Wasit Journal for Science & Medicine 2009 2 (1) : (132 - 143)

Measurement of Activities of Adenosine Deaminase and Superoxide Dismutase in Bladder Cancer Patients

Kareem Hamed Ghali - College of Medicine / University of Wassit

قياس فعالية انزيمي الادينوسين دي امينيز والسوبر اوكساید دسيميوتيز في مرضى سرطان المثانة .

كريم حمد غالي – جامعة واسط / كلية الطب

الخلاصة

تضمنت الدراسة قياس فعالية انزيمي الادينوسين دي اميينز والسوبر اوكساید دسيميوتيز في مصل ثلاثة وثمانين مريضا مصابا بسرطان المثانة وعشرين فردا من الاصحاء . اذ لوحظ انخفاض معنوي في فعالية انزيم ADA مقارنة بالأصحاء في المرضى (0.136 U/gm vs. 0.206 U/gm) بينما لوحظ ارتفاع معنوي في فعالية انزيم السوبر اوكساید دسيميوتيز (11.430 U / ml vs 8 . 585 U/ ml) ولاسيما في المراحل المتقدمة من المرض .

ABSTRACT

The biochemical study investigated the specific activity of two enzymes; adenosine deaminase (ADA) and superoxide dismutase (SOD) in the sera of eighty three blader cancer (BC) patients and twenty controls. A significant reduced specific activity of ADA was observed in the patients (0.136 U/gm *vs.* 0.206 U/gm), while SOD contrasted the picture, and instead, a significant elevation of the activity was observed (11.430 U/ml *vs.* 8 *585* U/ml)., especially in the advanced stages of disease

INTRODUCTION

Among different classifications patterns of tumour makers, there is one postulated by Roulston and Leonard (1993) (1) which is commonly used, include enzymes which play an important role in biological activities in the body. These are used widely as tumor marker in several cancerous including BC, to detect early prognosis or treatment. Some of these enzymes play an important role in immunosystems. Such as xanthin oxidase, superoxide dismutase and adenosine deaminase (2).

Adenosine deaminase (ADA , EC-3-5-4-4) is an enzyme catalyzing the irreversible hydrolytic deamination of adenosine to produce inosine and

ammonia (3). ADA plays an important role in the development of the immune system in human, and its deficiency is associated with severe combined immunedeficiency (SCID) (4). The main function of ADA in lymphocytes appears to confer protection to these cells from the toxic effects of high concentration of adenosine and deoxyadenosine (5) . However the important point in this field is that, ADA seems to be associated with differentiation of epithelial cells (6), which lined some organs like Urinary bladder. This enzyme was found in all tissues like peripheral lymphoid tissues (2): while T-cells have a higher ADA activity than B cells(7). The ADA enzyme is encoded by a gene located on ch.20 (8).

Changes in the levels of ADA were noticed in many diseases. High serum ADA activity were found in patients with tuberculosis (TB) (9) , in typhoid. (10) and liver cancer (11) .While several authors observed low ADA activity in momonuclear cells of carcinomas of non- bronchogenic origin (12) and in SCID disease (13).

Durak *et al*., (1994) (14) studied the activity of ADA in BC patients and showed an increasment in ADA compared with healthy control, differences were also found between enzyme activities in the bladder of different stages and grades. However another study performed by Juma (1999)(15) denoted a decrement in the activity of ADA in BC patients compared with a healthy control.

Superoxide distmutase (SOD , EC-1-15-1-1, superoxide: oxidoreductase) are metalloprotein that present in all oxygen metabolizing cells. SOD catalyzes the dismutation of superoxide anion ($O_2^{\cdot -}$) into hydrogen peroxide and oxygen, which is normally produced in every living cell. Thus protect cells form damage induced by free radicals (16), as explained below:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H_2 \xrightarrow{D} H_2O_2 + O_2$$

Two types of superoxide dismutase are known in human tissues. Manganese superoxide dismutase (MnSOD) which is found in the cytosole, as well as, in mitochondria of eukaryotes, and Copper- Zinc superoxide dismutase (Cu, Zn SOD) (17) which is found in cytosole of eukaryotic cell. The MnSOD encoded by a gene which is located on chromosome 6 (18).While the Cu, Zn SOD gene was pointed on chromosome 21 (19). Alteration in SOD has been postulated to play a role in the pathogenesis of a number of clinical disorders such as cancer(20,21).
The specific activity of Cu,Zn SOD is increased in erythrocytes from patients

with Down's Syndrom (22), and liver disease (23). In contrast copper zinc superoxide dismutase was found to be marginally lower in the gastric and esophageal carcinoma (24) and in the other types of cancer. However several studies have reported the decline in MnSOD activity during diseases including cancer, aging, progeria, asthma and transplant rejection (21).While numerous studies have been reported that serum MnSOD was elevated in 58% of patients with ovarian carcinoma (25) and other types of cancer.

