

# The Diagnostic Significance of Serum Alcohol Dehydrogenase Isoenzymes and Aldehyde Dehydrogenase Activity in Prostate Cancer Patients

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**Abstract.** *Background/Aim: The aim of this study was to investigate a potential role of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) as tumor markers for prostate cancer (PCa). Materials and Methods: Serum samples were obtained from 52 patients with PCa, 34 patients with benign prostatic hyperplasia (BPH) and 60 healthy subjects. Class III and IV of ADH and total ADH activity were measured by the photometric method. For measurement of class I and II ADH and ALDH activity, the fluorometric method was employed. Results: Significantly higher total activity of ADH, ADH III and ADH IV were found in the sera of both, PCa and BPH patients compared with healthy individuals. The diagnostic sensitivity for ADH III activity was 94.2%, specificity 100%, PPV (positive predictive value) and NPV (negative predictive value) were 100% and 95.2% respectively. Area under receiver-operating characteristics (ROC) curve for ADH III activity was 0.993. Conclusion: The results suggest a potential role of ADH III activity as a parameter included in the panel of markers for PCa.*

Prostate cancer (PCa) is one of the commonly diagnosed malignancies in men worldwide and the second leading cause of death from cancer disease (1). Patients in early stage of PCa can be cured with radical prostatectomy or radiotherapy, with or without androgen ablation. Metastatic PCa, with secondary lesions found mostly in the skeleton, has very high mortality and morbidity (2). The standard method for diagnosis of PCa involves prostate biopsy, but due to its diagnostic limitations and invasive nature, it has

only partial utility. Prostate specific antigen (PSA) is the most commonly used serum marker for the detection of prostate cancer. Because of its lack of specificity in PCA screening, there is a clinical need for new prostate cancer markers to decrease unnecessary prostate biopsies (3).

Epidemiological studies have demonstrated a correlation between ethanol consumption and the occurrence of cancers in many organs. Worldwide, about 3.6% of all cancers were attributable to alcohol drinking and the International Agency for Research on Cancer (IARC) classified alcohol consumption as group 1 of human carcinogens (4). A relationship has been established between alcohol and cancers of oral cavity, pharynx, larynx, esophagus, stomach, liver, pancreas, colon, rectum and breast. Significantly increased risk of prostate cancer caused by ethanol consumption have been also noticed in few cohort studies (5, 6, 7). The exact mechanism by which alcohol consumption enhances carcinogenesis is still unexplained. Alcohol is oxidized primarily by alcohol dehydrogenase (ADH) to acetaldehyde, a substance capable of initiating carcinogenesis by forming adducts with proteins and DNA and causing mutations. Next, acetaldehyde is metabolized by aldehyde dehydrogenase (ALDH) to acetate.

In tissues of many cancers, we can observe disproportion between alcohol dehydrogenase and aldehyde dehydrogenase activities in comparison to healthy cells. These disturbances could be the cause of elevated production of carcinogenic acetaldehyde and also disorders in the metabolism of many biologically important substances *e.g.* retinoic acid (8). Many studies have shown that cancer cells of many organs may release ADH isoenzymes to the blood raising the possibility of using the measurement of ADH activity in cancer diagnostics.

In the current study, we determined the differences in the activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in the sera of patients with PCa. Moreover, we defined the diagnostic utility (sensitivity, specificity, predictive value for positive and negative results, and receiver-operating characteristics (ROC) curve) of ADH isoenzymes in prostate cancer.

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*Key Words:* Alcohol dehydrogenase isoenzymes, aldehyde dehydrogenase, prostate cancer, cancer markers, diagnostic utility.

## Materials and Methods

**Materials.** Blood samples were obtained before cancer resection from 52 patients with prostate cancer (mean age 65 years, range=58-86 years), hospitalized in Clinical University Hospital in Białystok, Poland. None of the patients had received chemotherapy, radiotherapy or immunotherapy before sample collection. We determined two control groups from which serum samples were taken: 34 patients (mean age 55 years, range=52-69 years old) with benign prostatic hyperplasia (BPH) and 60 healthy men (mean age 58 years, range=51-67 years old). All of patients (both studied and control groups) had a history of occasional alcohol consumption.

The research protocol was approved by the Medical University of Białystok's Human Care Committee located in Białystok, Poland (Approval No R-I-002/185/2016). All patients gave their informed consent for the examination.

