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RESEARCH ARTICLE

EVALUATION OF SERUM ADENOSINE DEMINASE ADA IN PATIENTS WITH OVARIAN CANCER IN COMPARISON WITH HEALTHY SUBJECTS

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ABSTRACT

Adenosine deaminase (ADA) is an enzyme which involves in purine metabolism and catabolism of adenosine and deoxyadenosine into inosine and deoxyinosine. There are three isoenzymes; ADA1 and ADA2, ADA1+cp. Total ADA is very important in the tumor cell growth. In the present study the activities of ADA, its isoenzymes (ADA1 and ADA2) have been examined in the serum ovarian tumors of 30 patients and 30 normal serums. with autoanalyzer HITACHI 912 byspectrophotometry method by or presence or absence of erythro-9-2-hydroxy-3-nonyl Adenosine (EHNA). We found the mean values for tADA، ADA2 and ADA1 in healthy controls 14.35 U/L and 8.44 U/L and 5.91 U/L respectively and in Ovarian patients 29.56 U/Land 19.23 U/L and 10.39 U/L respectively. When compared to the healthy controls, serum total ADA and ADA1 and ADA2 levels were significantly higher ($P<0.001$) in Ovarian cancer patients.it, can be proposed that when we compared to the healthy controls serum total ADA and ADA1 and ADA2 levels with ovarian cancer patients like other Parameters of disease activity like tumor marker CA-125, it could be diagnostic value of the Pathophysiology. Also in this research molecular weight of ADA Isoenzymes was investigated ADA1=35KD and ADA2=110KD.

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INTRODUCTION

Ovarian cancer is the most common cause of death from cancer among women. It is the ninth common cancer in women. The incidence is usually over 40 years old, especially 70 years old(Goodarzi MT et al.2010). The disease is more common in advanced countries except Japan. It's estimated that in the United States between 1.5% and 2.5% of women, or one in 40 to 60 women, are at risk for their illness during their lives. Age aging has a direct relationship (Lai et al.1987). Although the disease is called a silent killer, however, a few months before the diagnosis, in 95% of the affected people, the patient has non-specific symptoms of frequent urination, constipation due to tumor growth, abdominal swelling, bleeding and vaginal pains. In addition, in patients with advanced disease, in addition to the above symptoms, ascites (fluid accumulation in the peritoneum cavity), bloating and anorexia have also been reported (Guppy AE et al.2005; Reynolds EA et al.2006). The adenosine deaminase enzyme called the old adenosine aminohydrolase and the enzyme number EC 3.5.4.4 is one of the most important purine catabolism enzymes.

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The adenosine deaminase enzyme, known as ADA, has the ability to adenosine and 2-deoxy-adenosine hydrolyze to inosine, 2-dezoxiazinosine and ammonia, respectively (. Nygaard P.1978). ADA exists in three molecular forms: ADA1 with a molecular weight of 35 kDa, ADA1 + cp composed of two ADA1 molecules plus a protein called cp, with a molecular weight of 280 kDa, and an ADA2 molecular weight of 110 kDa(Nalini G et al.1993).ADA1 is present in all cells, but the highest amount is found in lymphocytes and monocytes. The isoenzyme ADA2 is found only in monocytes. The activity of the enzyme is due to its two isoenzymes (ADA1 and ADA2), each of which has different pH and Km values (Niedzwicki JG et al.1991).Investigations in several cases indicate an increase in serum ADA activity in cells and cancerous tissues (Yildirim Z et al.1999).In this study, the activity of tADA and its isosomes, ADA1 and ADA2, was measured in the serum of patients with ovarian cancer and compared with healthy subjects. We also calculated the molecular weights of ADA isoenzymes by electrophoresis.

MATERIALS AND METHODS

A sample of the patient with ovarian cancer was obtained from Valiasr clinic of Imam Khomeini Hospital. From 30 patients and 30 healthy individuals, blood samples were taken.