The study which was made by Durak *et al*., (1994) (14) illustrated that the SOD activity decreased in bladder cancer patients compared with controls.

PATIENTS AND METHODS

Patients

Eighty three patients were included who were divided according to gender ,stage ,as well as twenty healthy individuals were examined .

Methods

Adenosine deaminase

The measurement of ADA activity, is performed according to Gusti method (1981). (26)

Adenosine deaminase is easily assayed by measuring the amount of ammonia formed during the 60 minutes of the incubation of alkaline solution, producing a deep blue.

The ammonia concentration is directly proportional to the absorbance (A) of the indophenol at 630 nm.

Procedure

All solutions can be stored at least 2 months, except daily requirement of buffered adenosine solution. The reaction is performed according to the following table:

Reagent Tube	Reagent Blank (ml)	Standard (ml)	Adenosine Blank (ml)	Sample blank (ml)	Sample (ml)
Phosphate buffer solution	1.0	—	—	1.0	—
Buffer adenosine solution	—	—	1.0	—	1.0
Ammonium sulphate solution	—	1.0	—	—	—
Sample	—	—	—	0.05	0.05
D.D.W	0.05	0.05	0.05	0.05	0.05
Then all tubes are mixed well and incubated in water bath for 60 min. at 37C°. Then the following solution were added:					
Phenol nitroprusside solution	3.0	3.0	3.0	3.0	3.0
Alkaline hypochlorite solution	3.0	3.0	3.0	3.0	3.0

The contents of each tube are mixed well, and incubated for further 30 min. in 37 C° water bath. The absorbance is read at 630 nm against DW.

Calculation

Volume activity = $\frac{A-B}{C} \times 50$ (U/L); 37C °

Specific activity = $\frac{\text{Volume activity}}{\text{Total protein}}$ (U/mg)

Where:

A = A sample – A sample blank
B = A adenosine blank – Reagent blank
C= A standard – Reagent blank

Super oxide dismutase

The measurment of SOD is carried out according to Beyer and Fredovich method (1987). (27)

p**rocedure**

The procedure is performed according to the following table:

Tube **Solution**	**Blank**	**Sample**
Reaction mixture solution	1 ml	1 ml
Sodium azide	50 μl	50 μl
Serum	—	100 μl
Mixed well immediately		
D.W	430 μl	330 μl
Mixed well		
Riboflavin	20 μl	20 μl
Mixed well immediately		

The absorbance of each tube is read at 560 nm, then all tubes were illuminated for 7 min. at 25C° in illuminated box, containing two 20 w florescent lamps. The absorbance is measured at 560 nm.

Calculation

The SOD enzymatic activity is expressed as the percentage of inhibition of NBT reduction, where one unit of SOD is defined as the amount of the plasma that cause 50% decreased in the SOD inhibitable NBT reduction .

RESUILTS

The mean levels of total serum ADA specific activity in BC patients was (0.136 U/gm) (table 1), than the mean levels of the control group (mean 0.206 U/gm). This decline was found to be proportional to the aggressiveness of the disease. The mean value of ADA specific activity of high grade (mean 0.100 U/gm) was significantly decreased ($P < 0.001$) compared to low grade (mean 0.152 U/gm). On the other hand no significant differences were noticed

between superficial and invasive cases, and between male patients and female patients ($P > 0.05$).

The total serum SOD activity in this study is shown in table (2). The results illustrate that the serum total SOD activity has a highly significant increase ($P < 0.0001$) in total patients (mean 11.430 U/ml) when compared with healthy controls (mean 8.585 U/ml). These elevated values increased whenever the disease become more aggressive, so the comparison between low grade (mean 10.89 U/ml) and high grade (mean 12.67 U /ml) showed a significant differences ($P < 0.05$).

While no statistically significant differences were seen in mean serum SOD activity among male patients vs. female patients and invasive vs. superficial cases ($P > 0.05$).

While no statistically significant differences were seen in mean serum SOD activity among male patients vs. female patients and invasive vs. superficial cases ($P > 0.05$).

