Adenosine deaminase, AMP deaminase, 5`-Nucleotidase, Xanthine oxidase and Xanthine dehydrogenase Activities in Prostate Cancer Patients.

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الخلا صه:

تم قياس الفعالية النوعية لـ بعض إنزيمات ايض البيورين وهي deaminase, ADA
AMP ,XO,5`-NT والأكسدة والاختزال لأنزيمات XO) في مصول مجموعة السيطرة
(n=45) و مجموعة مرضى سرطان البروستات (n=47). وقد اظهرت النتائج وجود انخفاض
معنوي (p<0.001) في فعالية ADA و AMP-deaminase في مصول مرضى سرطان
البروستات بالمقارنة مع فعالية هذه الأنزيمات في مصول مجموعة السيطرة. بينما كانت هنالك
زيادة معنوية (p<0.001) في فعالية إنزيم 5`-NT في مصول مرضى سرطان البروستات
بالمقارنة مع مجموعة السيطرة. وفيما يتعلق بفعالية الأكسدة والاختزال لأنزيمات XO وأظهرت
النتائج عدم وجود فعالية أكسدة محسوسة لأنزيم XO في مصول جميع النماذج لمرضى سرطان
البروستات ،بينما أظهرت النتائج وجود انخفاض معنوي(p<0.001) في فعالية الاختزال لأنزيم
XO في مصول مرضى سرطان البروستات بالمقارنة مع مجموعة السيطرة. وأظهرت عملية
الترحيل الكهربائي على متعدد الكريل امايد وبالاعتماد على صبغة الفعالية الإنزيمية لكل إنزيم
فقد ظهر شكلين لأنزيم ADA في مصول السيطرة وثلاثة أشكال في مصول مرضى سرطان
البروستات. وثلاثة أشكال لأنزيم 5`-NT في مصول السيطرة و مصول مرضى سرطان
البروستات. أما إنزيم AMP-deaminase فقد ظهر بشكل واحد في مصول السيطرة و
مصول مرضى سرطان البروستات. هذه الأنزيمات ADA ,
AMP deaminase ,XO,5`-NT من المحتمل أن تستخدم كعلامة كيميائيه حيوية لمرض
سرطان البروستات لإمكانية المساعدة في التشخيص المبكر.

ABSTRACT:

The specific activities of some purine metabolism enzymes [Adenosine deaminase (ADA), AMP-deaminase, 5`-nucleotidase (5`-NT) and xanthine oxidase (the two forms: xanthine oxidase (XO) and xanthine dehydrogenase (XDH)], were measured in sera of control (n=45) and patients with prostate cancer (n=47).Sera ADA and AMP-deaminase specific activities in prostate cancer were significantly decreased ($p<0.001$) when compared with control group. By contrast there was significant increase ($p<0.001$) in 5`-NT specific activity observed in sera of prostate cancer. The activities of xanthine oxidase (XO) (the oxidase and the dehydrogenase activities) were the results revealed no detectable oxidase activities of XO in sera of prostate cancer. Where as, the dehydrogenase activity of this enzyme show significant decrease ($p<0.001$) in comparism to control group. Two forms and three forms of ADA were found in sera of control and

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prostate cancer patients respectively as judged by polyacrylamide gel electrophoresis .Three forms of 5`-NT were found in sera of prostate cancer patients and control. One forms of AMP-deaminase was detected in the sera of control and prostate cancer patients. ADA, AMP-deaminase, 5`-NT and (XO, XDH) enzymes may be used as a biochemical markers in prostate cancer patients to help the early diagnosis.

