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Original Research Article

Measurement of Adenosine Deaminase Concentration in Serum of Breast Cancer by HPLC before and after Chemotherapy in Hilla City

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Abstract

Breast cancer (BC) is a type of malignancy appeared in breast tissue , occurs in both premenopausal and also in postmenopausal women's. Adenosine deaminase (ADA) is an enzyme participate in metabolism of purine. However, the level of adenosine deaminase increased in most cancers patients and it could be of value before and after chemotherapy.

A total of 280 patient with (BC) divided in two groups, first group (n=40) women with breast cancer without chemotherapy, second group (n= 240) women with breast cancer with chemotherapy, this group was divided into six groups according to the doses used (1st – 6th doses). The control group (n= 40) apparently healthy women's matched with patient group. Women with (BC) have a significant higher serum level of ADA than those who are not diseased ($p<0.001$) and even than those who received chemotherapy ($p<0.05$).So, this lead to a conclusion that; breast cancer women have higher level of ADA . ADA level decreased upon administration of chemotherapy. The objective of this study is to assess ADA status in women's BC receiving chemotherapy treatment.

Key words: breast cancer (BC); Adenosine deaminase (ADA), Chemotherapy (C). Higher Serum Level (HSL).

قياس تركيز إنزيم الادينوسين دي امينيز في مصل مرضى سرطان الثدي باستخدام كروموتوغرافيا السائل ذات الأداء العالي قبل وبعد العلاج الكيماوي في مدينة الحلة

الخلاصة

سرطان الثدي (BC) هو النوع من السرطان الذي ينشأ من أنسجة الثدي، والذي من الممكن ان يصيب النساء في كلتا الحالتين سواء كانت في فترة ما قبل انقطاع الطمث او في فترة انقطاع الطمث او ما يسمى (سن اليأس) . ان انزيم الادينوسين دي امينيز (ADA) هو من الإنزيمات المسؤولة عن عملية التمثيل الغذائي للبيورين (القواعد النتروجينية الثنائية الحلقة). ومع ذلك فقد زاد مستوى إنزيم الأدينوسين دي امينيز عند النساء المصابات بسرطان الثدي والذي من الممكن فيه ان يظهرن تلك القيمة وظيفيا قبل العلاج الكيميائي وبعده .

تم جمع عينات ٢٨٠ مريضة بسرطان الثدي قسمت لمجموعتين، المجموعة الأولى تضم النساء المصابات بسرطان الثدي بدون اخذ علاج كيماوي إما تضم المجموعة الثانية اعلاة فتظم النساء المصابات بسرطان الثدي اللواتي أخذن العلاج الكيماوي وتم تقسيم المجموعة الثانية اعلاة إلى ست مجموعات وفقا لجرعات العلاج المعطاة (الجرعة الأولى – الجرعة السادسة) .وبالنسبة لمجموعة المطابقة والتي تتضمن النساء الأصحاء اللواتي أدرجن كمستوى طبيعي لمقارنة قيم الإنزيم. اظهر انزيم الادينوسين دي امينيز زيادة بشكل كبير في النساء المصابات بسرطان الثدي BC بدون اخذ العلاج الكيميائي مقارنة مع النساء السليمات ($P<0.001$) ، وحتى مع النساء اللواتي أخذن العلاج الكيميائي ($p<0.05$) ومن ذلك نستنتج ان مريضات سرطان الثدي BC يعانون من مستوى عال من ADA مقارنة مع المستويات الطبيعية للنساء

السليمات . ومع ذلك، فإن مستوى ADA قد انخفض بشكل كبير عند اخذ العلاج الكيميائي. هدفت هذه الدراسة إلى تقييم حالة ADA في مريضات سرطان الثدي اللواتي استخدمن العلاج الكيماوي.BC
الكلمات المفتاحية: سرطان الثدي(BC) ؛ نازعة أمين الأدينوزين (ADA)

Introduction

Breast cancer is a type of neoplasm appeared in the breast tissue, mostly originated from the inner lining of milk ducts or the lobules that produce the milk and supply it to the ducts [1]. Type of treatment determined by signs and symptoms such as size, stage , rate of growth and other tumor stage. Type of treatment include drugs (hormonal and chemotherapy) surgery , radiation and / or immunotherapy [2].