Blood samples were collected from healthy individuals from the Blood Transfusion Organization. Blood samples were centrifuged for 10-15 minutes at 3,000 rpm (3000 rpm) and separated from the serum. Immediately after separating the serum, samples were stored at 80 ° C until freezing of ADA activity. Serum and red blood cells were also used by SDS-PAGE electrophoresis to determine the molecular weight of ADA2 and ADA1 as red blood cells are sources of ADA1. Red blood cells were used to measure ADA1 molecular weight.

To measure the amount of ADA activity, a Hitachi auto analyzer was used. The patient's serum was placed in the device and after the above-mentioned reactions, the total ADA activity was obtained for each patient as well as healthy subjects. In this method, the EHNA inhibitor was used. EHNA specifically prevents ADA1 activity, and since ADA2 has a higher kilometer for adenosine and a Ki higher for EHNA than ADA1. As a result, ADA1 activity is inhibited at a concentration of 0.1mM EHNA, whereas ADA2 is not affected by this concentration. Following the inhibition of serum ADA1 activity by EHNA using the autoanalyser, according to the method described for tADA, the ADA2 activity is measured by the device. By reducing ADA2 from tADA, ADA1 can be achieved.

RESULTS

The results of the measurement of adenosine deaminase (tADA) and its isoenzymes (ADA1 and ADA2) by the Hitachi 912 auto-analyzer are shown in Table 1.

Table 1. Descriptive statistics of tADA and ADA1 and ADA2 concentrations based on two groups

group	ADA1(U/L)	ADA2(U/L)	tADA(U/L)
Control Mean N Std.deviation	5.91 30 2.22	8.44 30 3.29	14.35 30 2.7
Patient Mean N Std.deviation	10.39 30 2.21	19.23 30 2.37	29.56 30 3.43

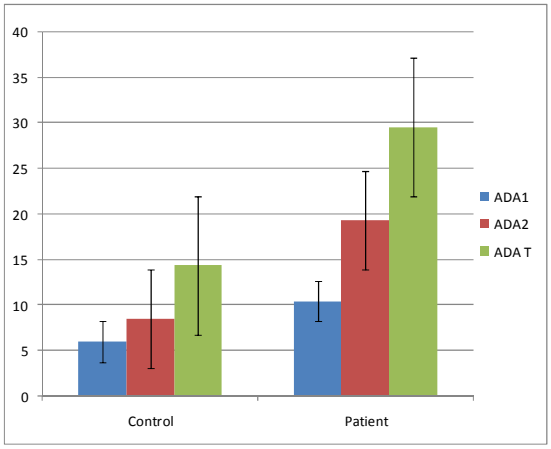

Diagram 1. Column Chart Comparison of ADA 1, ADA2 and ADAT concentrations based on two groups.

As shown in Figure 1, the activity level of tADA, ADA1 and ADA2 in the patient group shows a significant increase compared to healthy subjects.

Based on the hypothesis of this study, we claim that the level of activity of the adenosine deaminase enzyme in people with ovarian cancer is higher than healthy ones. We validate this claim by testing the statistical hypothesis to compare the variance of two societies based on statistical data. For the accuracy of this claim, the test statistic should be based on the following formula

$$z = \frac{(\overline{X_1} - \overline{X_2})}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

At a significant level of α = 0.001, the critical value is 3.08. That should be higher than the critical value in order to verify the test statistic (Z). In the above formula, the test statistic, 1X mean (Mean), 2 x Mean (Mean), Control group, 1 s, Std.deviation, Control group, S2, Std.deviation, Patient group, n1 represents the number of patient samples and The n2 represents the number of control samples.

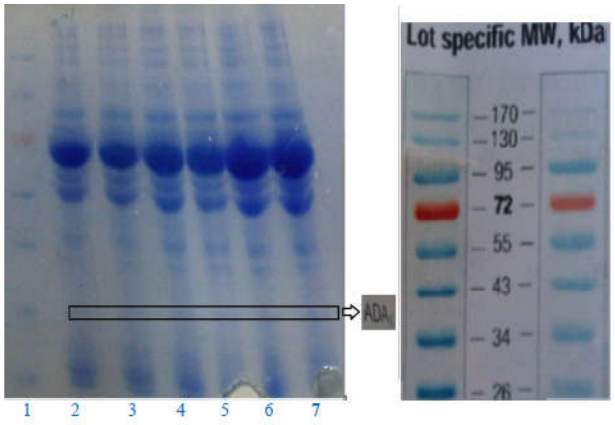

Figure 1. Determination of the molecular weight of the ADA1 isoenzyme on RBC on a 10% gel: From the left side of the first line, the weight Low molecular marker The second line of RBC in the healthy person and the rest of the RBC lines is in the ovarian cancer. The molecular weight of ADA1 is estimated at 35 kDa

