

The alcohol dehydrogenase isoenzyme alcohol dehydrogenase IV as a candidate marker of *Helicobacter pylori* infection

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Abstract

Introduction: *Helicobacter pylori* infection is associated with decreased alcohol dehydrogenase (ADH) activity in the gastric mucosa. The decrease in gastric ADH activity depends on the severity of inflammation and mucosal injury. This damage can be a reason of the release of enzyme from gastric mucosa and leads to the increase of the ADH activity in the sera of patients with *H. pylori* infection.

Material and methods: Serum samples were taken from 140 patients with *H. pylori* infection. Total ADH activity was measured by photometric method with *p*-nitrosodimethylaniline as a substrate and ALDH activity by the fluorometric method with 6-methoxy-2-naphtaldehyde. For the measurement of the activity of class I and II isoenzymes we employed the fluorometric methods, with class-specific fluorogenic substrates. The activity of class III ADH was measured by the photometric method with *n*-octanol and class IV with *m*-nitrobenzaldehyde as a substrate.

Results: The activity of ADH IV in the serum of patients with *H. pylori* infection increased about 42% (7.86 mU/l) in the comparison to the control level (4.52 mU/l). Total activity of ADH was 1105 mU/l in patients group and 682 mU/l in control. The diagnostic sensitivity for ADH IV was 88%, specificity 90%, positive and negative predictive values were 91% and 84% respectively. Area under ROC curve for ADH IV was 0.84.

Conclusions: *Helicobacter pylori* infection of gastric mucosa is reflected in the serum by significant increase of class IV and total ADH activity. The results suggest a potential role for ADH IV as a marker of *H. pylori* infection.

Key words: alcohol dehydrogenase isoenzymes, *Helicobacter pylori* infection.

Introduction

Helicobacter pylori (*H. pylori*) is a bacterium responsible for widespread infection of the stomach mucosa with more than 50% of the world's population infected, even though 80% of those infected have no symptoms [1]. *Helicobacter pylori* infection represents a key factor in the etiology of various gastrointestinal diseases, ranging from chronic active gastritis without clinical symptoms to peptic ulceration, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. Some virulence factors such as urease and flagella are present in all strains of *H. pylori* and are necessary for pathogenesis and colonization, but the

pathogenetic mechanism behind the harmful effects of *H. pylori* on the gastric mucosa is not fully clarified [2]. *Helicobacter pylori* contains cytosolic alcohol dehydrogenase (ADH) and consequently is capable of producing acetaldehyde from excess ethanol *in vitro* [3].

In the human stomach, ethanol is metabolized by alcohol dehydrogenase, and this metabolism contributes to the so-called first-pass metabolism of ethanol (FPM). The gastric mucosa contains three classes of ADH isoenzyme – I, III, and IV. Several isoenzymes of aldehyde dehydrogenase (ALDH) are also expressed in the gastric mucosa [4]. Many factors that influence the activity of ADH and ALDH have been identified in the stomach. It is well known that *H. pylori* infection causes atrophy and intestinal metaplasia in the human stomach. It is hypothesized that these histological changes also affect ADH activities. In our previous study we found the decrease of activity of class IV ADH isoenzyme in infected biopsies of gastric mucosa, although the total activity of ADH did not significantly differ between *H. pylori*-positive and negative subjects [5]. However, the total ADH activity has been elevated in the sera of patients with *H. pylori* infection. The increase of total serum ADH activity was positively correlated with class IV ADH and seems to be caused by the release of this isoenzyme from damaged gastric mucosa [6].

In the current study, which is a continuation of our previous investigations, we defined the diagnostic criteria such as diagnostic sensitivity, specificity, predictive value for positive (PVPR) and negative results (PVNR), and receiver-operating characteristic (ROC) curves of tested enzymes. These data may be used in the evaluation of ADH and ALDH as candidate markers of *H. pylori* infections.

Material and methods

Patients

The protocol was approved by the Human Care Committee of the Medical University of Białystok, Poland. All patients gave informed consent for the examination.