### Methods

**Determination of total ADH activity.** Total ADH activity was estimated by the photometric method using p-nitrosodimethylaniline (NDMA) as a substrate (9). The reaction mixture (2 ml) contained serum (0.1 ml), 1.8 ml of a 26  $\mu$ M solution of substrate in 0.1 M of sodium phosphate buffer, pH 8.5 and 0.1 ml of mixture containing 0.25 M n-butanol and 5 mM NAD. 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 (10). The reaction mixture contained 60  $\mu$ l of serum, 60  $\mu$ l of substrate, 20  $\mu$ l of 11.4 mM NAD and 2.8 ml of 50 mM of sodium phosphate buffer, pH 8.5. The mixture also contained 50  $\mu$ l of a 12mM solution of 4-methylpyrazole as a specific inhibitor of ADH activity. 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 activity was 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 Wierzchowski et al. (11). The assays were performed in a reaction mixture containing a serum (60  $\mu$ L), substrate (150  $\mu$ L of 300  $\mu$ M), NADH (100  $\mu$ l of 1 mM) and 0,1 M of sodium phosphate buffer, pH 7.6 (2.69 ml) using the conditions previously described (12). 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 assay mixture for class III alcohol dehydrogenase contained a serum (100  $\mu$ l), formaldehyde as a substrate (100  $\mu$ l of 1 mM), glutathione (100  $\mu$ l of 1 mM) and NAD (240  $\mu$ l of 1.2 mM) in 0.1 mol NaOH-pyrophosphate buffer pH 8.0 (13). The final volume was 2 ml. 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.** The assay mixture for class IV of ADH activity contained serum (50  $\mu$ l), m-nitrobenzaldehyde as a substrate (132  $\mu$ l of 80  $\mu$ M) and NADH (172  $\mu$ l of 86  $\mu$ M) in 0.1 M sodium phosphate buffer pH 7.5 (14). 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, diagnostic specificity, predictive value of positive (PPV) and negative (NPV) results and the ROC curve, were determined using GraphRoc Program for Windows (University of Turku, Turku, Finland) (15). For the diagnostic accuracy calculations and the ROC curve we used the total PCa patients group vs. control group.

**Statistical analysis.** Preliminary statistical analysis (Chi-square test) revealed that the distribution of ADH and ALDH activities did not follow a normal distribution. The differences between tested and control groups were evaluated by Mann-Whitney *U*-test. Data were presented as median and the mean values $\pm$ standard deviation. Statistically significant differences were defined as comparisons resulting in  $p < 0.05$ .

## Results

The activities of total ADH, ALDH and ADH isoenzymes in sera of patients with prostate cancer are presented in Table I. We have found that the total alcohol dehydrogenase activity was significantly higher in patients with prostate cancer and in the BPH group compared to healthy subjects. The mean total activity of ADH was 1.80 IU/l in sera of cancer patients, 1.82 IU/l in patients with benign hyperplasia and 0.95 IU/l in the control group. We also observed that ALDH activity is significantly lower in the PCa group (1.90 mIU/l) and BPH patients (1.52 mIU/l) in contrast to controls (7.23 mIU/l). We did not observe significant differences in ADH and ALDH activity between cancer and BPH group.

The comparison of ADH isoenzymes showed that both, ADH III and ADH IV activities are significantly higher in the sera of patients with prostate cancer and with benign lesions compared with healthy individuals. The activity of class III ADH was 75.99 mIU/l in PCa group, 68.79 mIU/l in BPH patients and 8.90 mIU/l in controls. The activity of ADH IV was lower than the activity of class III and reached 42.09 mIU/l in cancer, 54.72 mIU/l in BPH and 12.19 mIU/l in control group. Class III and IV of ADH did not indicate significant differences between BPH and control group. We have also observed that the activity of ADH I was significantly lower in the sera of patients with prostate cancer (0.94 mIU/l) and in BPH group (1.15 mIU/l) compared to controls (1.89 mIU/l). In contrast, the activity of class II ADH was also significantly lower only in prostate cancer patients in contrast to BPH and healthy individuals. The mean activity of this class ADH was 6.98 mIU/l in sera of cancer patients, 13.44 mIU/l in patients with benign hyperplasia and 14.01 mIU/l in the control group. We did not observe significant differences in ADH II activity between BPH and control group.

Table I. The comparison of ADH isoenzymes and ALDH activities in the sera of prostate cancer patients.

