Cytidine Deaminase Activity In Breast Cancer

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Abstract:

Cytidine deaminase (CTD) catalyzed the deamination of cytidine (CR) to uridine (UR) is a cytoplasmic enzyme existing in many kind of cells and widely distributed among mammalian tissues. It has been reported to be a sensitive indicator in several types of abnormalities. CTD activities in sera of thirty-eight females with breast cancer (BC) and twenty-five healthy females as a control were measured using a spectrophotometric method. Also electrophoretic pattern of sera of patients with BC and healthy females were assayed using polyacrylamide gel electrophoresis (PAGE) to differentiate between normal and abnormal proteins existing in the two cases.

Compared with healthy females, CDA activities found to significantly increase in sera of patients with BC (4.96± 2.34, 13.3 ± 3.72 U/L) ($P < 0.000$) respectively. PAGE profile of sera of patients with BC shows wide variety than that of normal females.

These results may consider a novel biochemical marker to aid in the diagnosis of BC.

الملخص:

أنزيم السايتدين دي أمينيز يحفز تفاعل إزالة أمين السايتدين ليحوله إلى اليوريدين. وهو إنزيم سايتوبلازمي موجود في العديد من الخلايا وموزع بشكل واسع في معظم الأنسجة ،وقد اعتبر في العديد من الدراسات السابقة بأنة مؤشر كيميائي حياتي حساس للعديد من الأمراض.

تم قياس فعالية أنزيم السايتدين دي أمينيز باستخدام طريقة طيفية في أمصال ثمان وثلاثون مريضة مصابة بسرطان الثدي إضافة إلى خمسة وعشرون أنثى سليمة ظاهريا كمجموعة سيطرة .

وتم ترحيل أمصال مجموعة من المريضات والسليمات ظاهريا كهربائيا بواسطة تقنية الترحيل الكهربائي على هلام البولي أكريل أميد (PAGE) لتمييز البروتينات غير السوية التي قد تظهر في الحالة السرطانية.

مقارنة مع الإناث السليمات فان فعالية إنزيم السايتيدين دي أمينيز أظهرت زيادة معنوية في أمصال المصابات بسرطان الثدي (4.96± 2.34, 13.3 ± 3.72) ($P < 0.000$) وحدة/لتر على التوالي . وبين نموذج (PAGE) فروق كبيرة بين أمصال الإناث المرضى والسليمات.

من النتائج المستحصلة في هذه الدراسة،فأنة قد تعتبر فعالية إنزيم السايتيدين دي أمينيز كمؤشر كيميائي حياتي جديد للمساعدة في الكشف وتشخيص سرطان الثدي .

Introduction:

Cytidine deaminase (CDA) or cytidine aminohydrolase, (EC 3.5.4.5)(1-3) is a cytoplasmic enzyme existing in many types of cell, [4] which catalyze the irreversible hydrolytic determination of cytidine, to uridine.(5-7)

It is can also catalyze the deamination of cytosine nucleoside analogues used as anticancer drugs to the corresponding uracil nucleosides analogues, such as, cytosine arabinoside (ARA-C) or (cytosar), [5,6] 5-azacytidine (5-AZA C) ,(8,9) 2`-2`-difluorodeoxycytidine or (gemcitabine) .(10)

This enzyme can deaminate and thus inactivate these nucleoside analogues ,(10,11) and is also responsible for degradation of 2`, 3`dideoxy-cytidine, a selective inhibitor of human immunodeficiency virus (HIV) reverse transcriptase.(12) To prevent the inactivation of these analogues, it must be used.CDA inhibitors such as tetrahydrouridine (THU),(10,13-15) Zebularin,(16) that could be an excellent candidate in combination chemotherapy with either ARA-C or AZA-CdR in patient with leukemia . (16)

CDA has been identified in the human liver, (17) kidney, brain(18) placenta,(19,20) spleen (21) and granulocytes (7,22) and in a variety of biological sources.(18) The very high levels of CDA present in the liver and spleen are responsible for the short plasma half-life (15-20 minutes) of cytosine nucleoside analogues in man.

