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Diagnostic Value of Adenosine Deaminase Activity in Benign and Malignant Breast Tumors

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Background and Aims. The present study was carried out to evaluate the activity of adenosine deaminase (ADA) and its isoenzymes ADA1 and ADA2 activities as a diagnostic tool in patients with benign and malignant breast disease.

Methods. Total ADA, ADA1, and ADA2 activities of serum and tumor were analyzed using 58 subjects including 20 patients with benign breast disease (BBD), 34 patients with primary breast cancer, and 20 patients as normal control subjects.

Results. The mean values for total ADA and ADA2 activities in the serum and tumor of BBD were significantly higher than those of healthy controls ($p < 0.01$). Furthermore, the mean values for total ADA and ADA2 activities of patients with breast cancer were significantly higher than those of the benign group ($p < 0.005$) and healthy subjects ($p < 0.0001$).

Conclusions. Based on the present results, it is concluded that the assessment of total ADA and ADA2 activities may be used as a reliable test for differential diagnosis of benign and malignant breast disease.

Key Words: Adenosine deaminase, Benign breast disease, Breast cancer, Isoenzyme.

Introduction

Benign breast disease (BBD) represents a group of histologically heterogeneous lesions, some of which are associated with increased risk for invasive breast cancer.

Breast cancer is now recognized as probably the most prevalent malignant transformation occurring in women (1). In order to investigate the diagnosis and characterization of this cancer with the aid of biochemical techniques, BBD may be used as a best representative model. Biochemical enzyme analyses have attracted great attention for better understanding of the biological nature of malignancies. Metabolic and anabolic processes of purine and pyrimidine are intensified in the cancerous tissues and cell lines and this manifests itself as a changed activity of a number of enzymes such as adenosine deaminase.

Adenosine deaminase (ADA, E.C.3.5.4.4) is an enzyme that catalyzes deamination of either adenosine or deoxyadenosine. Because of the irreversibility of the reaction by ADA, this reaction seems to be one of the rate-limiting steps in adenosine degradation (2). There are two isoenzymes of ADA in human tissues, ADA1 and ADA2 (3,4). ADA1 is present in all human tissues, and the majority of ADA activity is derived from ADA1. However, ADA2 is the predominant isoenzyme in the serum of normal subjects (5). Most human cells contain very small amounts of ADA2 and its major source is likely to be the monocyte−macrophage cell system (6). In several studies, ADA activities were found to be increased or decreased in cancerous tissues and cells (7−18) and may be of diagnostic value. Most of these studies have focused on purine enzymatic differences in cancerous tissues or cells as well as normal tissue. Furthermore some previous studies indicated that measurement of adenosine deaminase (ADA) may be useful in the diagnosis and/or monitoring of the malignancy (7,8,13). Although there are reports concerning the activity of total adenosine deaminase

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activity in patients with several malignant tumors including breast cancer, the patterns of ADA1, ADA2, and total ADA in patients with BBD and its comparison with those of breast cancer have not been elucidated.

In light of these data, this study was planned to investigate to what extent total ADA, ADA1, and ADA2 activities could serve as an appropriate and reliable diagnostic tool for malignant and benign cell proliferation in patients with newly diagnosed breast cancer and BBD and also to provide some contribution to the very limited data on this subject.

Materials and Methods

With local ethical approval and informed consent, 34 patients with breast cancer, 20 women with BBD, and 20 women as healthy controls were included in this study (Table 1). Five ml of blood was collected from each patient for complete blood examination. Tumor tissues were collected during surgery from September 2003 to April 2004. Patients received no therapy prior to surgery. Normal adjacent tissues were sampled away from the edge of the tumor region. Fresh surgical specimens were frozen immediately and stored at −80°C until assay. Tissues were washed and homogenized in equal amounts of phosphate buffer solution (pH 6.5). The homogenate was centrifuged at 15,000 × g for 60 min. Clear upper supernatant fluid was taken and the assays were carried out. All procedures were performed at 4°C.