Serum samples were taken for routine biochemical investigations from 140 patients (85 males and 55 females, mean age: 49 years, range: 34–73 years; mean age of men: 46, range: 40–73; mean age of women: 45, range: 34–66 years) with *H. pylori* infection. Serum samples were also obtained from 130 healthy subjects (control group, 70 males, 60 females, aged 35–65 years). The patients did not have any history of heavy alcohol consumption and none of them were receiving H₂ receptor antagonists, aspirin or proton pump in-

hibitors at least 1 week before serum collection. All of the patients drank alcohol occasionally and ethanol did not exist in serum samples of any subject when it was collected.

The mucosal alterations were established by histology using H + E staining. *Helicobacter pylori* infection was diagnosed by Giemsa staining of biopsy specimens. To assess the severity of gastric mucosal injury, the Sydney System for classification of gastritis was used. It has a grading scale of 0 to 3, where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The graded parameters were degree of inflammation, inflammatory activity, intestinal metaplasia, and mucosal atrophy [7]. In all tested patients, moderate inflammatory changes (degree 2) of gastric mucosa were present. Mucosal atrophy or metaplasia was not seen in any of the biopsy specimens.

Determination of total ADH activity

Total activity of ADH was estimated by the photometric method with 4-nitrosodimethylaniline (NDMA) as a substrate [8, 9]. The reduction of NDMA was monitored at 440 nm on a Shimadzu UV/VIS 1202 spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany).

Determination of total ALDH activity

Aldehyde dehydrogenase activity was measured using the fluorogenic method based on the oxidation of 6-methoxy-2-naphthaldehyde to fluorescent 6-methoxy-2-naphthoate [8, 10]. The fluorescence was read at an excitation wavelength of 310 and an emission wavelength of 360 nm on a Shimadzu RF-5301 spectrofluorophotometer (Shimadzu Europa GmbH, Duisburg, Germany).

Determination of class I and II ADH isoenzymes

Class I and II ADH isoenzyme activities were measured using fluorogenic substrates (4-methoxy-1-naphthaldehyde for class I and 6-methoxy-2-naphthaldehyde for class II) in a reduction reaction according to Wierzychowski *et al.* [8, 11]. The measurements were performed on a Shimadzu RF-5301 spectrofluorophotometer at an excitation wavelength of 316 nm for both substrates and emission of 370 nm for class I and 360 nm for class II isoenzymes.

Determination of class III ADH isoenzyme

The activity of class III ADH isoenzyme was estimated by the photometric method with formaldehyde as a substrate [8, 12]. The reduction of NAD was monitored at 340 nm and 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

Determination of class IV ADH isoenzyme

Class IV ADH isoenzyme activity was measured using the photometric method with 3-nitrobenzaldehyde as a substrate [8, 13]. The oxidation of NADH was monitored at 340 nm and 25°C on a Shimadzu UV/VIS 1202 spectrophotometer.

Diagnostic values calculation

The diagnostic criteria, such as the diagnostic sensitivity, specificity, predictive and negative value and the ROC curve were determined using GraphRoc Program for Windows (University of Turku, Turku, Finland) [14].

$$\text{Sensitivity} = \frac{\text{Number of true-positive results}}{\text{Number of true-positive results} + \text{Number of false-negative results}} \times 100\%.$$

$$\text{Specificity} = \frac{\text{Number of true-negative results}}{\text{Number of true-negative results} + \text{Number of false-positive results}} \times 100\%.$$

$$\text{PVPR} = \frac{\text{Number of true-positive results}}{\text{Number of true-positive results} + \text{Number of false-positive results}} \times 100\%.$$

$$\text{PVNR} = \frac{\text{Number of true-negative results}}{\text{Number of true-negative results} + \text{Number of false-negative results}} \times 100\%.$$

Statistical analysis

A preliminary statistical analysis (χ^2 test) revealed that the distribution of ADH and ALDH activities did not follow a normal distribution. Con-

sequently, Wilcoxon's test was used for statistical analysis. Data are presented as median, range and mean values. Statistically significant differences were defined as comparisons resulting in $p < 0.05$.