DISCUSSION

Serum Specific Activity of ADA in Bladder Cancer

Adenosine deaminas is an immune enzyme, which catalyses deamination of adenosine and deoxyadenosin to inosine and
2-deoxyinosione and amonia in purine metabolism pathway (28)

The results of the present study demonstrate significant ($P<0.001$) lower values of serum ADA activity in BC patients than in healthy controls (Table 1). This relationship was also observed by Juama (1999) (15) in Iraqi BC patients. While Durak *et al* (1994) (14)) showed increased ADA in BC patients from Turkey. On the other hand jukaddes *etal* (2003) observed not any statistatics differences in ADA activities between patient with BC and healthy controls (29)

The difference may be attributed to race, otherwise, the disease heterogeneity may have some role to play. Further more tumour grading may also have some effect. In this respect, patients with low grade tumour recovered more ADA activity than high grade patients (0.152 vs. 0.100 Ulgm). Therefore, ADA activity may be considered as a marker of a disease progression and aggressivety , and may help in the therapeutic management of BC.

A further point is worth to be highlighted , ADA is present in all cells of mammals, but its deficiency affects the lymphocytes patients only Therefore, a

reduction in ADA activity may lead to malfunction the immune response negatively, and consequently, the tumour progression may escape the immunosurveillance.

Levels of Total SOD in Bladder Cancer.

Superoxide dismutase plays a key role in the detoxification of superoxide radicals, and thus protect cells from damage induced by free radicals (16). The enzyme catalyzes the dismutation of two superoxide radicals ($O_2^{\cdot -}$ into O2 and H_2O_2). During the past years, SOD activity in tumour cells has received an increasing attention. Total SOD is decreased in many, but not in all tumours (30). Changes in SOD activity of blood of leukemia patients have been reported. Erythrocytes SOD also decreased in patients with malignant lymphoma and acute myeloid leukemia (31).
Cu,Zn SOD was found to be marginally lower in gastric and esophageal carcinoma (24).

However, total SOD activity is significantly greater in acute myeloblastic leukemia than in normal individuals (32) , Mn SOD was elevated in ovarian carcinoma (25). In this study, serum SOD activity in patients with bladder cancer showed a significant ($P<0.001$) increase compared with healthy controls (Table 2).the results reported here is in contrast with results of Mujan *etal* (2007) (33) , but its contradictory to that reported by the results of Durak *et al*. (1994).(14) This may be attributed to the following:

1- The generated free radicals was not supperoxide anion. The increased level of Cu and Fe supports this suggestion.
2- The increase may reflect the defence against toxocity. The increase of SOD in high grades disease supports this view.
3- The increase in the isoenzyme SOD (e.g. Mn SOD) results from exposure to radiation and biological agents (e.g. viruses and bacteria). In addition this enzyme is considered as a defence mechanism against pro- apoptotic stimuli.

Table (1): The adenosine deaminas activity in the sera of total patients with bladder cancer, healthy controls, and different grades and stages.

Comparison / Variable:	Total		Sex		Stage		Grade	
	Healthy controls N (20)	Patients N (83)	Male patients N (64)	Female patients N (19)	Superficial N (34)	Invasive N (49)	Low grade N (58)	High grade N (25)
Mean ± SD	0.206 ± 0.03	0.136 ± 0.06	0.142 ± 0.06	0.117 ± 0.07	0.152 ± 0.07	0.125 ± 0.05	0.152 ± 0.07	0.100 ± 0.03
SE	0.008	0.007	0.081	0.016	0.012	0.008	0.009	0.007
t= value		4.457		1.424		1.873		3.464
P <		H.S. P < 0.001		N.S.		N.S.		H.S. P< 0.001
95% C.I lower s	0.187	0.121	0.125	0.083	0.126	0.108	0.133	0.085
95% C.I Upper	0.224	0.151	0.158	0.151	0.178	0.142	0.170	0.115
Minimum	0.145	0.047	0.052	0.047	0.052	0.047	0.052	0.047
Maximum	0.315	0.337	0.337	0.314	0.337	0.284	0.337	0.173

C.I:Confidenceinterv

Table (2): Serum total SOD activity in total patients with bladder cancer healthy controls and different grades and stages.