Table1. Demographic characteristic of study populations

	Malignant (n = 34)	Benign (n = 20)	Normal (n = 20)
Age (years)	46.82 ± 10.56 (28–70)	44 ± 7.83 (30–56)	41 ± 6.38 (26–60)
Menopause status			
Premenopause	19 (56%)	11 (55%)	12 (60%)
Postmenopause	15 (44%)	9 (45%)	8 (40%)
Depth of tumor invasion (T)			
T1	13 (38%)		
T2	11 (32 %)		
T3	7 (21%)		
T4	3 (9%)		
Lymph node status (N)			
N0	16 (47%)		
N1	11 (32%)		
N2	7 (21%)		
Distant metastases (M)			
M0	18 (53%)		
M1	16 (47%)		
Tumor grade (G)			
G1	2 (6%)		
G2	15 (44%)		
G3	17 (50%)		
Tumor size			
≥2 cm	19 (56%)		
<2 cm	15 (44%)		

Blood samples were collected in a 5 mL tube without any anticoagulant. To obtain serum, the blood was then centrifuged at 3000 × g for 10 min at 4°C.

Protein was determined by the method of Bradford (19). Total ADA activity and its isoenzymes (ADA1, ADA2) were assayed spectrophotometrically by the method of Giusti (20). ADA2 activity was measured in the presence of selective ADA1 inhibitor, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) (200 μM/L) (9). Total ADA activity was measured in the absence of EHNA and ADA1 activity was then calculated by subtracting the ADA2 from the total ADA activity (9). Sensitivity of the assay was 0.5 U/L, with intra- and inter-assay coefficients variation (CV) of 3.9 and 6.4%, respectively.

Data were expressed as mean ± standard deviation (SD). Mann-Whitney U test and Spearman analysis were used for the analysis of differences among the groups; p value <0.05 was considered significant. Statistical analysis was performed by Statistical Package for Social Science (SPSS).

Results

Total Tumor ADA Activity and Its Isoenzymes

As illustrated in Figure 1, total ADA, ADA1 and ADA2 activities in the benign tumors were found to be higher than those of normal breast tissues (non-cancerous). Differences are statistically significant ($p<0.001$). However, these activities are lower than those of malignant breast tumors (Figure 2). The differences are also statistically significant ($p<0.0001$).

Total Serum ADA Activity and Its Isoenzymes

Serum total ADA and ADA2 activities in patients with breast cancer and those with benign tumors are significantly

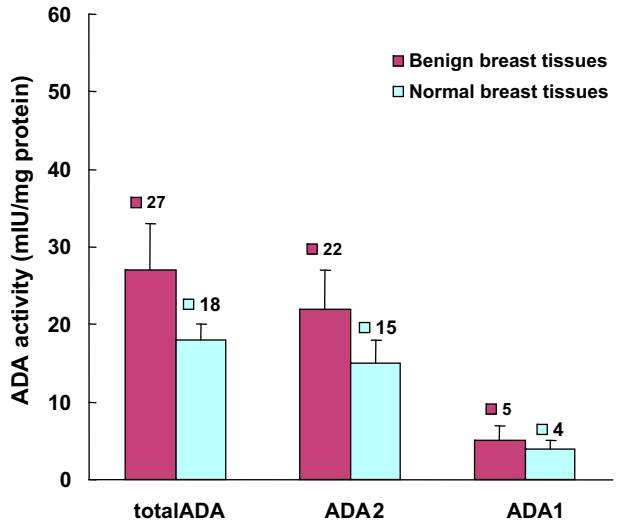

Figure 1. Total ADA, ADA1 and ADA2 activities in benign breast tissues in comparison with normal tissues. Color version of this figure available online at www.arcmedres.com.

