The Diagnostic Value of Alcohol Dehydrogenase Isoenzymes and Aldehyde Dehydrogenase Measurement Sera of Cervical Cancer Patients

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Abstract. *Aim: The aim of this study was to investigate a potential role of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) as tumor markers for cervical cancer. Materials and Methods: Blood samples were obtained from 43 women with cervical cancer. Isoenzymes class III, IV of ADH and total ADH activity were measured in the sera by the photometric method and class I, II ADH and ALDH activity by the fluorometric method. Results: The total activity of ADH and ADH I was significantly higher in the serum of patients with cervical cancer than in control groups. The diagnostic sensitivity for ADH I was 61,76%, specificity 65,7%, PPV and NPV were 70 and 62,16% respectively. AUC for ADH I was 0,654 and for total ADH 0,618. Conclusion: The results suggest a potential role of ADH I as a marker for cervical cancer.*

Cervical cancer represents one of the major issues of oncology, being the fourth leading cause of cancer-related deaths in women worldwide (1). Survival rates amount to 80% for patient with localized cancer, 47% for those with regional disease, and 21% for women with metastasis at time of diagnosis (2). Human papillomavirus (HPV) infection is the main risk factor for cervical cancer and 80% to 90% of these neoplasms are caused by HPV contagion (3). Evidence suggests a possible association between alcohol consumption and risk of cervical cancer (4, 5).

As it was established by the International Agency for Research on Cancer (IARC), consumption of alcoholic beverages is causally associated with cancers of the oral cavity, pharynx, larynx, oesophagus, colorectum, liver and female breast (6). The main hypothesis of alcohol-related carcinogenesis assumes participation of acetaldehyde, the toxic and mutagenic product of ethanol metabolism (7). The formation and degradation of acetaldehyde depend mostly on the activity of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Human alcohol dehydrogenase and aldehyde dehydrogenase, enzymes responsible for ethanol metabolism, exist in multiple molecular forms that have been grouped into several classes.

In our previous study, we showed that the activities of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase were present in normal cervical epithelium. Moreover, the total activity of ADH and its isoenzyme class I were significantly increased in cervical cancer tissues with the tendency toward increased, in accordance with the advancement of the disease (8). Studies of Jelski *et al*. showed that changes of ADH isoenzymes and ALDH activities can be reflected in sera of cancer patients, what creates a possibility for its application as tumor markers (9-11). Serum markers can assist in early diagnosis of cancer, determine high-risk patients, monitor response to the therapy and detect early occurrence of tumor. Furthermore, they should be non-invasive, simple, easy tool and low-cost procedure of determination.

In this study we investigated the activity of ADH and its isoenzymes and the total activity of ALDH in the sera of patients with cervical cancer. We hypothesized that the changed activity of these isoenzymes in cancer cells would be reflected in the serum and could thus be helpful for diagnosis of cervical malignancies.

Materials and Methods

Materials. Blood samples were obtained before surgery from 43 women (mean age=52 years, range=36-75 years) with cervical cancer. Patients were diagnosed as stage I (20 women), stage II (13 women) and stage III (10 women); 25 suffered from planoepitheliale carcinoma and 18 from adenocarcinoma. None of the patients had received chemotherapy or radiotherapy before sample collection. We determined two control groups from which serum samples were taken: 31 women (mean age 42 years, range 30-69 years old) with

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cervical intraepithelial neoplasia (CIN) and 52 healthy women (mean age 58 years, range 51-67 years old). All of women (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 Bialystok, Poland (Approval Nr R-I-002/179/2009). All patients gave their informed consent for the examination.