Tested group	ADH I Mean±SD Median	ADH II Mean±SD Median	ADH III Mean±SD Median	ADH IV Mean±SD Median	ADH Total Mean±SD Median	ALDH Total Mean±SD Median
Prostate cancer (n=52)	0.94±0.85 0.66	6.98±5.09 6.11	75.99±46.96 60.00	42.09±35.00 30.23	1.80±1.78 1.17	1.90±2.74 1.03
BPH (n=34)	1.15±0.84 0.97	13.44±7.41 15.35	68.79±43.44 61.41	54.72±78.98 18.01	1.82±1.18 1.94	1.52±0.95 1.55
Control group (n=60)	1.89±0.92 1.88	14.01±3.37 14.26	8.90±2.53 8.56	12.19±3.87 12.47	0.95±0.48 0.84	7.23±2.45 7.31
	$p^a < 0.001$ $p^b = 0.113$ $p^c < 0.001$	$p^a < 0.001$ $p^b < 0.001$ $p^c = 0.786$	$p^a < 0.001$ $p^b = 0.527$ $p^c < 0.001$	$p^a < 0.001$ $p^b = 0.137$ $p^c < 0.001$	$p^a = 0.047$ $p^b = 0.539$ $p^c < 0.001$	$p^a < 0.001$ $p^b = 0.325$ $p^c < 0.001$

SD: Standard deviation; BPH: benign prostate hyperplasia. Statistically significant differences were defined as comparisons resulting in  $p < 0.05$ . Data are expressed as mIU/l (ADH total- IU/l).  $p^a$ , cancer patients vs. controls;  $p^b$ , cancer patients vs. BPH patients;  $p^c$ , BPH patients vs. controls.

Table II. Diagnostic criteria for ADH total and ADH isoenzymes for prostate cancer.

	Cut-off (mIU/l)	Diagnostic sensitivity (%)	Diagnostic specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
ADH III	17.36	94.2	100	100	95.2	97.3
ADH IV	14.79	90.4	76.7	77.0	90.2	83.0
ADH total	2.21	32.7	61.0	62.0	63.2	68.8

Table II shows the diagnostic criteria for ADH III, ADH IV and ADH total. The sensitivity (94.2%) and specificity (100%) of class III ADH were higher than that for isoenzyme class IV (90.4% and 76.7% respectively). Both positive and negative predictive values were also the highest for the activity of ADH III (100% and 95.2%). The accuracy was 97.3% for the ADH III activity and only 83% for ADH IV. All diagnostic criteria for the total activity of ADH were much lower than that for isoenzymes class III and IV.

The relationship between diagnostic sensitivity and specificity was illustrated by a ROC curve (Figure 1). It shows that areas under the ROC curve for ADH III (0.993) and for ADH IV (0.904) activity were much higher than the ROC area of the total ADH (0.609).

## Discussion

The role of alcohol consumption on prostate cancer remains uncertain. There have been conflicting reports of possible associations of ethanol use with PCa, emphasizing on the need for research in this field. There are a number of

biological mechanisms that may explain the link between alcohol consumption and cancer development. One theory concerns high acetaldehyde exposure, causes mutations and sister chromatid exchanges in human cells. Moreover, acetaldehyde interferes at many sites with DNA synthesis and repair, and by binding to DNA and cellular protein, forms adducts that result in cancer development (16). Chronic ethanol consumption generates production of reactive oxygen species (ROS), that result in oxidative damage of proteins, lipids and nucleic acids (17). Furthermore, alcohol causes disturbances in retinoic acid biosynthesis, leading to an increased risk for cell proliferation and malignant transformation (18).

Data have shown that cancer cells have the ability to release alcohol dehydrogenase (ADH) isoenzymes to the blood of patients (8). This suggests that increased ADH activity in the sera of prostate cancer patients may be due to enzyme released from cancer cells. We have shown a significant increase in total alcohol dehydrogenase activity and a significant decrease in aldehyde dehydrogenase in the sera of patients with prostate cancer. These changes in enzymes activity provide evidence that there is a tendency to higher acetaldehyde production and lower