Results

The activities of alcohol dehydrogenase, aldehyde dehydrogenase and isoenzymes of alcohol dehydrogenase in the sera are presented in Table I. The comparison of ADH isoenzymes activities showed that a large difference was exhibited by class IV ADH. The median activity of this class isoenzyme in the serum of patients with *H. pylori* infection increased by about 42% (7.86 mU/l) in the comparison to the control level (4.52 mU/l). This increase was statistically significant. The other tested classes of ADH isoenzymes had higher activities in the sera of patients with *H. pylori* infection, but the differences were not statistically significant. The total alcohol dehydrogenase activity was significantly higher (42%) in patients with infection than in the healthy subjects. The median total activity of ADH was 1105 mU/l in the patient group and 682 mU/l in the control group. The analysis of ALDH total activity did not show a significant difference between *H. pylori* patients and healthy persons.

Table II shows the fulfillment of diagnostic criteria for total ADH and ADH IV. The sensitivity (88%) and specificity (90%) of ADH IV were higher than values for total ADH. Both the positive predictive values and negative predictive values were also the highest for ADH IV.

The relationship between diagnostic sensitivity and specificity was illustrated by a ROC curve (Figure 1). It shows that the area under the ROC curve for ADH IV (0.84) was higher than the ROC area of total ADH (0.73).

Discussion

The spiral-shaped, gram-negative bacterium *H. pylori* is found in colonized gastric mucosa or adherent to the epithelial lining of the stomach. Although most *H. pylori*-positive people are

Table I. Activity of ADH isoenzymes in the sera of *H. pylori* infected patients

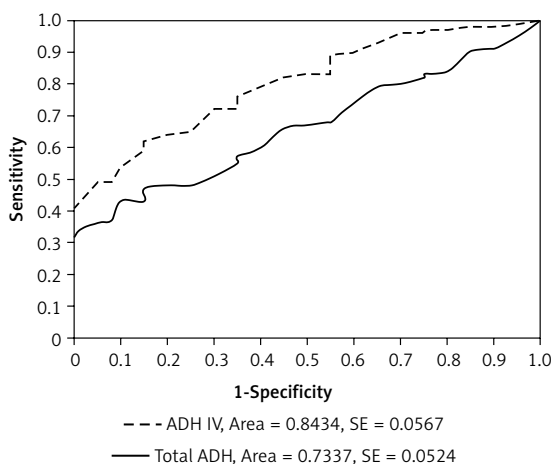
Group	ADH total Median, range, mean	ALDH total Median, range, mean	ADH I Median, range, mean	ADH II Median, range, mean	ADH III Median, range, mean	ADH IV Median, range, mean
Infected patients (n = 140)	1105, 491–184, 1086	3.23, 1.78–6.97, 3.04	1.53, 1.12–2.94, 1.41	14.32, 5.88–22.98, 13.96	12.90, 7.75–20.12, 12.35	7.86, 4.08–13.94, 7.44
Noninfected patients (n = 130)	682, 395–1547, 657	3.03, 1.62–6.75, 2.68	1.40, 1.06–2.67, 1.33	13.95, 4.46–20.66, 13.74	12.42, 7.14–19.54, 12.05	4.52, 2.79–9.85, 4.24
Value of p	< 0.001	0.483	0.374	0.526	0.289	< 0.001

Data are expressed as mU/l; p, *H. pylori* infection vs. noninfection.

Table II. Diagnostic criteria for total ADH and ADH IV for *H. pylori* infection

	Cut-off [mU/l]	Diagnostic sensitivity (%)	Diagnostic specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Total ADH	1324	72	78	79	78
ADH IV	9.65	88	90	91	84

The cut-off points were obtained from a study of a healthy population (95th percentile) [14].