CDA activity has been found to be very high in synovial fluids (SF) from rheumatoid arthritis (RA) and, crystal pyrophosphate disease (CPPD) followed by psoriatic arthropathy, reactive arthritis, spondyl arthropathy,(23) and in active systemic lupus erythematosus (SLE) rather than in active SLE.(24) However, increase serum CDA activity in patients with non-Hodgkin's lymphoma (25),placenta cancer, liver cancers (26), gout and articular chondrocalcinosis has been reported, as well.(27)

On the basis of National Cancer Institute incidence and National Center for Health Statistics mortality data, the American Cancer Society has estimated that breast cancer (BC) will be the most commonly diagnosed cancer among women in the US in 2002(28). Breast cancer is expected to account for 31% (203 500) of all new cancer cases among women, and 39 600 will die from this disease.(29) The incidence and mortality rates for BC are approximately five times higher in North America and north Europe than they are in many Asian and African countries (30). Carcinoma of the breast may occur at any age but is rare before 25 years and most frequent between 40 and 70 years.(31)

About 5% to 10% of BC cases hereditary. Recently the BRCA1 gene has been cloned. It is located on chromosome 17q21. Mutation of this gene is believed to occur in 45% of cases of genetically transmitted BC. Women with a mutated BRCA1 gene have an 80% risk of developing BC by age 65 years. A second susceptibility gene known BRCA2 has recently been cloned. It is located on chromosome 13q12-13.(30)

The impetus for early detection of BC is the improved survival that results when it is discovered and treated in its less advanced stages. Screening for BC involves the application of different types of techniques in order to discover it in asymptomatic women. The methods of screening include physical examination, self-examination, biochemical test, thermography, mammography, and ultrasonography.(32)

Unfortunately, only _50% of the breast cancers are localized at the time of diagnosis (33). Despite the availability and recommended use of mammography as a routine screening method for women 40 years of age and older, its effectiveness in reducing overall population mortality from breast cancer is still being investigated (34)

Recently, the diagnosis of breast lesions or neoplasms has traditionally been made by open breast biopsy, although fine-needle aspiration cytology is helpful.(30)

Currently, serum tumor markers, such as CA15.3, that have been investigated for use in breast cancer detection still lack the adequate sensitivity (23%) and specificity (69%) to be applicable in detecting early-stage carcinoma in a large population.(35) The Food and Drug Administration approved tumor markers, such as CA15.3 and CA27.29, are recommended only for monitoring therapy of advanced breast cancer or recurrence . New biomarkers that could be used individually or in combination with an existing modality for cost-effective screening of breast cancer are still urgently needed.(36)

In the present study, we try to add a reliable biochemical marker, which may be useful for the screening and diagnosis of.BC.

Material and Method:

Patients and Controls:

Thirty-eight females with breast cancer admitted to oncology unit in Merjian teaching hospital in Hilla city, have been subjected to present study, as well as twenty five females apparently healthy individuals as a control, after having been asked about their health.

Determination of Serum CDA Activity :

The CTD activity was routinely determined by a direct spectrophotometric assay based on the loss of absorbance when cytidine is converted to uridine at 282 nm with Milton Roy sepctronic 21 where, $\Delta\varepsilon = -3600$ in 0.1M HCl solution with quartz cuvettes for a 1-cm light path at pH 7.4.(1)

The standard assay solution contained enzyme (that exist in serum), cytidine 0.002 M and Tris-HCl buffer (0.05M pH=7.4), in a final volume of 1.0 ml ^{1}. One unit of activity was defined as the amount of enzyme that required to deaminate 1μ mole of cytidine per minutes in the standard assay solution at 25°C.

Separation of Serum Proteins Using PAGE:

The separation of serum proteins was conducted by using conventional 3.5% and 5ml slots PAGE with (LKB 2117 multiphor, Sweden) to avoid the precipitation of some proteins of the gel surface in the slot.(37)

Statistical Analysis :

All values were expressed as mean ± SD Student's t-test was used to estimate differences between the groups and differences were considered significant when the probability was ($p < 0.05$).

Results:

In the present study, the average age of patients with breast cancer and healthy controls were (46.15 ± 11.08, 31.32 ± 11.55) years respectively, as shown in Figure 1.