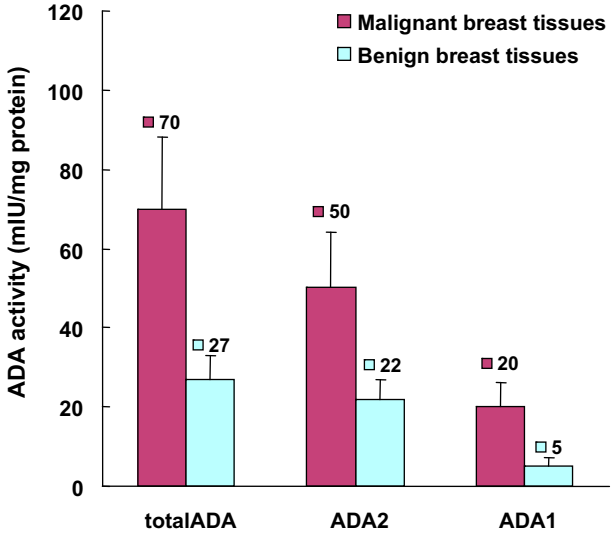

Figure 2. Comparison of total ADA, ADA1 and ADA2 in benign breast disease (BBD) and malignant breast tumors. Color version of this figure available online at www.arcmedres.com.

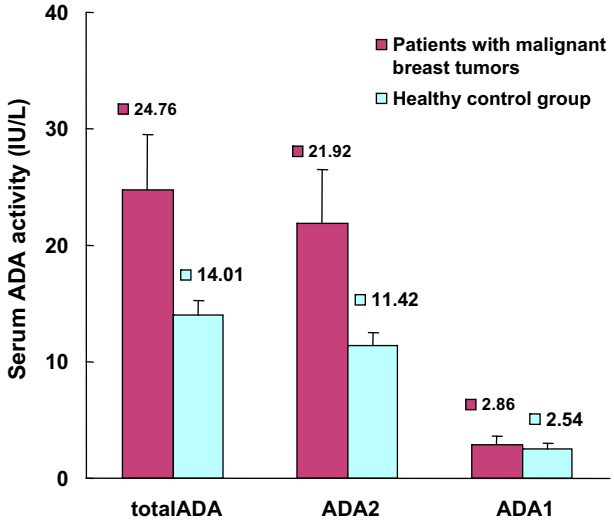

Figure 4. Serum total ADA, ADA1 and ADA2 activities in patients with malignant breast tumors and healthy controls. Color version of this figure available online at www.arcmedres.com.

higher ($p < 0.0001$) than those of normal subjects (Figure 3 and 4). However, ADA1 activity was not significantly increased ($p > 0.5$). In addition, as shown in Figure 5, serum total ADA and ADA2 activities in BBD are lower than those observed for breast cancer patients. However, the differences are not statistically significant ($p > 0.5$). No significant differences were shown between serum ADA1 activity of BBD and breast cancer patients.

Discussion

In recent years there has been expanding interest in the use of antigens, hormones, and enzymes for the diagnosis and prognosis of benign and malignant tumors and also for the assessment of treatment response. Several experiments have been carried out to assess the role of various enzymes in breast cancer, and it has been known that there are important interrelations between the carcinogenic process and the activities of some enzymes in malignant tumors.

Although BBD has been studied extensively, the etiology of this disease is still poorly understood. The status of total ADA activity and the pattern of its isoenzymes in benign tumors and their relationships to those of malignant tumors have not been examined. There are several reports indicating that increase or decrease of ADA1 and ADA2 activities in the serum are due to the stimulation of cellular immunity (21–25).

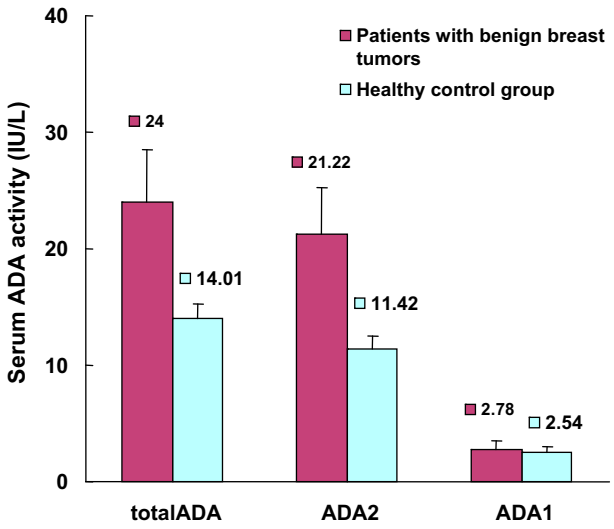

Figure 3. Serum total ADA, ADA1 and ADA2 activities in BBD and healthy controls. Color version of this figure available online at www.arcmedres.com.