Determination of total ADH activity. Total ADH activity was estimated in sera of patients by the photometric method using p-nitrosodimethylaniline (NDMA) as a substrate (12). The reaction mixture (2 ml) contained serum (0.1 ml), 1.8 ml of a 26 μM 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-naphtaldehyde to fluorescent 6-methoxy-2-naphtoate (13). The reaction mixture contained 60 μl of serum, 60 μl of substrate, 20 μl of 11.4 mM NAD and 2.8 ml of 50 mM of sodium phosphate buffer, pH 8.5. The mixture also contained 50 μl of a 12 mM 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

Determination of class I and II ADH isoenzymes. Class I and II ADH isoenzymes 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*. (14). The assays were performed in a reaction mixture containing a serum (60 μl), substrate (150 μl of 300 μM), NADH (100 μl of 1 mM) and 0,1 M of sodium phosphate buffer, pH 7.6 (2.69 mL) using the conditions previously described (15). 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 μL), formaldehyde as a substrate (100 μL of 1 mM), glutathione (100 μl of 1 mM) and NAD (240 μl of 1.2 mM) in 0.1 mol NaOH-pyrophosphate buffer pH 8.0 (16). 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 μL), m–nitrobenzaldehyde as a substrate (132 μL of 80 μM) and NADH (172 μL of 86 μM) in 0.1 M sodium phosphate buffer pH 7.5 (17). The oxidation of NADH was monitored at 340 nm and 25˚C on a Shimadzu UV/VIS 1202 spectrophotometer.

Diagnostic value calculation. The diagnostic criteria, such as the diagnostic sensitivity, 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) (18).

$$\text{sensitivity (\%)} = \frac{\text{Number of true-positive results} \times 100\%}{\text{Number of true-positive results} + \text{Number of false-negative results}}$$

$$\text{specificity (\%)} = \frac{\text{Number of true-negative results} \times 100\%}{\text{Number of true-negative results} + \text{Number of false-positive results}}$$

$$\text{PPV (\%)} = \frac{\text{Number of true-positive results} \times 100\%}{\text{Number of true-positive results} + \text{Number of false-positive results}}$$

$$\text{NPV (\%)} = \frac{\text{Number of true-negative results} \times 100\%}{\text{Number of true-negative results} + \text{Number of false-negative results}}$$

Statistical analysis. Preliminary statistical analysis (Chi-square test) revealed that the distribution of ADH and ALDH activities did not follow a normal distribution. Consequently, the Wilcoxon test was used for statistical analysis. Data was presented using median, range and mean values. Statistically significant differences were defined as comparisons resulting in $p<0.05$.

Results

The activities of total ADH, ALDH and ADH isoenzymes in the sera of patients with cervical cancer are presented in Table I. The comparison of ADH isoenzyme activities shows that the highest activity was exhibited by class III ADH in all examined groups. The median activity of this class in the sera of cancer patients was 10.537 mIU/l, 10.488 mIU/l in patients with CIN and 10.412 mIU/l in healthy women. The activity of class II ADH was about 1 to 2 times lower and the activity of class IV 2-times lower than that of class III in every studied group.

The only difference between the tested groups was achieved by class I ADH. The median activity of this class ADH was 1.862 mIU/l in cancer patients, 1.347 mIU/l in women with CIN and 1.312 mIU/l in healthy ones. The increase of ADH I in cervical cancer was statistically significant in comparison to both control groups. The other tested classes of ADH isoenzymes had higher activities in the sera of patients with cancer but the differences were not statistically significant ($p>0.05$). There was no significant differences between CIN patients and healthy women, although there was a tendency toward increased activity of every class ADH in CIN group.

The total activity of alcohol dehydrogenase was significantly higher (approximately by 34%) in the sera of patients with cervical cancer than in women with CIN and 45% higher than in healthy ones. The median total activity

Table I. *Activity of ADH isoenzymes and ALDH in sera of patients with cervical cancer and healthy women.*

Tested group	ADH I Median Range Mean	ADH II Median Range Mean	ADH III Median Range Mean	ADH IV Median Range Mean	ADH Total Median Range Mean	ALDH Total Median Range Mean
Cervical cancer (n=43)	1.862 0.745-2.622 1.638	8.533 3.873-12.972 8.423	10.537 6.70-14.225 10.463	5.146 3.30-6.827 5.063	0.804 0.124-1.422 0.773	2.537 1.625-3.43 2.527
CIN (n=31)	1.347 0.560-2.033 1.297	8.505 4.235-12.562 8.399	10.488 6.778-14.075 10.427	5.104 3.407-6.545 4.976	0.602 0.172-1.325 0.577	2.551 1.58-3.455 2.518
Control group (n=52)	1.312 0.544-1.951 1.248	8.412 4.688-11.965 8.327	10.412 6.812-13.779 10.296	5.033 3.041-6.732 4.886	0.553 0.095-0.938 0.516	2.465 1.591-3.384 2.487
	p^a<0.001 p^b<0.001 p^c=0.358	p^a=0.356 p^b=0.276 p^c=0.348	p^a=0.583 p^b=0.474 p^c=0.378	p^a=0.402 p^b=0.474 p^c=0.683	p^a<0.001 p^b<0.001 p^c=0.367	p^a=0.402 p^b=0.378 p^c=0.406

