antibody but also react with the non-specific antibodies in the present study, which eventually led to some *H. pylori*-negative subjects being classified KatA or AhpC as 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 KatA may serve as a novel biomarker for GC screening. Combined analysis of KatA and FlaA could improve the 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 unrealistic to carry out 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 is unsuitable 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 KatA may serve as a novel biomarker for GC screening. Besides, combination for KatA and flagella protein A could improve the 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.

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**P-Reviewer:** Aoyagi K, Vorobjova T **S-Editor:** Ma YJ **L-Editor:** **E-Editor:**

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Virulence factors**  **serostatus** | **All subjects** | | | |  | ***H. pylori* positive subjects** | | | |  | ***H. pylori* negative subjects** | | | |
| **Case** | **Control** | **OR (95%CI)** | ***P******-*value**1 |  | **Case** | **Control** | **OR (95%CI)** | ***P-*value**1 |  | **Case** | **Control** | **OR (95%CI)** | ***P-*value**1 |
| 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) |  |

1The*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.

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

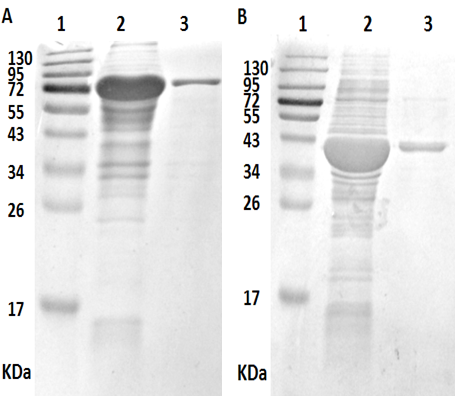
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **All subjects** | | | | |  | ***H*. pylori positive subjects** | | | | |  | **O *H*. pylori negative subjects** | | | | |
| **Antibody**  **level (OD)1** | **Case** | **Control** | **OR (95%CI)2** | ***P*-value for trend** |  | **Antibody**  **level (OD)** | **Case** | **Control** | **OR (95%CI)2** | ***P*-value for trend** |  | **Antibody**  **level (OD)** | **Case** | **Control** | **OR (95%CI)2** | ***P*-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.

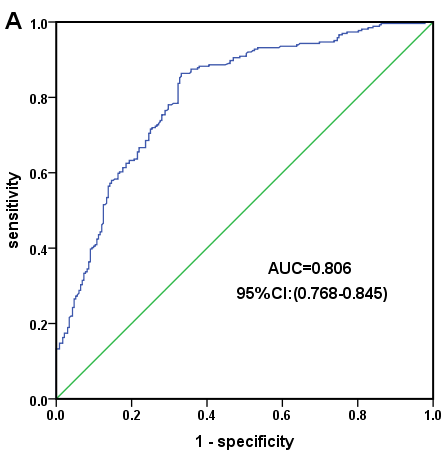
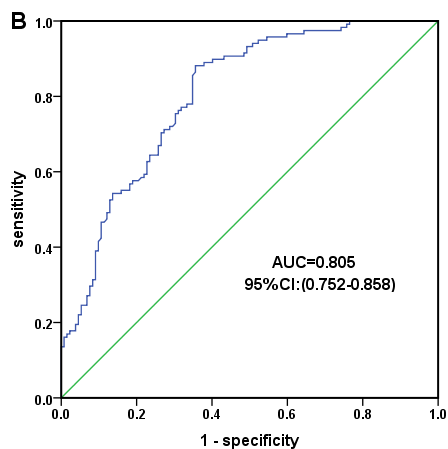
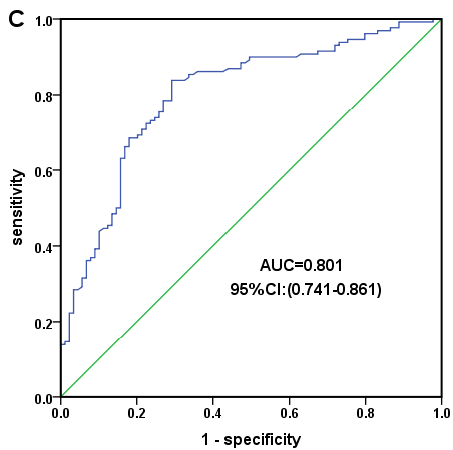
**Table 3 Sensitivity and specificity of different catalase and alkyl hydroperoxide reductase critical values**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **All subjects** | | |  | ***H. pylori* positive subjects** | | |  | ***H. pylori* negative subjects** | | |
| **Percentile1** | **Critical**  **value (OD)2** | **Sensitivity**  **(%)** | **Specificity**  **(%)** |  | **Critical**  **value (OD)2** | **Sensitivity**  **(%)** | **Specificity**  **(%)** |  | **Critical**  **value (OD)2** | **Sensitivity**  **(%)** | **Specificity**  **(%)** |
| KatA |  |  |  |  |  |  |  |  |  |  |  |
| Optimal cutoff pointb | 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 | 100.0 | 8.47 |  | 0.7873 | 97.75 | 14.62 |
| AhpC |  |  |  |  |  |  |  |  |  |  |  |
| Optimal cutoff pointb | 0.3647 | 75.65 | 45.49 |  | 0.3613 | 75.76 | 48.31 |  | 0.233 | 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 |