Comparison	Total		Sex		Stage		Grade	
Variable: SOD (U/ml)	Healthy controls N (20)	Patients N (83)	Male patients N (64)	Female patients N (19)	Superficial N (34)	Invasive N (49)	Low grade N (58)	High grade N (25)
Mean ± SD	8.585 ± 3.16	11.430 ± 3.32	11.195 ± 3.38	12.225 ± 3.08	10.82 ± 2.92	11.850 ± 3.55	10.89 ± 2.83	12.67 ± 4.04
SE	0.64	0.36	0.42	0.70	0.50	0.50	0.37	0.80
t= value		3.727		1.188		1.386		2.294
P <		H.S. P < 0.001		N.S.		N.S.		S. P < 0.05
95% C.I lower s	7.24	10.70	10.34	10.73	9.80	10.83	10.14	11.00
95% C.I Upper	9.92	12.15	12.04	13.71	11.84	12.86	11.64	14.34
Minimum	1.66	1.58	1.58	7.14	5.82	1.58	5.82	1.58
Maximum	16.14	18.59	18.59	18.09	17.19	18.59	17.19	18.59

C.I: Confidence interval

REFERENCES

1- Roulston, J.E. and leonard, R.C. F. (eds):(1993). Serological tumour marker, 1 st (ed). Longman group, U.K. p 87.

2- Moriwaki, Y.; Yamamoto, T. and Higashino, K.:(1999). Enzymes involved in purine metabolism- A review of histochemical localization and functional implications, Histol. Histopathol., 14: 1321-1340.

3- Martin, B., van Dear, w. and William, N. (1976). Human adenosine deaminase. J. Bioc. Chem. 251 (18): 5448-5456.

4- Giblett, E.R.; Anderson, J.E.; Cohen.; Pollara, B. and Meuwiseen, H.J.:(1972). Adenosine deaminase deficiency in two patient with severely impaired cellular immunity. Lancet. 2, 1067-1096.

5- Fox, I.H. and Kelley, W.N.:(1978). The role of adenosine and 2- deoxy adenosine in mammalian cells. Annu. Rev. Biochem. 47, 655-686.

6- Trotta,P ,P , and Balis, M, E, ,: (1997).Structural and kinetic alterations in adenosine deaminase associated with the differentiation of rat intestinal cells ,Cancer Res. 37 , 2297 – 2305

7- Sullivan, J.L.; Osborn, W.R.A. and Wedgewood, R.J.:(1997). Adenosine deaminase activity in lymphocytes, Br. J. Haematol. 37, 157-158.

8- Robert, K.; Daryle, K.; Peter, A. and Victor, W. (eds): (2000). Harper's biochemistry, 25 th (ed) Mc Graw- Hill, New York, U.S. P. 1205

9- Hassan,F.;Sharaf El Din,M;El Mougy,F.and Hussein,I.: (1993).A pilot study of diagnosis and therapeutic value of serum mycobacteial antigen(A60) Ig G and adinosine deaminase in pulmouary tuberculosis. New Egyption J.Med. 8(4):1020-1026

10- Mishra,O.P;Gupta,B.L.;nath,G.and Chandra,L.:(1994). Adenosin deaminase activity in typhoid fever.India Pedi:. 3\(11):1379-1384

11- Koloayashi,F.;Ikeda,T.;Marumo,F.and Siato,C.:(1993).Adenosine deaminase isoenzymes in liver disease .Am.J.Gast. 88(2):266-71

12- Robani, M.Y.; Cheong, Y.M. and Rani, J.M.:(1995). The use of adenosine deaminase activity as a biochemical marker for the diagnosis of tuberculous meningitis. Malay. I. Pathol. 17 (2): 67-71.

13- Murray, R.K.; Granner, D.K.; Smayes, P.A. and Rodwell, V.W. (ed): Nishimura, N.; Ho, Y.; Aduchi, T.; Hirano, K.; Sugiura, M. and Sawaki, S. (1982). Enzyme immunoassay for cuprozinc- superoxide dismutase in serum and Urine. J. Pharm. Dyn. 5: 394-402.

14- Durak, I., Perk, H.; Kavutcu, M.; Canbolat, O., Akyol, O. and Beduk, Y.:(1994) Adenosine deaminase, 5'nucleotidase, xanthine oxides. Superoxide dismutase, and catalase activities in cancerous and

noncancerous human bladder tissues. Free- Radic. Biol. Med. 16 (6): 825-31.

15-. Juma, A.S.M.:(1999). Cytogenetic, Biochemical and bacteriological studies on patients infected with schistosoma haematobium and host with bladder cacinoma. Ph.D. Thesis. Saddam University, Baghdad- Iraq,

16- Mitrunen, k.; Sillanpaa, P.; kataja, v.; Eskelinen, M.; kosma, V.M.; Benhamon, S.; Uusitupa, M. and Hirvonen, A.: (2001). Associated between manganes superoxide dismutase (Mn SOD) gene polymorphism and breast cancer risk. Carinogenesis. 22 (5): 827-9.

