SHORT COMMUNICATIONS

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EVALUATION OF SERUM ADENOSINE DEAMINASE AND ITS ISOENZYMES IN PATIENTS WITH OVARIAN CANCER

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Ovarian cancer is the most lethal gynecological cancer worldwide. There are great relationships between the activities of adenosine deaminase (ADA), one of the enzymes in purine nucleotide pathway and carcinogenic process. In the present study the activities of the total ADA, ADA1 and ADA2 were measured in the sera of the patients with ovarian cancer. The activities of tADA, ADA1 and ADA2 were assessed in sera of 30 patients with ovarian cancer and 30 normal control individuals, using a modified Ellis method in which only ADA2 activity was measured in the present of a specific inhibitor, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). Our results showed that the tADA, ADA1, and ADA2 serum activities of patients were found to be significantly increased ($P < 0.05$) than those of healthy control group. Although, ADA and its isoenzymes were not the specific markers for diagnosis of ovarian cancer, measurement of their activities may be used as a diagnostic means in ovarian cancer as well as the other analytical procedures.

K e y w o r d s: adenosine deaminase (ADA), ADA isoenzymes, ovarian cancer, EHNA.

Ovarian cancer is usually diagnosed in the late stage, and overall, 5-year survival rates are not more than 20% [1-3]. Early stage of the disease is mostly asymptomatic, the clinical manifestations of ovarian cancer are mostly associated with the advanced disease. [4].

Several circulating markers have been detected and quantified in blood, peritoneal fluid or homogenized neoplastic specimens in earlier stages of tumor progression. There are significant associations between the carcinogenic process and the activities of some enzymes in malignant tumors.

Adenosine deaminase (EC 3.5.4.4) is one of the enzymes in purine salvage pathway which catalyzes the irreversible hydrolytic deamination of adenosine and 2′deoxyadnosine to form inosine and 2′deoxyinosine respectively [5-7]. ADA is an important enzyme in the differentiation and proliferation of lymphocytes and the monocyte–macrophage system [8, 9]. It consists of two major isoenzymes: ADA1 and ADA2 [10]. Each of them has different optimal pH (pH 6.5 and 7.5 for ADA2 and ADA1 respectively), Michaelis constants, and relative substrate specificity [7]. ADA1 exists in two forms: a small form that is a monomer with molecular weight of 33,000 Da and a large form which is a dimer/combining protein complex and with a total molecular weight of 280,000 Da, but ADA2 exists only as a monomer with a molecular weight of 100,000 Da [10].

The ADA1 exists in every cell, but the major activity of this isoenzyme is in lymphocytes and monocytes. The main ADA in the serum of normal subject is ADA2 [8]. Most cells contain small

amounts of ADA2 and its main source is the monocyte–macrophage cells [11, 12]. The present survey was carried out to assess the activities of the total ADA, ADA1, and ADA2 in the sera of patients with ovarian cancer and in comparison with those of healthy subjects.

Materials and Methods

Patients: From February 2014 until April 2015, must be deleted blood samples were collected from 30 women aged 20-60 years, who were referred to the Emam Khomeini Hospital, Tehran, Iran. The blood samples were drawn from patients before starting any treatment. Also blood samples of 30 women of similar age were obtained as controls. Informed consent was obtained in all cases. The study was approved by the Ethics Committee of Pasteur Institute of Iran.

Measurement of serum ADA activity. 5 ml of venous blood in the tube without anticoagulant. was centrifuged at 3000 rpm for 10 minutes at 4 °C, and then the sera were stored at -70 °C until enzyme analysis.

tADA was measured using a modified Ellis method [6, 12] using AVITEX kit and model 7070 type Automatic Analyzer (Hitachi Co. Ltd, Tokyo, Japan). This assay is based on the indirect quantitation of the decrease in NADH by the release of ammonia and inosine from adenosine. One ADA unit is defined as the amount of enzyme required to convert 1 mM adenosine into inosine and ammonia per minute under standard conditions and is expressed as IU/l.

The ADA2 activity was analyzed using the same procedure with 0.1mmol/l of erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) obtained from Sigma–Aldrich (St. Louis, MO, USA.), but only the ADA2 isoenzyme is active in the presence of EHNA. Then, ADA1 activity was calculated by subtracting the activity of ADA2 from the tADA activity [8].

Statistical analysis. All the results were expressed as mean ± standard deviation (SD). Comparison between the control and the patient groups were carried out by one-way ANOVA test. A P value < 0.05 was considered statistically significant. The statistical analysis was done by Statistical Package for Social Sciences (SPSS, version10.0).

Results and Discussion

The results of measuring the activities of tADA, ADA1and ADA2 are shown in Table 1.

Table 1. Adenosine deaminase and its isoenzymes levels in ovarian cancer (n = 30)

Group	Isoenzymes, IU/l		
	tADA	ADA1	ADA2
Control	14.35±2.70	5.91±2.22	8.44±3.29
Patient	29.56± 3.43	10.39±2.21	19.23±2.37

tADA, ADA1 and ADA2 serum activities in patients with ovarian cancer were significantly increased ($P < 0.005$) as compared to healthy control subjects. In this study ,analyzed CA125 serum marker levels were also analyzed in patients with ovarian cancer and healthy persons. The data of CA125 levels are shown in Table 2.