Chemotherapy is anti-cancer drugs , used to destroy and eliminate the fast-growing cancer cells and stop the growth and division. Cancer cells grow and multiply and divide rapidly, chemotherapy thereby acting to block the process of division of cancer cells and eliminate them. These drugs have been used in the treatment of many cancerous diseases for the past 40 years, and currently there are more than 30 types in use [3].

The choice of which chemotherapy has to be used differ from patient to patient according to several factors include the cancerous tumor ,place of cancer, health status of patient and age. chemotherapy has the advantage over radio therapy in that it destroy cancerous cell in every part of the body, while the radio therapy destruct the malignant cells in specific part of the body [3].

Adenosine deaminase (ADA) (EC3.5.4.4) is an enzyme participate in metabolism of purine [4]. It is needed for the degradation of adenosine from food and for the turnover of the nucleic acids in tissue [5]. ADA irreversibly deaminates adenosine, converting it to the related nucleoside inosine by the substitution of the amino group for a hydroxyl group [4, 5].

It can then ribosylated inosine (removal of ribose) by another nucleoside phosphorylase enzyme called purine (PNP), converted to hypoxanthine [4]. ADA exists in almost of all mammalian cells , acts in humans in the developing and maintaining of the immune system [5]. However, It seem to have another function as epithelial cell differentiation, neurotransmission, and gestation maintenance [5, 6]. Severe combined immunodeficiency (SCID) result from its deficiency [7].

Materials and Methods

Patients

study groups consist of (280) subjects divided into two groups, first groups consist of (40) women with breast cancer without chemotherapy while second groups (240) women with breast cancer treated with chemotherapy divided into six groups, each groups consist of (40) subjects according to chemotherapy doses from the first dose to the sixth dose and their age ranged from (37 - 75) years. All samples were collected from Oncology Center in Marjan teaching hospital in Hilla city. All patients underwent medical history and physical examinations including age, gender, history of breast cancer, BMI, hypertension and its duration diabetes mellitus and its duration, history of smoking and family history of breast cancer . The tests were performed at the Laboratory of the Department of Biochemistry, College of Medicine, University of Babylon. Decision agreements was obtained for this study (315 10/1/2015).

Control

control group consist of (40) females. Collected from medical staff whom were apparently free from signs and symptoms of breast cancer , age ranged from (35 - 77) years, all of them were non-smokers, free from DM, hypertension and no family history of breast cancer.

Sample collection

Blood sample were obtained from subject group in fasting state. Blood put

in gel tube and separated at 3000 xg for 10 min at 4ºC to obtain serum. Consequently , serum was divided into aliquots in eppendorf and stored at (-20º C) until time of analysis.

Determination of Adenosine Deaminase Activity in Serum Using Reversed- Phase High-Performance Liquid Chromatography

Samples were analyzed by High Performance Liquid Chromatography (HPLC) system, model Shimadzu 10AV-LC equipped with binary delivery pump model LC-10AV, the eluted peaks were monitored by UV/V detector SPD-20A. The condition of separation are listed in table (1). Standards of suspected compound were run similarly for identification and quantification, the concentration of each isolated compound [8, 9].

Table 1 : Separation conditions of High performance liquid chromatography

Parameter	Characteristic for ADA identification
Mobile phase	A= (50 Mm KH2PO4) B= (50 Mm KH2PO4 and 20% MeOH)
Type of Column	C18 – ODS (25cm x 4.6 mm X 10µL)
Volume injection sample	20 µl
Detector	UV Spectrophotometer at 280 nm
Flow Rate	1 ml / min
Temperature	35ºC

Calculation

The area under a peak is used for calculating the concentration of a sample according the following formula:

Concentration of sample (µg/ml) = (the area of the sample/area of the standard) × Standard Conc.× Dilution factor.