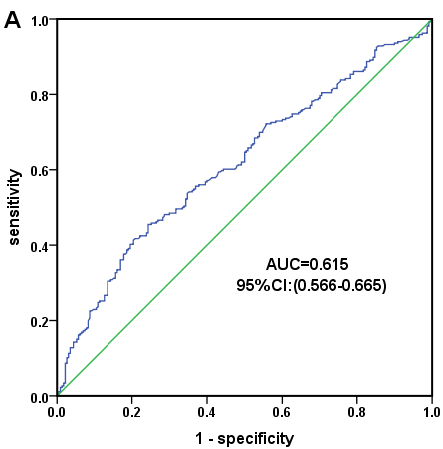
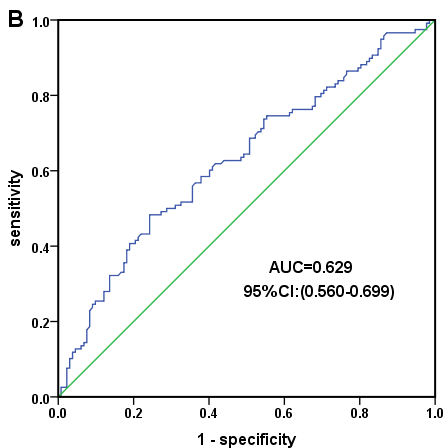
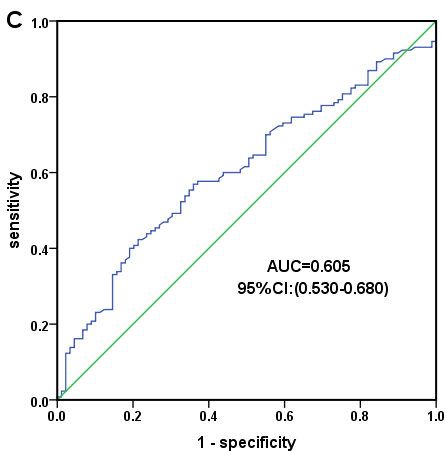
1Percentiles of serum KatA and AhpC antibody levels in controls; 2Optimal cutoff point in the different parameters was identified according to the maximum Youden’s index (sensitivity + specificity –1). *H. pylori*: *helicobacter pylori*; KatA: catalase; AhpC: alkyl hydroperoxide reductase.



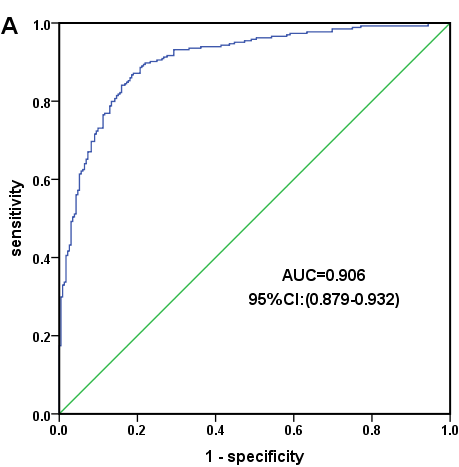
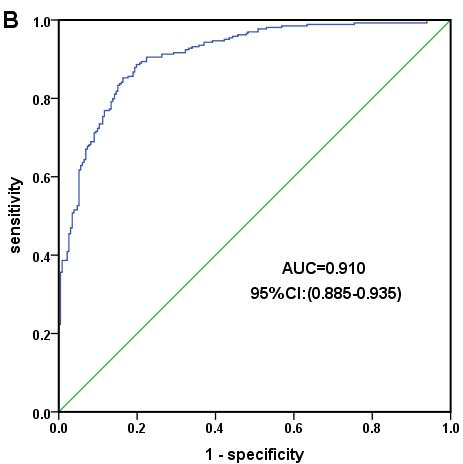
**Figure 1 SDS-PAGE analysis of purified recombinant proteins.** A: KatA; B: AhpC. 1: Marker; 2: Unpurified protein; 3: Purified protein. KatA: catalase; AhpC: alkyl hydroperoxide reductase.

**Figure 2 Receiver operating characteristic curvefor 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 curvefor serum alkyl hydroperoxide reductase antibody.** A: All subjects; B: *H. pylori*-positive subjects; C: *H. pylori*-negative subjects**.** *H. pylori*: *helicobacter pylori*.

**Figure 4 Receiver operating characteristic curvefor combined analysis in all subjects.** A: KatA + FlaA; B: KatA + FlaA + AhpC.

KatA: catalase; AhpC: alkyl hydroperoxide reductase; FlaA: flagella protein A.