**Figure 1.** Areas under ROC curves for ADH IV and total ADH

asymptomatic, the presence of *H. pylori* may lead to mucosal injury, and it is the cause of most peptic ulcer disease and a primary risk factor for gastric cancer [15]. Eradication of the organism results in ulcer healing and reduces the risk of ulcer recurrence and complications. It is known that *H. pylori* infection is associated with the decrease of alcohol dehydrogenase activity and first-pass metabolism of ethanol in the stomach [16]. Class I, III and in particular IV ADH isoenzymes present in the human stomach, which have been termed "gastric" ADH, contribute to total ADH activity. Some investigations have shown that the decrease of ADH activity (especially ADH IV) in *H. pylori*-positive specimens depended on the severity of gastric mucosal injury [5, 17]. Eradication of *H. pylori* normalizes alcohol dehydrogenase activity within 2 months [3]. It is commonly accepted that changes of enzyme activity in the cells of gastric mucosa are reflected by an increase of the corresponding enzyme activity in the serum. We found that the total activity of alcohol dehydrogenase was significantly higher in the sera of *H. pylori*-positive patients than in the controls. The increase of total ADH was positively correlated with ADH IV so the cause for the increase of serum total alcohol dehydrogenase in the case of *H. pylori* infection is an elevation of class IV ADH isoenzymes. The activities of other ADH isoenzymes were higher in the serum of infected patients than in *H. pylori*-negative patients, but the differences were not statistically significant. In many studies

gastric ADH activity was measured with ethanol concentration about 100 mM that saturated class I and IV ADH [18, 19]. In our study we determined the total alcohol dehydrogenase activity with 4-nitrosodimethylaniline as a substrate and the activity of each class ADH isoenzymes separately using sensitive and specific substrates. These are new methods of determination of the activity of alcohol dehydrogenase. These methods allow evaluation of the activity of each ADH isoenzyme more specifically than by using ethanol as the substrate.

A large number of invasive and non-invasive methods have been used for diagnosis of *H. pylori* infection in humans. Culture from gastric biopsies is a standard method for the diagnosis of *H. pylori* infection [20]. Determination of alcohol dehydrogenase activity (especially ADH IV) in serum can provide an alternative and less invasive method for diagnosis of *H. pylori* infection. Higher serum activity of ADH in patients with *H. pylori* infection might be a result of enzyme release from inflamed gastric mucosa cells and could be helpful for diagnosing infection. The diagnostic criteria for disease markers are sensitivity, specificity and area under the curve (AUC). The ideal method should possess very high specificity and very high sensitivity. The criteria for 100% sensitivity and 100% specificity have not as yet been fulfilled by any of the known methods. Since the discovery of *H. pylori* many tests have been designed for its diagnosis. But no test is accurate enough to be the 'gold standard' [21]. The urea breath test had the highest sensitivity and specificity: 98% and 89% respectively [22]. However, the rapid urease test, culture, and histology require endoscopic biopsy of gastric mucosal tissue, which is expensive, inconvenient for the patient and available only at specialized centers. Serological tests that detect anti-*H. pylori* IgG antibodies are non-invasive, and less expensive, but a positive serological test does not mean active infection. Performance of serological tests depends on the antigen preparation used, and as *H. pylori* strains differ among geographic locations, local validation of the test is necessary. Serological tests are widely used for non-invasive diagnosis, but they cannot differentiate a current infection from a past exposure [23]. We believe that the measurement of ADH IV activity in serum can be used to monitor the effectiveness of treatment of *H. pylori* infection.

In the present study, the diagnostic sensitivity and specificity were the highest for ADH IV (88% and 90% respectively) and total ADH (72% and 78% respectively). These values are relatively high but lower than for serological tests. The predictive value for positive results indicates the probability with which the infection of *H. pylori* exists in the case of positive test results. The negative predictive value for negative results predicts the probability that disease exists in the case of negative test results. In this investigation, ADH IV has high predictive values for positive and negative results (91% and 84% respectively).

The most important criterion for disease markers is the sensitivity/specificity diagram – the ROC curve. The area under the ROC curve indicates the clinical usefulness of tested substances. A larger area under the ROC curve signifies a better tumor marker. In this study the ADH IV (0.84) area under the ROC curve was higher than that for total ADH (0.73).

This is the first study showing all the diagnostic criteria for alcohol dehydrogenase and aldehyde dehydrogenase in *H. pylori* infection. These results strongly suggest a potential role for ADH (especially ADH IV) as markers for *H. pylori* infection, but further investigations and confirmation by a prospective study are necessary.

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