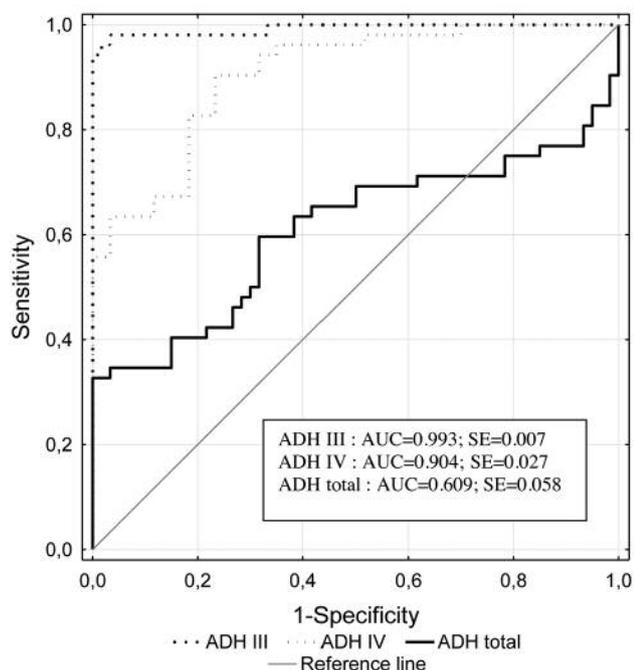


Figure 1. Receiver-operating characteristic (ROC) curves for alcohol dehydrogenase class III (ADH III), class IV (ADH IV) and total alcohol dehydrogenase (ADH total) activity. AUC: Area under curve; SE: standard error of the mean.

ability to remove this carcinogenic compound. The significantly elevated ADH activity was found in the sera of patients with malignant diseases of the esophagus, stomach, liver, pancreas, colorectum, kidney, urinary bladder, endometrium, cervix or brain (8).

The increase of ADH activity in prostate patients is a result of significantly elevated activity of isoenzymes class III and IV ADH. Class IV reveals the main role of ethanol oxidation so increased activity of this class causes elevated production of acetaldehyde. Moreover, this class of ADH takes part in the metabolism of lipid peroxidation products, that may also intensify prostate carcinogenesis. The end products of lipid peroxidation may damage DNA through formation of exocyclic adducts. The main products of lipid peroxidation are: crotonaldehyde, acrolein, 4-hydroxy-2-nonenal and malondialdehyde (19). Moreover, the activity of ADH IV in retinol metabolism is 6 times higher than for ADH II and 14 times higher than for ADH class I so in the case of lower ALDH activity retinoic acid biosynthesis pathway is also disturbed (20). ADH III participates mainly in metabolism of endogenous long-chain alcohols and aldehydes. This class of ADH catalyzes the oxidation of S-hydroxymethylglutathione and disturbances in its activity may lead to depletion of glutathione, which is a strong antioxidant compound that is important for the maintenance of redox state in cells. The

consequence of decreased glutathione concentration may be generation of reactive oxygen species and induction of oxidative stress leading to cancer development (21).

Molecules associated with prostate cancer may serve as a basis for designing non-invasive diagnostic tests. The ideal cancer marker should be characterized by high specificity, high sensitivity and suitable value of area under ROC curve. PSA has been widely used, is the preferred assay for prostate cancer diagnosis and represents the gold standard marker for urologists worldwide. However, this test is not prostate cancer specific but could also be elevated in benign prostate hyperplasia, prostatitis and in prostate manipulations. The literature data show that diagnostic significance of the prostate cancer biomarkers is the subject of many researches. The Prostate Health Index (PHI) is a mathematical formula which relies on the different proportions of specific biomarkers (tPSA, fPSA, -2proPSA). PHI provides additional information in differentiation of benign prostatic conditions and PCa. PHI was found to have greater AUC (0.73) than PSA alone (0.382) (22). In our study, we defined that the area under ROC curve for ADH III and ADH IV was higher in prostate cancer than for PHI (0.993 and 0.904 respectively). The 4Kscore test is determined on serum levels of 4 human kalikreins (tPSA, fPSA, intact PSA, human kalikrein 2) in combination with clinical information. The AUC value of 4Kscores is higher than for PSA alone and PHI but lower than for ADH isoenzymes (0.821) (23). Urinary biomarker – Pca antigen 3 (PCA3) has been shown to have a variable sensitivity, specificity, PPV and NPV. PCA3 sensitivity was 58-82%, specificity 58-76%, PPV and NPV 67% and 87% respectively (24, 25). All diagnostic criteria for ADH class III and IV was higher than for PCA3. AUC value for PCA3 in prostate cancer was 0.68-0.87 and was also lower than for ADH isoenzymes. %  $\alpha$ 2,3-sialic acid test identifies PSA glycoforms containing  $\alpha$ 2,3-sialic acid, which have been linked to PCa aggressiveness. Data have shown that combination of both markers, %  $\alpha$ 2,3-sialic acid and PHI gave better values of diagnostic criteria (sensitivity 100%, specificity 94%, AUC 0.985), similar to ADH class III and IV (26).

This is the first study showing all the diagnostic criteria for alcohol dehydrogenase and aldehyde dehydrogenase in prostate cancer. These results highlight that the future of prostate cancer diagnosis might rely on the combination of a panel of markers where alcohol dehydrogenase isoenzymes activity might also be included.

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