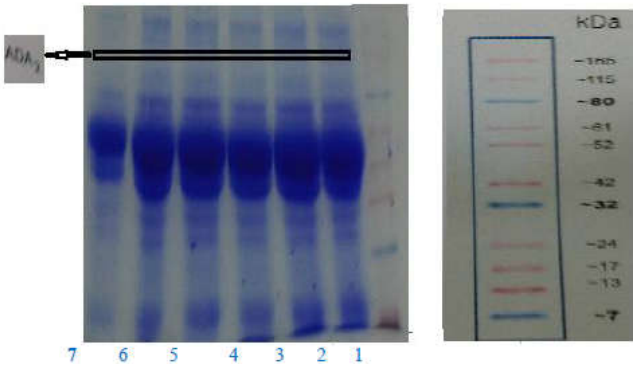

Figure 2. Determination of ADA2 molecular weight in serum on 8% gel. From the right side of the first line, the high molecular weight marker of the second and third lines of the control group (healthy) with dilution of 1 to 20, and the fourth, fifth, sixth and seventh serum lines of individuals With ovarian cancer, dilutions are 1 to 20, 1 to 30, 1 to 40, and 1 to 50. Given the above figure, the molecular weight of ADA2 is estimated to be 110 kDa.

After the numbering, the following results were obtained. MThe test statistic for ADA1 was 7.84841, ADA2 was 14.5 and ADA T was 1818.98.

All three test stanzas are more critical (3.08) and show a significant difference, and this confirms the veracity of the hypothesis.

DISCUSSION

The amount of ADA activity in the blood is the result of a balance between the rate of release from cells or other sources and the rate of purification or inactivation. Factors such as enzyme leakage from cells, induction of enzymes in a particular tissue, and the proliferation of certain types of cells expressing the enzyme can affect the amount of enzymes in the plasma. However, increasing ADA activity can be a sign of faster growth and development of cancer cells (Yildirim Z et al.1999). Many studies have shown that ADA plays an important role in the proliferation and development of various types of malignant cells in relation to the relationship between the level of key enzymes of the Purine nucleotide pathway and some of the clinical signs of tumor progression (Canbolat O et al.1996). Given that the ADA activity source is multiple, changes in the activity of this enzyme can be due to several factors. It also applies to changes in the activity of ADA in ovarian cancer, and can be due to changes in the ADA of a tumor or blood cells such as monocytes, lymphocytes and erythrocytes and underlying diseases such as inflammatory diseases. Some studies have suggested that increased levels of ADA in cancer patients may be due Turn-over DNA in malignant cells, but this issue has not yet been clearly determined (Pragathi, P et al.2005). Lai has proven that the increase in serum ADA activity is directly related to the stage of the cancer, and it has been shown that this increase in serum ADA activity is directly proportional to the size of the primary tumor Connected (Lai H et al.1987)When we placed erythrocytes on a 10% concentration SDS-PAGE gel (Fig. 1), a bond was found in the 35 kDa region, which is believed to contain ADA1 because ADA1 is a specific enzyme of erythrocytes. Then, we placed the serum sample on a SDS-PAGE gel with a concentration of 8%. We found a band in the 110 kDa region, which we believe would have ADA2 (Figure 2), because ADA2 is the dominant isoenzyme present in the serum (Ben Shooshan I et al.2002).

Conclusion

The level of ADA1, ADA2 and total ADA activity in the serum of people with ovarian cancer and its comparison with healthy people can have a diagnostic value, as well as other active parameters such as the tumor marker CA-125, in terms of pathophysiology.

Meanwhile, the molecular weights of ADA isoenzymes were evaluated by electrophoresis and ADA1 = 35 kDa and ADA2 = 110 kDa were estimated.

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