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Serum adenosine deaminase activity in Iraqi patients with breast cancer on tamoxifen therapy

Tamoksifen tedavisi gören meme kanserli Irak'lı hastalarda serum adenozin deaminaz aktivitesi

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Abstract
Breast cancer (BC) is a type of cancer originating from breast tissue, Tamoxifen is the usual endocrine therapy for breast cancer in pre-menopausal women, and also in post-menopausal women. Adenosine deaminase (ADA) is an enzyme involved in purine metabolism, and also increased in most cancers. It is aimed to assess the status of ADA in women with BC on tamoxifen therapy in this study. The present study is a cross-sectional study (2010/2011) done at Al-Kadhumia Teaching Hospital. The procedure includes measurement of ADA in sera of women with BC who were estrogen positive (whether on tamoxifen treatment or not). This measurement was done using ELISA Kit for Adenosine Deaminase (ADA) E91390Hu. A total of 160 patients with BC were involved in this study, they were classified as newly diagnosed premenopausal women with BC G1: (n=40); newly diagnosed postmenopausal women with BC G2: (n=40); Premenopausal women with BC on tamoxifen therapy G3: (n=40); Postmenopausal women with BC on tamoxifen therapy G4: (n=40). A matching group of eighty apparently healthy women who were included as controls (n=80), who were classified as Premenopausal women G5: (n=40); Postmenopausal women with G6: (n=40). Serum ADA was significantly reduced in women with BC receiving tamoxifen therapy when compared with newly diagnosed patients with BC ($p < 0.001$) and even with controls ($p < 0.05$). In conclusion, patients with BC have high level of serum ADA compared with controls; however, the level of ADA was significantly reduced upon tamoxifen treatment. The above results were supported by the significant alteration in levels of s. ADA indicating high rate of malignant cell-turn-over resulting in high rate of purine catabolism which is limited by tamoxifine adminstration.
Keywords: Adenosine deaminase; breast cancer; tamoxifen.

Özet
Meme kanseri meme dokusundan kaynaklanan bir kanser tipidir. Tamoksifen hem postmenapozal hem de premenapozal kadınlardaki meme kanserleri için kullanılan yaygın bir endokrin terapisidir. Adenozin deaminaz(ADA) purin metabolizmasında yer alır ve aynı zamanda çoğu kanserde artmıştır. Bu çalışmada tamoksifen terapisi alan meme kanserli hastalardaki ADA durumunu değerlendirilmesi amaçlanmıştır. Mevcut çalışma kesitsel bir çalışma olup 2010-2011 yılları arasında Al-Kadhuma Eğitim Hastanesi'nde yapılmıştır. Bu prosedür östrojen pozitif (tamoksifen tedavisi alan veya almayan) olan meme kanserli hastaların serumlarındaki ADA ölçümünü içermektedir. Bu ölçüm ELISA Kit for Adenosine Deaminase (ADA) E91390Hu kullanılarak yapılmıştır. Toplam 160 hasta çalışmaya dahil edilmiş olup bunlar yeni teşhis edilmiş premenopozal kadınlar BC G1: (n=40); yeni teşhis edilmiş postmenopozal kadınlar BC G2: (n=40); tamoksifen terapisindeki meme kanserli premenopozal kadınlar G3: (n=40); tamoksifen terapisindeki meme kanserli postmenopozal kadınlar G4: (n=40) olarak sınıflandırılmışlardır. Karşılaştırma grubu olarak 80 sağlıklı kadın, kontrol grubu olarak (n=80) çalışmaya dahil edilmiş olup premenopozal kadınlar G5: (n=40); postmenopozal kadınlar G6: (n=40) olarak sınıflandırılmışlardır. Yeni teşhis edilmiş meme kanserli kadınlarla karşılaştırıldığında, serum ADA seviyesi tamoksifen alan hastalarda önemli ölçüde düşmüştü ($p<0.001$), hatta kontrol grubu ile karşılaştırıldığında da oldukça düşmüştü ($p<0.05$). Sonuç olarak kontrol grubu ile karşılaştırıldığında meme kanserli hastalar yüksek ADA serum seviyesine sahiplerdi. Öte yandan tamoksifen tedavisini takiben ADA seviyeleri ciddi oranda düşmüştür. Yukarıdaki sonuçların da desteklediği ADA seviyelerindeki ciddi değişim gösteriyor ki tamoksifen tedavisi yüksek orandaki malign hücre dönüşümünü yüksek oranda pürin katabolizması ile sonuçlanmasını sağlıyor.
Anahtar kelimeler: Adenozin deaminaz; meme kanseri; tamoksifen

Introduction

Breast cancer (malignant breast neoplasm) is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk (1).