Data are expressed as mIU/l (except ADH total - IU/l). p^a, cervical cancer *vs*. control group; p^b, cervical cancer *vs*. CIN; p^c, CIN *vs*. control group.

of ADH was 0.804 IU/l in the cervical cancer group, 0.602 IU/l in CIN and 0.553 IU/l in the women without changes in cervix. The analysis of ALDH activity did not indicate significant differences between all tested groups. We did not also find any significant differences in the activities of ADH and ALDH between both control groups.

Table II shows the diagnostic criteria for ADH total and ADH I. The sensitivity (61.76%) and specificity (65.70%) of ADH I were higher than values for ADH total (54.28% and 60.00%, respectively). Both the positive predictive value and negative predictive value were also the highest for ADH I.

The relationship between diagnostic sensitivity and specificity was illustrated by a ROC curve (Figure 1). It shows that area under the ROC curve for ADH I (0.654) was higher than the ROC area of ADH total (0.618).

Table II. *Diagnostic criteria for ADH total and ADH I for cervical cancer.*

	Diagnostic sensitivity [%]	Diagnostic specificity [%]	Positive predictive value [%]	Negative predictive value [%]
ADH I	61.76	65.70	70.00	62.16
ADH total	54.28	60.00	57.57	57.75

Discussion

Alcohol consumption is a critical factor in carcinogenesis and immune suppression associated also with increased risk of HPV infection and persistence (19). Ethanol metabolism leads to acetaldehyde production and oxidative stress occurrence, that act synergistically to HPV load, causing initiation and promotion of cervical carcinogenesis. Moreover, alcohol consumption elevates the estrogen level, which increases HPV expression *via* up-regulation of the progesterone receptor and the response to growth factors and to facilitate cell proliferation (20, 21).

In our previous study, we showed that the activity of alcohol dehydrogenase and its isoenzyme class I were significantly higher in cervical cancer tissue then in healthy cervix and the activity of aldehyde dehydrogenase did not differ between tissues types (8). These results suggest that cancerous cells have a greater capability for ethanol oxidation and lesser ability to remove highly carcinogenic acetaldehyde. Studies *in vitro* have shown that acetaldehyde interferes at many sites with DNA synthesis and repair what can result in tumor development. Acetaldehyde also binds to proteins, resulting in structural and functional alterations and by binding to DNA, it forms stable DNA adducts (22). Moreover, acetaldehyde inhibits O6-methyl-guanyltransferase, an enzyme necessary for reactivation of adducts caused by alkylating agents (23). Furthermore, class I of ADH plays an important role in maintaining homeostasis of retinoid. Retinol is first oxidized to retinal by alcohol dehydrogenase and then retinal is rapidly converted to retinoic acid (RA) by ALDH (24). Retinoic acid is particularly important because of its effect on cellular growth, differentiation and apoptosis. Choo *et al*. confirmed that retinoids inhibit the proliferation and differentiation of cervical cells infected with HPV (25). Therefore, changes in the activities of ADH and ALDH could perturb retinoid

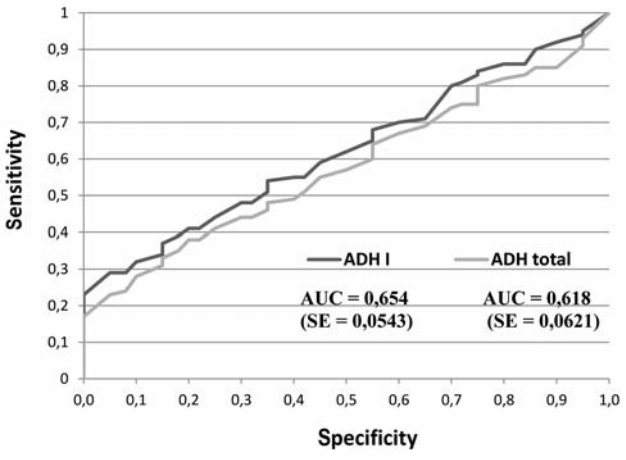

Figure 1. *Areas under ROC curves for ADH I and ADH total.*

homeostasis and alter the intracellular RA concentration, leading to abnormal differentiation and high susceptibility to HPV in the cervical epithelium.