Preparation of Standard Solution

A volume of 20 µL of adenosine deaminase (ADA) was dissolved in the methanol for HPLC to the desired concentrations [10].

Preparation of (EHNA) (200 mmol/L)

A weight of 0.5 gm of erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) was dissolved in absolute methanol grade for HPLC and brought to a final volume of 40 ml. (This Solution is stable for at least one weeks at 4°C) [10].

Preparation of Sample

1. A volume of 20 µL of serum was added to (10 µL) of (EHNA).
2. After 1 min vortex 10 µL from sample was taken and injected into the HPLC.

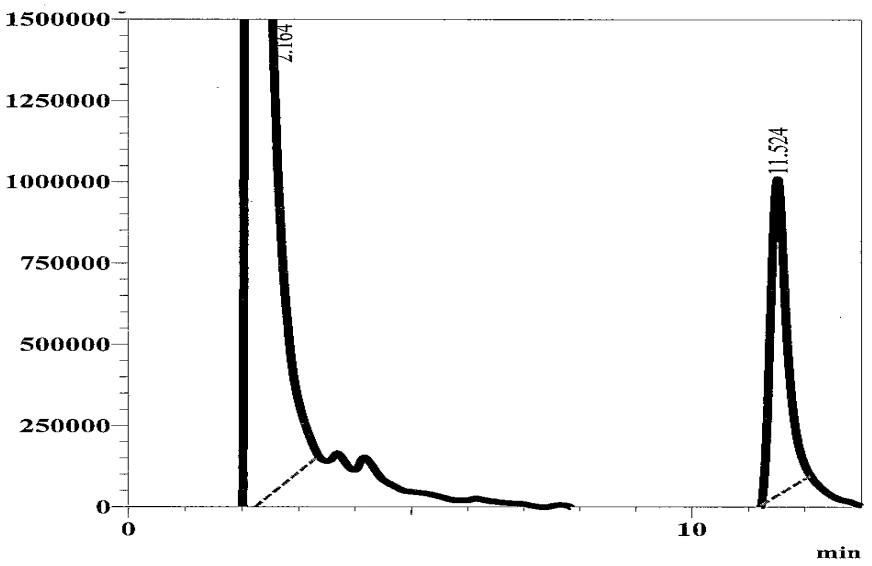
Result and Discussion

In this study, samples were divided into two groups, the first group women with breast cancer without chemotherapy, and the second group women with breast cancer with chemotherapy, this group in turn was divided into six groups according to doses of chemotherapy first dose to sixth, as described in Table (1).

Table 1 : patients & Control groups

Group	No. of sample	Description
G1	40	Healthy women (control)
G2	40	Women with breast cancer without chemotherapy
G3	40	Women with breast cancer first dose chemotherapy
G4	40	Women with breast cancer Second dose chemotherapy
G5	40	Women with breast cancer third dose chemotherapy
G6	40	Women with breast cancer fourth dose chemotherapy
G7	40	Women with breast cancer fifth dose chemotherapy
G8	40	Women with breast cancer sixth dose chemotherapy

In this study, it is developed an accurate assay to assess ADA activity in serum of breast cancer patient . Figure (1) shows that a complete baseline separation was obtained within adenosine deaminase by HPLC .