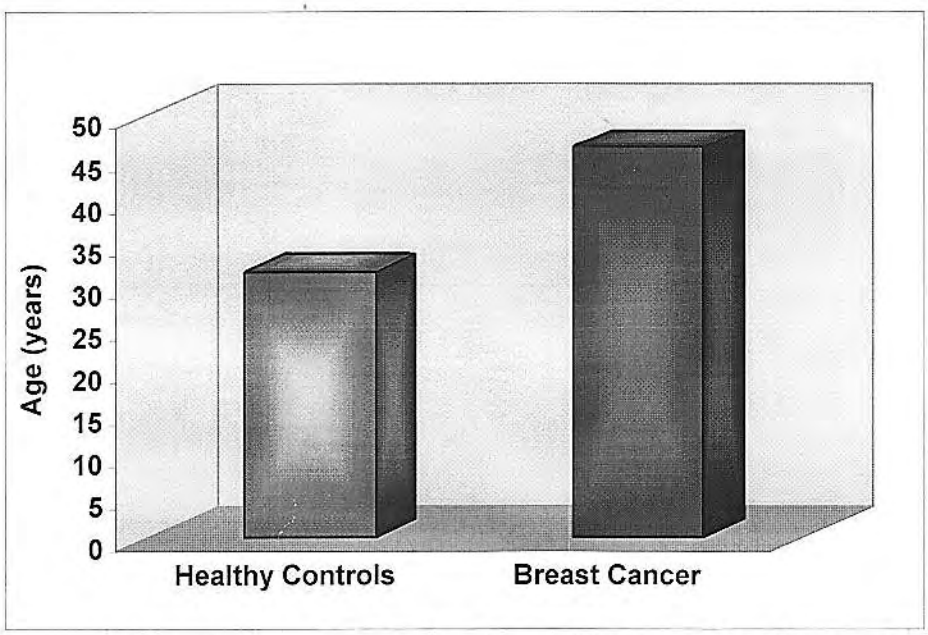

Figure 1 Average Age of Patients with BC and Healthy controls

As shown in Figure 2, significantly high levels of serum CTD activities were found in the patients BC (13.3 ± 3.72 U/ L) when compared with healthy controls (4.96 ± 2.34U/L).

The usefulness of serum CTD activities as a biochemical marker in detecting and screening of BC evaluated by measures of clinical utility in the terms of clinical sensitivity and specificity.

High sensitivity 94.73 % to BC, which represent the proportion of positive test results, and specificity 96 %, which represent the proportion of negative test results in healthy controls were obtained.

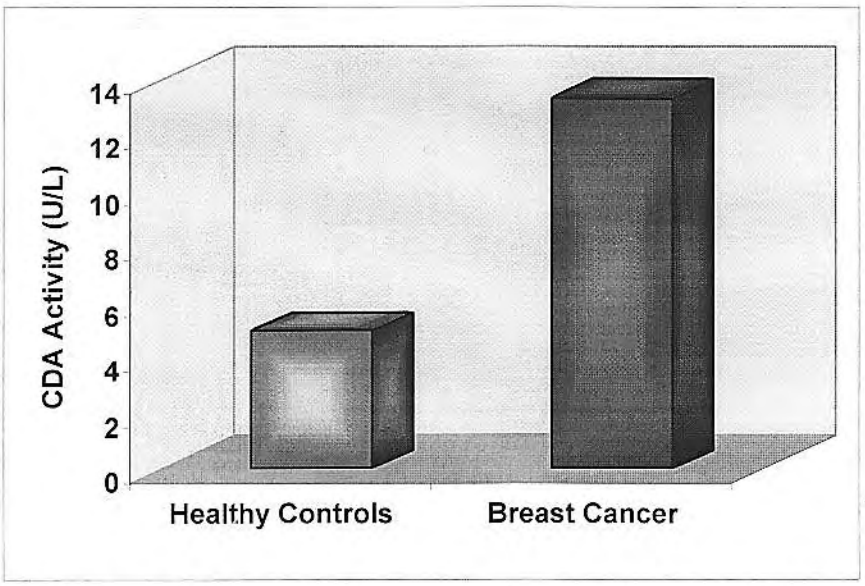

Figure 2 CDA Activity in Sera of Patients with BC and Healthy controls

Discussion:

Normally, CDA is existing in serum, although at relatively low activity,(38) but an extremely elevated in serum CDA activity in abnormal pregnancies due to a damaged placenta under progressive deterioration, (18) therefore, it is reported to be a sensitive indicator of abnormal pregnancy.(18,39)

Serum CDA activity was found to exaggeratedly increase in patients with abruptio placenta (46.3 U) and amnionitis associated with intrauterine fetal death (55.9U).(39) The cells of the infected amnion and the damaged placenta, both of which contained high CDA levels, appeared to leak CDA into the maternal circulation. High levels of CDA activity were observed in abnormal pregnancy such as pre-eclampsia, intrauterine death and cholestasis of pregnancy when compared with normal pregnancy.(18) Also, increase serum CDA activity has been reported in patients with non-Hodgkin's lymphoma (25, placenta, and liver cancers in our laboratory.(26)

Massively high levels of CDA were reported in gastric tissues from patients with gastric cancer and various types of solid tumors cell line.(40,41)

An increase in spontaneous release of CDA was noted in active SLE PMN (10.003 ± 2.637 U/5×10^6 PMN) that equal to 10.003 ± 2.637 U/L of PMN, compared with inactive SLEPMN (5.358 ±1.65 U/5×10^6 PMN) or normal group (5.449 ± 1.358 U/5×10^6 PMN).(24)

Several observations were noted in the present study:

(1) Increase in CDA in sera of patients with BC may be attributed to the presence of CDA in mammary glands in high level, and the leak out of CDA from damaged cells may be reflect to the turnover rate of these cells. When such cells are involved in the uncontrolled cell proliferation (cancer), they appear to leak out CDA into the circulation.