Moreover, isoenzymes of ADH and ALDH can be secreted from cancer cells and be found in the sera of the patients, thus creating the possibility of its application in cancer diagnostics. Isoenzymes of ADH as tumor markers in different malignant diseases are the subject of research (10, 11, 26). In our study we found that total activity of alcohol dehydrogenase and the activity of ADH I were significantly higher in the sera of patients with cervical cancer in comparison to the control group and to patients with CIN. But the analysis of ALDH activity did not indicate significant differences between the tested groups. Studies of ADH and ALDH activities in endometrial cancer revealed similar results. The activities of total ADH and its class I isoenzyme were significantly higher in sera of endometrial cancer patients than in women with myoma uteri and healthy ones (10). Another female cancer, in which changes in ADH activity were found, is breast cancer. In the serum of patients with breast cancer, activity of ADH I is also elevated. Moreover, the serum activity of ADH I isoenzymes appeared to have a tendency to increase in accordance with advancement of the disease (27).

SCC antigen (squamous cell carcinoma antigen) is the most commonly used serum marker for cervical cancer but is not suitable for its early diagnosis. In several studies, SCC-Ag levels were elevated in approximately 24-53% of patients with stage Ib or IIa (28, 29). Similar results were found according to CYFRA 21-1 levels (29). Thus, both markers have a poor sensitivity in early stage of cervical cancer. The other tumor-associated antigens, such as CA 125, CEA and CA 19.9 are sometimes raised in the sera of patients with cervical cancer but have been found to be significantly higher in patients with adenocarcinoma than in those with SCC (30). Taking into consideration all advancement stages of cervical cancer, SCC antigen (71.8%) had the best sensitivity. In our study, the sensitivity of ADH I was lower than for SCC antigen (61.76%) but much higher than for CEA (32.4%). The specificity of ADH I was 65.7% and was lower than for SCC antigen and CEA (96.6% and 90% respectively). The predictive value of positive results was also higher for SCC antigen and CEA in comparison to ADH I but predictive value of negative results was higher for ADH I compared to both markers (31). According to Chmura *et al.,* the sensitivity of CYFRA 21-1 ranged from 6% (stage Ib) to 49% (stage IV) and was lower than sensitivity of total ADH and ADH I activities in cervical cancer (32).

Study of diagnostic significance of ADH isoenzymes in endometrial cancer revealed similar results like that from cervical cancer. The sensitivity of ADH I in endometrial cancer was 69% and was equal to that of the classic endometrial cancer marker CA125 (10, 33). It is interesting that we did not find any significant differences between the activity of ADH in patients with CIN and healthy woman. Related results were at endometrial cancer patients - ADH did not differ between women with myoma uteri and healthy ones (10). On the other hand, data have shown that CA125 could be also increased in the sera of patients with benign changes in the uterus (34). The most important criterion for tumor markers is a ROC curve and the area under curve (AUC) value. AUC for ADH I in cervical cancer was higher than for the total ADH activity (0,654 and 0.618 respectively). The similar diagnostic utility of ADH I was stated in endometrial cancer patients (AUC=0.682), suggesting the possibility of using ADH isoenzymes as tumor markers of uterus cancers (10).

In conclusion, we can state that the activity of class I ADH isoenzymes and the total activity of ADH were elevated in the sera of patients with cervical cancer compared to the healthy control and the group of patients with CIN. This activity is probably delivered from cervical cancer cells. Total ADH activity or ADH I activity in the serum may be approved as candidate markers of cervical cancer. Diagnostic utility of ADH I is limited, but a combination with other tumor markers may help identify even early-stage cervical cancer.

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