The size, stage, rate of growth, and other characteristics of the tumor determine the kinds of treatment. Treatment may include surgery, drugs (hormonal therapy and chemotherapy), radiation and/or immunotherapy (2). Some breast cancers are sensitive to hormones such as estrogen and/or progesterone, which make it possible to treat them by blocking the effects of these hormones (2). Tamoxifen is an antagonist of the estrogen receptor in breast tissue via its active metabolite, hydroxytamoxifen. In other tissues such as the endometrium, it behaves as an agonist, and thus may be characterized as a mixed agonist/antagonist. Tamoxifen is the usual endocrine therapy for hormone receptor-positive breast cancer in pre-menopausal women, and is also a standard in post-menopausal women although aromatase inhibitors are also frequently used in that setting (3). While the hormone estrogen promotes the growth of breast cancer cells, tamoxifen works by blocking estrogen from attaching to estrogen receptors on these cells. By blocking the estrogen receptors, it is believed that the growth of the breast cancer cells will be halted (3).

Adenosine deaminase (ADA) is an enzyme (EC3.5.4.4) involved in purine metabolism (4). It is needed for the breakdown of adenosine from food and for the turnover

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of nucleic acids in tissues (4). ADA irreversibly deaminates adenosine, converting it to the related nucleoside inosine by the substitution of the amino group for a hydroxyl group (4). Inosine can then be deribosylated (removed from ribose) by another enzyme called purine nucleoside phosphorylase (PNP), converting it to hypoxanthine (4).

ADA is present in virtually all mammalian cells; its primary function in humans is the development and maintenance of the immune system (4). However, ADA association has also been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance (6). It has also been proposed that ADA, in addition to adenosine breakdown, stimulates release of excitatory amino acids and is necessary to the coupling of adenosine receptors and heterotrimeric G proteins (5). However, the full physiological role of ADA has not yet been completely understood (4).

Some mutations in the gene for adenosine deaminase cause it not to be expressed. The resulting deficiency is one cause of severe combined immunodeficiency (SCID) (7). Conversely, mutations causing this enzyme to be overexpressed are one cause of hemolytic anemia (8). Elevated levels of ADA have also been associated with AIDS (8,9). There are 2 isoforms of ADA: ADA1 and ADA2. ADA2 is the predominant form present in human blood plasma and is increased in many diseases, particularly those associated with the immune system: for example rheumatoid arthritis, psoriasis and sarcoidosis. The plasma ADA2 isoform is also increased in most cancers. ADA2 is not ubiquitous but co-exists with ADA1 only in monocytes-macrophages (4).

Materials and Methods

Subjects

The study was a cross-sectional study carried out at Oncology Department at Al-Yarmouk Teaching Hospital, during the period from October, 2010 till the end of September, 2011. The protocol for the study was approved by the Ethical committee of Al-Nahrain Medical College, and informed signed consent was given by each subject.

Blood samples

Five milliliters of random venous blood were withdrawn from each patient, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged at 1800 x g for 10 minutes at 4°C, and the separated serum was transferred into Eppendrof tube and was used for measurement of ADA. The tubes were stored at –20°C until analysis, which was done within one month after collection (10).

Methods

Measurement of serum ADA was done by ELISA Kit for Adenosine Deaminase (ADA) E91390Hu. manufactured by Life Science Inc (10).

Principle of the Method

The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to ADA.

Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for ADA. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain ADA, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of ADA in the samples is then determined by comparing the O.D. of the samples to the standard curve (10).

Statistical analysis

Statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (t-test) to test the significancy of mean difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results

Subjects

A total of 160 patients with BC were enrolled in this study: Eighty of them were newly diagnosed to have BC who receives no therapy for cancer; they were further subdivided into 40 premenopausal women with BC (G1) and other 40 postmenopausal women with BC (G2).

The remaining 80 patients were women with BC who receive Tamoxifen as hormonal therapy; they also full into two classes: G3 consists of 40 premenopausal women with BC who receive tamoxifen and G4 consists of 40 postmenopausal women with BC who receive tamoxifen as in Table 1.

The study included another 80 apparently healthy subjects , they were neither alcoholic nor smoker with no family history of any type of cancer who serve as healthy controls (G5 and G6); they were matched with patients` groups for age, sex, age of menarche, and body mass index as in Table 1: G5 consists of 40 apparently healthy premenopausal women who serve as healthy controls for G1 and G3. G6 consists of 40 apparently healthy postmenopausal women who serve as healthy controls for G2 and G4.