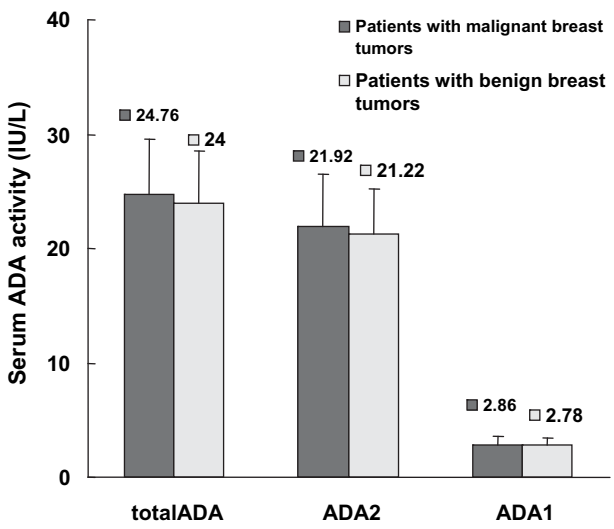

Figure 5. Serum total ADA, ADA1 and ADA2 activities in malignant breast tumor in comparison to BBD. Color version of this figure available online at www.arcmedres.com.

Very few published clinical studies about the value of adenosine deaminase activity in breast cancer patients are available to date. In one of the preliminary studies performed on a small number of breast cancer patients, the activity of adenosine deaminase was significantly increased in the serum (8). In another study, the activity of total ADA was also found to be significantly elevated in the mammary tumors of breast cancer patients (13).

Our earlier study has shown that total ADA and ADA2 activities increased in serum and malignant tissues of breast cancer (7). The results of the present study revealed that total ADA and its ADA2 isoenzyme activities were significantly increased in both benign and malignant tumors significantly. However, the activities in BBD are significantly lower than those of breast cancer tumors. To discuss these findings, some authors suggest that high ADA activities play an important role in the salvage pathway (10,26), whereas others propose that increased ADA activity may be a compensatory mechanism against toxic accumulation of its substrates, adenosine (27,28). There are many possible sources of adenosine in tumor cells including accelerated purine and pyrimidine metabolism, cell death and nucleotide degradation, ischemia and ATP breakdown, AMP release and hydrolysis of S-adenosyl homocysteine. Furthermore, high ADA activity is against the high toxicity of deoxyadenosine and its derivatives dAMP, dADP and dATP, which are potent inhibitors of nucleic acid biosynthesis (29). High ADA activity in malignant tumors may give selective advantage to the cancer cells via production of high amounts of hypoxanthine, substrate of hypoxanthine guanine phosphoribosyl transferase (HGPRT). It is a key enzyme for the salvage pathway (30).

Patient serum ADA values are significantly higher than those of controls. The results are also shown that ADA activity in the serum of breast cancer patients is higher than those of patients with BBD, although not significantly. The reason is not clear and needs further investigation but may be due to the presence of an abnormal ADA variant that is unable to secrete into the plasma, or ADA may originate from macrophage infiltration to the malignant breast tumors (31).

In conclusion, the more pronounced activity of adenosine deaminase in breast cancer patients than that of healthy subjects and BBD patients strengthened the suggestion that the measurement of these enzyme activities may be useful in the differential diagnosis of benign and malignant proliferation. To the best of our knowledge, this is the first report concerning the measurement of total ADA, ADA1, and ADA2 activities both in patients with breast cancer and with BBD.

Furthermore, according to the results obtained in this study, it can be concluded that in patients with high ADA2 and total ADA activities, the probability of the presence of malignancy may also be high and, therefore, this test may be valuable as a biochemical technique in combination with other established markers for the identification of breast tumors.

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