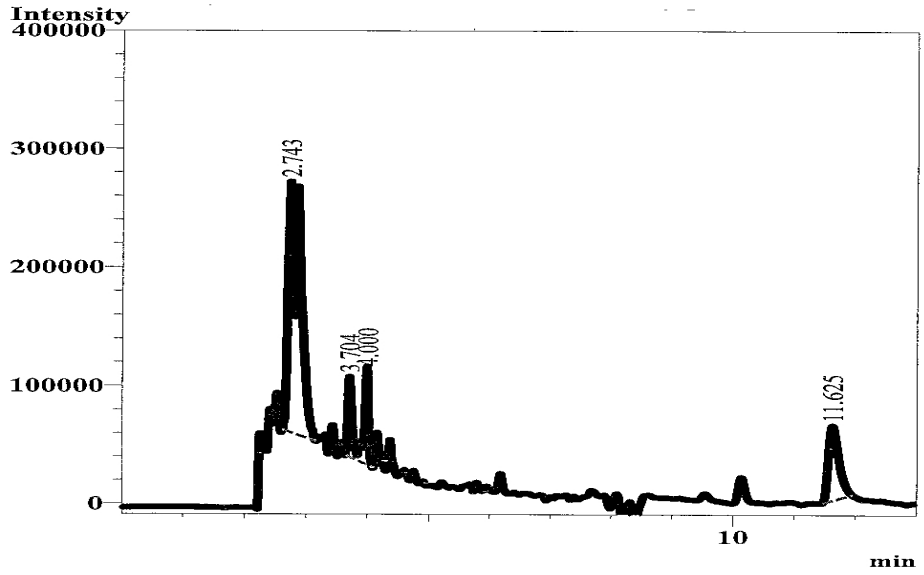


Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	2.164	125731096	86.5317	5279367	
2	11.524	19569467	13.4683	973859	
Total		145300563	100.0000	6253226	

Figure 1: HPLC chromatograph of standard ADA, its retention time (RT) is 11.52

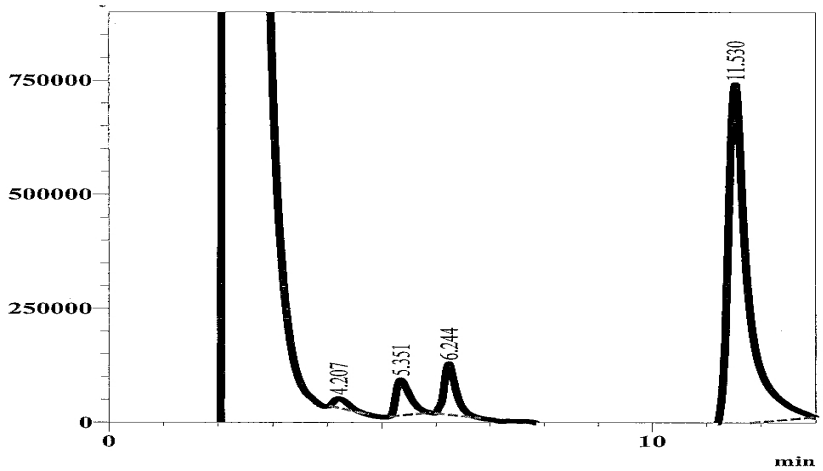
As it shown in Figures (2 ,3 , 4, and 5) distinctly difference in the curves peaks between patients and healthy controls, which were compared with the standard material ADA . Curved of peaks the standard material appeared when ADA analysis at minute (RT =11.52 min) which its retention time (RT) for the emergence of substance analysis using the above mentioned conditions. At same time the concentration of ADA in women with breast cancer without chemotherapy much more than healthy women .



Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	2.743	2797829	65.5663	200673	
2	3.704	329463	7.7209	63646	
3	4.000	385553	9.0353	80654	
4	11.625	754331	17.6775	63197	
Total		4267176	100.0000	408170	