Serum adenosine deaminase

Serum ADA was highly significantly reduced in BC groups who receive treatment with tamoxifen (G2 & G 4) when compared with newly diagnosed BC groups whom receive no therapy (G1, G3) [$p < 0.001$] and even with healthy controls (G5 & G6) [$p < 0.05$] as in Table 2.

Table 1. Clinical criteria of patients` groups with Breast Cancer & Control (presented as range and mean + SD).

Group	No.	Age [range] , Mean (SD) in years	Age at Menache [range],mean (SD) in years	BMI [range],mean(SD)
G1	40	[30-40], 35(3.5)* §	[11-13], 12.5 (0.5)* §	[17-25.3], 20.2 (3.3)* §
G2	40	[57-69], 62(7)** §§	[11-13.5], 11.7 (0.6) **§§	[23.5-32-2], 28.1 (3.5) **§§
G3	40	[32-42], 36(4) ***	[12.5-14.5], 13.4(0.3) ***	[14.8-27.4], 20.9(6.6) ***
G4	40	[46-55], 60(6) ****	[12.5-14], 13.6 (0.3) ****	[21.5-33.2],27.1 (6) ****
G5	40	[28-39], 34(5)	[12-13], 12.8 (0.8)	[19.6-25.6], 21 (3)
G6	40	[55-66], 61(5)	[12-14], 13.2 (0.6)	[20-30.5], 26(4.3)

(G1): Premenopausal women with BC: newly diagnosed, on no treatmen; (G2): Postmenopausal women with BC: newly diagnosed, on no treatment; (G3): Premenopausal women with BC: on Tamoxifen; (G4): Postmenopausal women with BC: on Tamoxifen; (G5): Premenopausal Healthy Controls; (G6): Postmenopausal Healthy Controls.

** t-test: G1 versus G5, p > 0.05, ** t-test: G2 versus G6, p>0.05, *** t-test: G3 versus G5, p>0.05, **** t-test: G4 versus G6, p>0.05, § t-test: G1 versus G3, p>0.05, §§ t-test: G2 versus G4, p>0.05*

Table 2. The mean serum Adenosine deaminase in different women with Breast Cancer and controls (presented as mean + SEM).

Variable	G1	G2	G3	G4	G5	G6
No	*40*	*40*	*40*	*40*	*40*	*40*
Serum ADA (IU/L)	30.4 ± 1.2*§	28.5 ± 0.8**§§	11.1 ± 0.6***	11.5 ± 0.34****	13.5 ± 0.4	13.0 ± 0.5

** t-test: G1 versus G5, p < 0.001, ** t-test: G2 versus G6, p<0.001, *** t-test: G3 versus G5, p<0.05, **** t-test: G4 versus G6, p<0.05, § t-test: G1 versus G3, p<0.001, §§ t-test: G2 versus G4, p<0.001*

Discussion

ADA catalyzes the irreversible deamination of deoxyadenosine and adenosine and thereby plays a role in maintaining cellular pools of these important purine bases. ADA is expressed in all human tissues; and estrogen induces ADA mRNA in human breast cancer cells (11).

In cancer there is an increased turnover of malignant cells and an associated increase in nucleotide metabolism leading to an increase in purine metabolizing enzymes. ADA is particularly sensitive to stimulation by growth factors and cytokines during rapid tissue proliferation (12). The reduction in ADA activity following tamoxifen therapy can be explained by the fact that tamoxifen induces a significant apoptosis in estrogen-sensitive cells. One possible mechanism might be through the inhibition of ADA and subsequent accumulation of toxic adenosine and deoxyadenosine that causes inhibition of ribonucleotide reductase and also inactivation of s-adenosyl homocysteine hydrolase, which results in apoptosis (13). Numerous studies have documented an increase of ADA in very rapidly growing malignancies like breast cancer, where it has been documented as a tumor marker.

In the present study serum ADA was found to be significantly elevated in breast cancer; this was in accord with other studies like Aghaei et. al.(14) , Shatova et. al.(15) and Aghaei et. al.(16) studies.

In conclusion, the present study indicates the usefulness of measuring serum ADA activity for assessing BC. The simplicity of measuring ADA activity combined with its cost effectiveness gives an added advantage to consider ADA as a tumor marker in BC.

Further studies on these lines in a larger number of patients are needed to evaluate the role of ADA as a tumor marker, for monitoring disease activity or response to therapy, also in the early detection of BC which remains the goal of identifying a tumor marker which can be included in the diagnostic panel..

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