17- Yi, S., Larry, W.O. and Ying, L.:(1988). A simple method for clinical assay of superoxide desmutas. Clin. Chem. 34 (3) 497-500.

18- Mates, M. and Sanchez- Jimenez , F.:(1999). Frontiers in Bioscience ,4th,(ed), 339-345. (Cited by Al- Habal,2001)

19- Schwartz, P. (1997). Ph.D. thesis, John Hopkins Univ., Baltimore, 157-158Maryland. (Cited by Al- Habal,2001).

20- Koshida, K.; Adachi, T.; Yamamoto, H.; Uchibayashi, T. and Hirano, K.:(1996). Serum and tissue levels of manganese superoxide dismutase in testicular germ cell tumours, Euro. Urol.29: 487-490.

21- Macmillan, C.L.A. and Cruthirds, D.L.:(2001). Invited review manganes superoxide desmutase in disease. Free Radic. Res. 34 (4): 325-36.

22-Nishimura, N.; Ho, Y.; Aduchi, T.; Hirano, K.; Sugiura, M. and Sawaki, S. (1982). Enzyme immunoassay for cuprozinc- superoxide dismutase in serum and Urine. J. Pharm. Dyn. 5: 394-402.

23- Nishimura, N.; Ho, Y.; Aduchi, T.; Hirano, K.; Sugiura, M. and Sawaki, S. : (1982).Enzyme immunoassay for cuprozinc- superoxide dismutase in serum and Urine. J. Pharm. Dyn. 5: 394-402.

24-Janssen, A.; Bosman, C. ; van-Duijn, W.; Oostendrop-van-de-Ruit, M.; Kubben, F.; Griffioen, G.; Lamers, C.; van-Krieken, J.; van-de-velde, C. and Verspaget, H.: (2000). superoxide dismutase in gastric and esophageal cancer and the prognostic impact in gastric cancer. Clin. Cancer Res. 6 (8): 3183-92.

25- Ishikawa, M.; Yaginuma, Y.; Hayashi, H.; Shimizu, T.; Endo, Y. and Taniguchi, N.:(1990). Reactivity of a mono clonal antibody to manganese superoxide desmutase with human ovarian carcinoma. Cancer Res. 50: 2538-42.

26- Gusti, G.: Adenosine deaminase. In: bergmeyer, H.V. (ed).(1981). Methods of enzymatic analysis vol-2, 2 nd (ed). Academic press, new York. pp. 1092.

27- Beyer, W.F. and Fridovich, I.:(1987). Assaying for superoxide dismutase activity. Some large consequences of minor changes in condition. Analy. Biochem.; 161: 559-566.
28- Harriman, G. and Abusharab, E.:(1994). Adenosine deaminase inhibitors. Synthesis and biological evaluation of 4-Amino-1-2 (S)-hydroxy-3 ® nonyle-IH- imidazo [4, 5, C] pyrimidine derivatives. J.M ed. Chem. 37 (2): 304-8.
29- Jukaddes G. ; Haluk A. ; Useyin Y. ; Erturk E . and Omar a.(2003). Adenosine deaminase and xanthine oxidase activities in bladder washing fluid from patients with BC a preliminary study . Clin . Bioch. 36 (3) . 193 – 96 .
30- Oberley, L.W. and Baettner, G.R.:(1979). Role of superoxide dismutase in cancer: a review. Cancer Res. 39: 1141-9.
31- Saito,T.; Kurasuki, M.; Kaji, I.I. and Saito, K.:(1984). Deficiency of erythrocyte superoxide desmutase and catalase activities in patients with malignant lymphoma and acute myeloid leukemia. Cancer lett. 24: 141-6.
32- Yoshimitsu, K.; Kobayashi, Y. and Usui, T.:(1984). Superoxide desmutase in leukemia blasts of children with acute leukemia. Acta paediatr scand. 37: 92-6 (Cited by Sun, 1988).
33- Mujan C. ; ahment O. ; Anil C. ; and Calip D. : (2007).the investigation of GSTT1 , GSTM and SOD polymorphisun in BC patients . Iner. Urol . and Morp . Springer Netherlands . 39 (4) . 1043 – 8 .

Recived ……..…….……. (23/6/2008)
Accepted ……...……... (26/1/2009)