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

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ABSTRACT

The biochemical study investigated the specific activity of two enzymes; adenosine deaminase (ADA) and superoxide dismutase (SOD) in the sera of eighty three bladder 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 immunodeficiency (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), typhoid, (10) and liver cancer (11). While several authors observed low ADA activity in mononuclear 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 increment 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 ($O_2^-$) into hydrogen peroxide and oxygen, which is normally produced in every living cell. Thus protect cells from damage induced by free radicals (16), as explained below:

$$O_2^- + O_2^- + 2H_2 \rightarrow H_2O_2 + O_2$$

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:
Table

<table>
<thead>
<tr>
<th>Tube</th>
<th>Reagent blank (ml)</th>
<th>Standard blank (ml)</th>
<th>Adenosine blank (ml)</th>
<th>Sample blank (ml)</th>
<th>Sample (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer solution</td>
<td>1.0</td>
<td>_</td>
<td>_</td>
<td>1.0</td>
<td>_</td>
</tr>
<tr>
<td>Buffer adenosine solution</td>
<td>_</td>
<td>_</td>
<td>1.0</td>
<td>_</td>
<td>1.0</td>
</tr>
<tr>
<td>Ammonium sulphate solution</td>
<td>_</td>
<td>1.0</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Sample</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>D.D.W</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Then all tubes are mixed well and incubated in water bath for 60 min. at 37°C. Then the following solution were added:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Phenol nitroprusside solution</th>
<th>Alkaline hypochlorite solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

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); 37°C

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

Procedure

The procedure is performed according to the following table:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction mixture solution</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
<tr>
<td>Serum</td>
<td>_</td>
<td>100 μl</td>
</tr>
<tr>
<td>Mixed well immediately</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.W</td>
<td>430 μl</td>
<td>330 μl</td>
</tr>
<tr>
<td>Mixed well</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>20 μl</td>
<td>20 μl</td>
</tr>
<tr>
<td>Mixed well immediately</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

RESULTS

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 statistics 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 (\( \text{O}_2^- \text{O}_2 \) into O2 and \( \text{H}_2\text{O}_2 \)). 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 et al (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 superoxide anion. The increased level of Cu and Fe supports this suggestion.
2- The increase may reflect the defence against toxicity. 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>
<thead>
<tr>
<th>Comparison</th>
<th>Healthy controls</th>
<th>Male patients</th>
<th>Female patients</th>
<th>Superficial invasive</th>
<th>Low grade</th>
<th>High grade</th>
<th>Stage</th>
<th>Sex</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (86)</td>
<td>100'000</td>
<td>70'007</td>
<td>30'073</td>
<td>7%</td>
<td>94%</td>
<td>2%</td>
<td>1</td>
<td>M</td>
<td>4.64</td>
</tr>
<tr>
<td>N (69)</td>
<td>100'000</td>
<td>70'007</td>
<td>30'073</td>
<td>7%</td>
<td>94%</td>
<td>2%</td>
<td>1</td>
<td>F</td>
<td>4.64</td>
</tr>
<tr>
<td>N (64)</td>
<td>100'000</td>
<td>70'007</td>
<td>30'073</td>
<td>7%</td>
<td>94%</td>
<td>2%</td>
<td>1</td>
<td>M</td>
<td>4.64</td>
</tr>
<tr>
<td>N (63)</td>
<td>100'000</td>
<td>70'007</td>
<td>30'073</td>
<td>7%</td>
<td>94%</td>
<td>2%</td>
<td>1</td>
<td>F</td>
<td>4.64</td>
</tr>
</tbody>
</table>

Table (1): The adenosome deaminase activity in the sera of total patients with bladder cancer; healthy controls, and different grades and stages.
### Table (2): Serum Total SOD activity in total patients with bladder cancer, healthy controls and different grades and stages.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Stage</th>
<th>Sex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>invasive</td>
<td>male</td>
<td>N (68)</td>
</tr>
<tr>
<td>Low</td>
<td>invasive</td>
<td>female</td>
<td>N (49)</td>
</tr>
<tr>
<td></td>
<td>superficial</td>
<td>male</td>
<td>N (19)</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>female</td>
<td>N (44)</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>female</td>
<td>N (63)</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>female</td>
<td>N (20)</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>male</td>
<td>N (25)</td>
</tr>
</tbody>
</table>

**Grades and Stages:**
- **High Grade:**
- **Low Grade:**
- **Invasive:**
- **Superficial:**
- **Male:**
- **Female:**
- **Control:**
- **Healthy:**

**Comparison:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Minimum</th>
<th>Maximum</th>
<th>95% CI Upper</th>
<th>95% CI Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>62.59</td>
<td>17.91</td>
<td>62.94</td>
<td>17.19</td>
</tr>
<tr>
<td>Group B</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Group C</td>
<td>11.64</td>
<td>11.64</td>
<td>11.64</td>
<td>11.64</td>
</tr>
<tr>
<td>Group D</td>
<td>1.14</td>
<td>1.14</td>
<td>1.14</td>
<td>1.14</td>
</tr>
</tbody>
</table>

**P-values:**

- p < 0.05
- *S* = significance

**Mean ± SD:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>12.67</td>
<td>8.58</td>
</tr>
<tr>
<td>Group B</td>
<td>12.67</td>
<td>8.58</td>
</tr>
<tr>
<td>Group C</td>
<td>12.67</td>
<td>8.58</td>
</tr>
<tr>
<td>Group D</td>
<td>12.67</td>
<td>8.58</td>
</tr>
</tbody>
</table>
REFERENCES
14- Durak, I., Perk, H.; Kayutcu, M.; Canbolat, O., Akyol, O. and Beduk, Y.:( 1994 ) Adenosine deaminase, 5’nucleotidase, xanthine oxides. Superoxide dismutase, and catalase activities in cancerous and


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