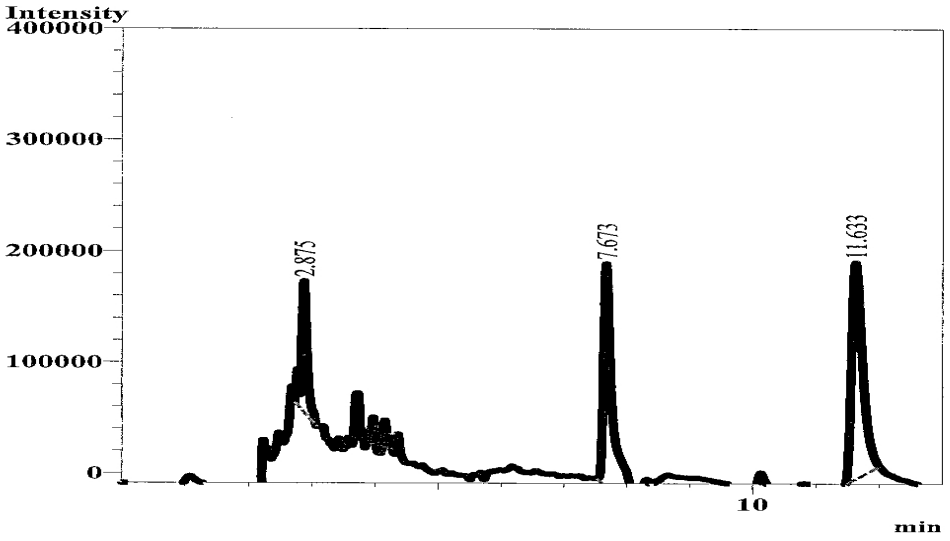
Figure 2: (ADA) in serum of healthy women its retention time (RT= 11.62)



Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	4.207	389384	1.7624	20235	
2	5.351	1396888	6.3226	77874	
3	6.244	1718367	7.7777	111743	
4	11.530	18588798	84.1372	745463	
Total		22093437	100.0000	955315	

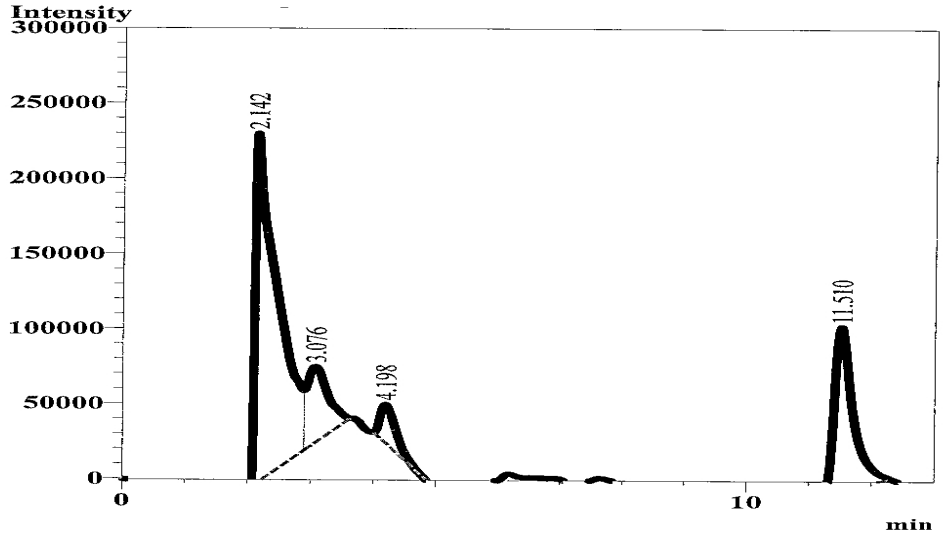
Figure 3: ADA in serum of women with breast cancer and without chemotherapy, its retention time (RT= 11.53)



Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	2.875	751303	14.1511	116338	
2	7.673	2185387	41.1626	200784	
3	11.633	2372472	44.6864	193963	
Total		5309162	100.0000	511085	

Figure 4 : ADA in serum after Fourth dose chemotherapy, its retention time (RT= 11.63)



Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	2.142	5616154	59.6152	232989	
2	3.076	1194649	12.6811	50501	
3	4.198	458734	4.8694	25415	
4	11.510	2151143	22.8343	106537	
Total		9420680	100.0000	415442	

Figure 5: ADA in serum after Sixth dose chemotherapy, its retention time (RT=11.51)