(2) Oxidative stress associated with cancer may be due to CDA release from cell in which plasma membrane is damaged by reactive oxygen species (ROS) or reactive nitrogen species (RNS).

(3) In addition to the above suggestions, it may be the serum factors such as autoantibodies or immune complexes or specific antigens, or the influence of drug therapy that stimulate the release of CDA.

The classical approach for discovering disease-associated proteins is two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).[29] Although 2D-PAGE is unchallenged in its ability to resolve thousands of proteins, it is labor- intensive, requires large quantities of protein, and is not easily converted into a diagnostic test. [28] Therefore, we try to use conventional one-dimensional PAGE to differentiate between normal and abnormal proteins existing in the two cases.

The electrophoretogram of serum proteins is helpful in detecting changes in individual protein fractions and as well as abnormal bands in certain disease conditions.(42)

Bands of α_1 globulins in sera of patients with BC were found to slightly increase when compared with healthy control. This is due to increase in acute phase proteins including α_1–antitrypsin and α_1–acid glycoprotein, as shown in Figure 3.

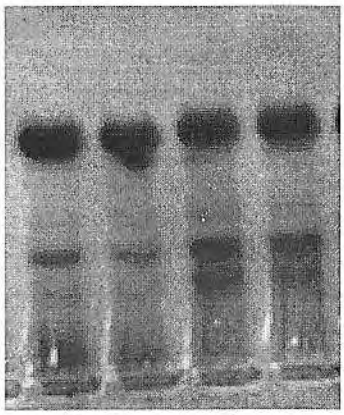

Figure 3 Electrophoretogram of Serum Proteins of Patients and Healthy Controls

Compared with healthy control, α_2-globulin bands of sera of patients in the present study were found to be clearly increased. This result may be due to increase in acute phase proteins which rise in malignancy, including heptoglobin, antihemophilicglobin, ceruloplasmin and macroglobulin.(43)

β-globulin bands were found to be apparently increased in sera of all types of patients subject to present study, when compared with healthy controls. This might be due to the rise in transferrin or high levels of estrogen, and increase in immune complex.

A visible increase in γ-globulin bands was observed in sera of patients with BC, when compared with these bands of healthy control. Such increase was observed in patients with chronic inflammatory disease and liver disease, or disseminated neoplasms, suggesting a polyclonal γ-globulin increase is associated with immune reaction.[42,43]

Serum CTD activity may consider a novel biochemical marker to aid in the diagnosis of BC. The electrophoretogram of serum proteins is not helpful in the detection of changes in individual protein in the case of BC.

References:

1- Cohen R. W., and Wolfenden R., J. Biol. Chem., (1971), 246, 7561.
2- Cohen R. W., and Wolfenden R., J. Biol. Chem., (1971), 246, 7566.
3- Ashley G. W., and Bartlett P.A., J. Biol. Chem., (1971), 259, 13615.
4- Thompson P. W., Jones D. D. and Currey H. L. F., Ann. Rheum. , (1986), 45, 9.
5- Camiener G. W., Biochem. , Pharmac. , (1965), 16, 1681.
6- Camiener G. W. and Smith C. G., Biochem. , Pharmacol. , (1965), 14, 1405.
7- Chabner B.A, Johns D. G., Drake J. C., and Evans W. H., J. Clin. Invest. , (1974) 53 ,922.
8- Chabner B.A., Drake J. C. and Johns D.G., Biochem. Pharmaccol. , (1973), 22, 2763.
9- Chabot G.G., Bouchard J. and Momparler R.L., Biochem. Pharmacol., (1983), 32 ,1327.
10- Eliopoulos N., Cournoyer D., Momparler R.L., Cancer Chemother. Pharmac., (1998), 42, 373.
11- Momparler R.L. and Labiberte J., Leu K., Res., (1990), 14, 751.
12- Faivre-Nitschke S. E., Grieneberger J. M., and Gualberto J. M., Eur. J. Biochem. , (1999), 263, 869.
13- March J.H., Kreis W., Barile B., Akerman S., Schulman P., Allen S.L., DeMarce L.C., Schuster M.W., and Budman D.R., Cancer Chemother. Pharmacal. , (1993), 31, 481.
14- Kreis W., Budman D.R., Chan K., Allen S.L., Schulman P., Lichtman S., Weiselberg L., Schuster M., Freeman J., Akerman S., Attas L. and Vinciguerra V., Leukemia, (1991), 5, 991.
15- Wentworth D.F. and Wolfenden R., Biochemistry, (1975), 14, 5099.
16- Laliberte J., Marqaes V.E., and Momparler R.L., Cancer Chemother. Pharm., (1992),30,7.
17- Wentworth D. F. and Wolfenden R., Biochemistry, (1975), 14, 5099.
18- Okamura T., Kigasawa K ., Takeuchi T. and Ohta C., Int . J. Gynecol . Obstet., (1993) , 41, 53.
19- Vita A., Vincenzetti S., Amici A., ferretti E., and Magni G., Adv. Exp. Med. Biol., (1991), 309, 45.
20 -Laliberte J., and Momparler R. L., Cancer Res., (1994), 54, 5401.
21- Vita A., Cacciamani T., Natalini P. Ruggieri S. and Magni G, in " Purine and pyrimidine metabolism in man VI, part B ", Mikanagi K, Nishioka K. and Kelley W.N. (eds.), Plenum Publishing Corporation, New York (1989).
22- Boyum A., Lovhaug D., Seeberg E. Nordlie E. M., Exp. Hematol. , (1994), 3, 18.
23-Geborek P., Månsson B., Hellmer G., Saxne T., Brit. J. Rheumatol. , (1992), 31, 235.
24-Yu C. -L., Tsai C. -T., Sun K. –H., Chen Y. -S., Lin W. -M., Liao T. -S., Brit. J. Rheumatol. , (1992), 31, 675.
25- Abdulsamie H. Alta'ee, Mufeed J. Ewadh, Oda M. Y. Al-Zamely,Nat.J.Chem., (2003),11,484,.
26- Abdulsamie H. Alta'ee, Mufeed J. Ewadh, Oda M. Y. Al-Zamely Nat.J.Chem., (2004)13,711.
27-Revonsky J., Stancikova M., Bosmansky K., Kovalancik M., Ann. Rheum. Dis. (letter, comment), (1991) 50, 659.
28-Jinong L., Zhen Z., Jason R., Young Y. W., and Daniel W. C.,Clinical Chemistry , (2002), 48, 1296.
29- Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, Cancer J Clin (2002),52,23.
30- Bassett L.W. Oncology ,John Wiley and Sons, (1982),USA.
31- 6MaoSween R.N., and Whaley K., Muir's Textbook of Pathology, (13th ed.), El. Bs. With Edward Arnold, UK, (1992).
32-Hacker N.F. and Moore J.G.,Essential of Obstetrics and Gynecology,3rd .Ed.W.B. Sanders company, (1998),USA.
33- National Cancer Institute. Cancer Net PDQ cancer information summaries. Monographs on " Screening for breast cancer" . http://www.cancer.gov/cancer_information/pdq/ (Updated Feb 2002).
34- Antman K., Shea S. , JAMA (1999),281,1470.
35- Chan D.W., Sell S. Tumor markers. In: Burtis C.A., Ashwood E.R., ed.Tietz fundamental of clinical chemistry, 5th ed. Philadelphia: W.B. Saunders,(2001).
36- Chan D.W., Beveridge R.A., Muss H., Fritsche H.A., Hortobagyi G., Theriault R., J Clin Oncol (1997),15,2322.

37- Fehrnstrom H. and Moberg U., (1977), LKB, Application Notes 306, Sweden.
38-Gandhi V., Amer. Soc. Hematol. , (1999),7, 463.
39-Okamura T. and Kigasawa K., Prenatal Diag., (1994), 14, 213.
40-Boyum A., Tennfjord V.A., Gran C., Lovhaug D., Oktedalen O., and Braudtzaeg P., J. Infect. Dis., (2000), 182, 1784.
41- Watanabe S., and Ushida T., Biochem. Biophs. Acta. , (1996), 1312, 99.
42-Mayne P.D., Clinical Chemistry in Diagnosis and Treatment, 6th ed., London, Arnold a member of the Hodder headline Group plc. (1998).
43-Bishop M.L., Duben-Engelkirk, and Fody E.P., Clinical Chemistry, 4th ed. Philadelphia, Lippincott Williams & Wilkins (2000).