The survival in ovarian cancer considerably diminishes if cancer is diagnosed at the late-stage. Unfortunately, most women are presented with advanced disease. Transvaginal ultrasound (TVUS) and the CA-125 blood test are used most often for the screening of ovarian cancer. Analysis of serum proteins by the method of proteomics and combinations with other tumor markers may increase the sensitivity and specificity of screening [4].

The activities of various enzymes of purine nucleotide pathway are known to be associated with carcinogenesis [13]. In particular, increased ADA activity has an important role in the salvage pathway of cancer cells [3]. Likewise, increased ADA activity may be compensatory against a toxic accumulation of adenosine and deoxyadenosine substrates produced by enhanced purine metabolism in the cancer cells [14, 15] .

Results on the ADA activity in the cancer patients are contradictory. ADA activity was found to increase in some tumors while in other tumors ADA activity is decreased or unchanged [16] .

Table 2. CA125 serum levels in patients with ovarian cancer and healthy subjects (n = 30)

Statistics	CA125, U/ml	
	Normal controls	Patients
Mean	15.676	63.544
Median	15.700	29.475
Mode	5.80	199.00
Minimum	3.10	6.36
Maximum	36.40	396.00

In this research, ADA1, ADA2 and total ADA serum activities in patients with ovarian cancer were significantly elevated as compared to normal subjects ($P < 0.05$), and the increase can be attributed to proliferation of cancer cells.

It has been also proved that the ADA levels in blood are the result of the balance between the rate of ADA entering to the circulation and rate of its removal or inactivation. There are several factors affecting the serum enzyme levels: leakage of enzymes from cell, enzyme induction in a specific tissue by some factors, and proliferation of a specific cell type. Some studies suggested that the increased ADA levels in patients with cancer may be related to the increased DNA turnover of malignant cells, but it is still unclear Pragathi et al. indicated that ADA level is elevated only in ovarian cancer, but not in the benign tumor. Thus, this parameter can serve as a tumor marker, and may be effective in differentiating ovarian cancer from benign tumors of the ovary [18].

Conclusion. The measurement of serum activities of tADA and its isoenzymes may be a useful supplementing ovarian cancer diagnosis. However, a detailed investigation is needed to detect ADA at different stages of the disease to confirm the clinical value of this assay.

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ВИЗНАЧЕННЯ АКТИВНОСТІ АДЕНОЗИНДЕЗАМІНАЗИ ТА ЇЇ ІЗОЕНЗИМІВ У ПАЦІЄНТІВ ІЗ РАКОМ ЯЄЧНИКІВ

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Рак яєчників – гінекологічне захворювання з найвищим рівнем смертності в усьому світі. Існує зв'язок між активністю аденозиндезамінази (ADA), одним із ензимів пуринового нуклеотидного обміну, і канцерогенезом. У цій роботі було досліджено активність загальної ADA, ADA1 і ADA2 в сироватці крові пацієнтів із раком яєчників. Активність ADA, ADA1 і ADA2 визначали у 30 хворих на рак яєчників і у 30 здорових пацієнтів контрольної групи з використанням модифікованого методу Елліса, тільки активність ADA2 визначали в присутності специфічного інгібітора – еритро-9-(2-гідрокси-3-ноніл) аденіну (EHNA). Одержані результати показали, що активність загальної ADA, ADA1 і ADA2 в сироватці крові пацієнтів була значно збільшена ($P < 0,05$) порівняно з контрольною групою. Хоча ADA й її ізоензими не є специфічними маркерами раку яєчників, визначення їхньої активності може служити додатковим показником в діагностиці раку яєчників, а також в інших аналітичних процедурах.

Ключові слова: аденозиндезаміназа (ADA), ізоензими ADA, рак яєчників, EHNA.

ОПРЕДЕЛЕНИЕ АКТИВНОСТИ АДЕНОЗИНДЕЗАМИНАЗЫ И ЕЕ ИЗОЭНЗИМОВ У ПАЦИЕНТОВ С РАКОМ ЯИЧНИКОВ

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Рак яичников – гинекологическое заболевание с самым высоким уровнем смертности. Существует связь между активностью аденозиндезаминазы (ADA), одним из энзимов пуринового нуклеотидного обмена, и развитием канцерогенеза. В настоящей работе была исследована активность общей ADA, ADA1 и ADA2 в сыворотке крови пациентов с раком яичников. Активность ADA, ADA1 и ADA2 определяли у 30 больных с раком яичников и 30 здоровых пациентов контрольной группы с использованием модифицированного метода Эллиса, только активность ADA2 определяли в присутствии

специфического ингибитора – эритро-9-(2-гидрокси-3-нонил) аденина (EHNA). Полученные результаты показали, что активность общей ADA, ADA1 и ADA2 в сыворотке крови больных была значительно увеличена ($P < 0,05$) по сравнению с контрольной группой. Хотя ADA и ее изоэнзимы не являются специфическими маркерами рака яичников, измерение их активности может служить дополнительным показателем в диагностике рака яичников, а также в других аналитических процедурах.

Ключевые слова: аденозиндезаминаза (ADA), изоэнзимы ADA, рак яичников, EHNA.

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