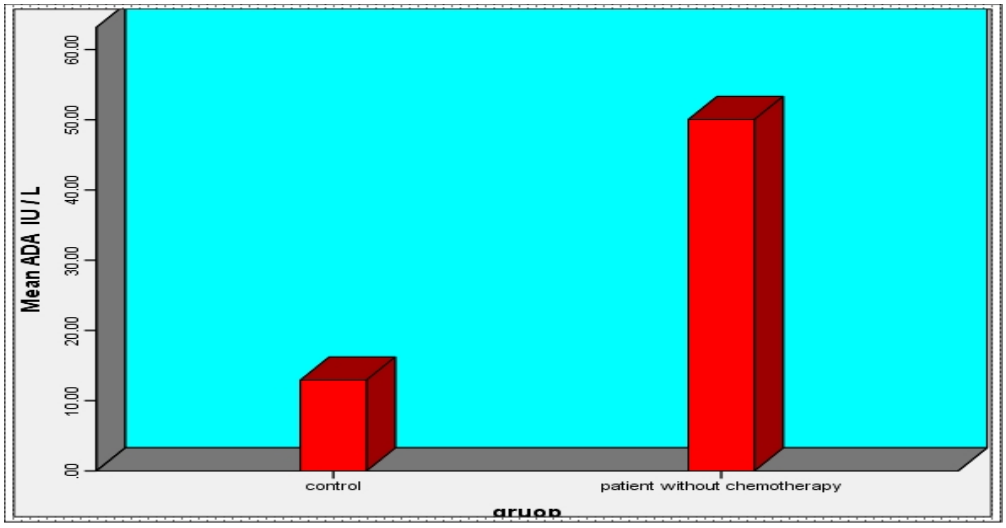


Figure 6 : ADA of women with breast cancer without chemotherapy and healthy women as mean

The mean ± SD of adenosine deaminase in serum had shown a increase in the patient with breast cancer without chemotherapy in comparison to that of control group as table (2) reveals. While table (3) reverted to the difference in (Mean ± SD) (ANOVA) of ADA concentration between groups of patient them self-according to division that mention in table (1)

Table 2: Mean of ADA in women with breast cancer compared with healthy control group

Group	No. of samples	Mean (IU/L) ± S D	P value
Control	40	12.07 ± 0.475	p<0.001
Patient without chemotherapy	40	49.96 ± 4.350	

According to t-test, there were significance difference between the mean of ADA in serum of patient and mean of ADA in serum of control ($p < 0.001$)

Table 3: Mean ± SD (ANOVA) of ADA (IU/L) in serum of breast cancer without, with chemotherapy group and healthy women

Description	No. of sample	Mean±SD (IU /L)	P Value
Control	40	12.07±0.47	P<0.001
Women with breast cancer without chemotherapy	40	49.96±4.35	
Women with breast cancer first dose chemotherapy	40	47.99±4.02	
Women with breast cancer Second dose chemotherapy	40	35.40±2.13	
Women with breast cancer third dose chemotherapy	40	26.47±1.74	
Women with breast cancer fourth dose chemotherapy	40	18.19±1.51	
Women with breast cancer fifth dose chemotherapy	40	15.19±0.53	
Women with breast cancer sixth dose chemotherapy	40	12.02±0.41	

In cancer ; rapid tissue proliferation is stimulated by growth factors and cytokines resulted in increased malignant cells turnover which is associated with increase in nucleotide metabolism, where the purine metabolizing enzymes is a part of it and ADA is a member of this metabolic pathway [11].

ADA activity decrement after chemotherapy administration may be due to programmed cell death of large cancerous cells. A hypothesized mechanism of this decrement may be through this sequence: Toxic adenosine and deoxyadenosine accumulated as a result of ADA inhibition , which leads to inhibition of ribonucleotide reductase and also inactivation of S-adenosyl homocysteine hydrolase, leading to apoptosis [12].

ADA documented as being a tumor marker in many tumors grow quickly like breast cancer . In current study it's found that ADA has been significant elevated in breast cancer patients . This was in agreement with other studies such as Aghaei et al [13], Shatova et. Al [14] and Aghaei et. Al [15] studies

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.

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