INTRODUCTION:

Adenosine deaminase (ADA, adenosine amino hydrolase E.C. 3.5.4.4) is a cytosolic enzyme. ADA participates in the purine metabolism where it degrades either adenosine or 2-deoxyadenosine producing inosine or 2`-deoxyinosine, further metabolism of these deaminated nucleosides leads to hypoxanthine which can be either transformation into uric acid by xanthine oxidase [1, 2] or salvage bathway into mononucleotides by action of hypoxanthine guanine phosphoribosyl transferase [3].Different laboratories have shown that ADA may appear also on the cell surface (ecto ADA) [4]. Its mean physiological activity is related to lymphocytic proliferation and differentiation. Also a marker of cell mediated immunity, its activity is found to be elevated in those diseases in which there is a cell-mediated immune response [2, 5].

Adenosin monophosphate deamionase (AMP deaminase) catalyzes the conversion of adenosine monophosphate to inosine monophosphate and ammonia. Two form of AMP deaminase were reported to be present (soluble and membrane bound).It is found in a variety of animal tissue and cells. AMP deaminase plays crucial role in the inter conversion of purine nucleotides [6], and is stabilizing the adenylate energy charge.

5`-Nucleotidase (5`-NT) activity controls intracellular levels of nucleoside 5-monophosphate, surface located 5`-NT is the major contributor to the cascade that completely hydrolyze extracellular ATP to adenosine [7] and thus of major pharmacological interest [8].

Xanthine oxidase (XO E.C.1.1.3.22) is the enzyme catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid, which are the terminal biochemical reactions of the purine degradation pathway [9]. Therefore, any defect in purine metabolism results in an increase in the level of uric acid. This eventually leads to the deposition of sodium hydrogen urate monohydrate crystals in joints. The disease associated with this phenomenon is known as gout [10]. Moreover, increase in the level of XO also causes renal stone formation and

ischemic myocardium and reactive oxygen species (ROS) induced diseases [11].

Prostate cancer is the most common human cancer and the second leading cause of cancer death; it is a major public health problem in the developed world .The oxidative damage may play a role in prostate cancer [12, 13].

THE AIM OF THIS STUDY:

The aim of our study is to evaluate the some purine metabolic enzymes activities (Adenosine deaminase (ADA), AMP deaminase, 5`-Nucleotidase, Xanthine oxidase and Xanthine dehydrogenase activities in sera of healthy (control) and patients with prostate cancer. Detection of changes in the enzymes by separation with conventional-PAGE from sera healthy and prostate cancer patients. Detailed knowledge of the enzymatic pathways of purine nucleotide metabolism is important in elucidating any biochemical differences that may exist.

MATERIALS AND METHODS:

Chemicals: All laboratory chemical and reagent in this work were of analar grade and imported from BDH Co. and SIGMA Co.(Na_2CO_3,$CuSO_4.2H_2O$,sodium potassium tartrate, SDS, NaOH,Folinreagent,BSA,H_2SO_4,$KMnO_4$,adenosine,anhydrous ammonium sulphate, ammonia, ammonium sulfate, phenol , sodium nitroprusside , NaOCL,
5`-AMP ,sodium barbiturate, manganes sulphate, deionized water, HCL,$NiCL_2.6H_2O$,$SnCL_2$,ammonium molybdate,KH_2PO_4,$NaHPO_4$,Na_2HPO_4,
Xanthine, EDTA-Na_4, gelatine, PMS, tetrazolium salt.

Samples: (47) patients with prostate cancer were included in this study. Admitted to merjan teaching hospital in Hilla city, over period of about (7) months from March 2006 to September 2006 The diagnosis of prostate cancer was established by clinical analysis. All the subjects were examined clinically and information pertaining to age, habits and health status was recorded in special case proforma. Other group included (45) healthy (control). Blood samples were collected from both controls and patients for the estimation of serum ADA, AMP deaminase, 5-Nucleotidase, Xanthine oxidase and Xanthine dehydrogenase Activities.

Adenosine deaminase (ADA) Activity Assay: The serum was assayed immediately for ADA activity at 37 ^{0}C by a spectrophotometric method using adenosine as the substrate. This method is based on the Bertholet reaction, that is, the formation of colored indophenol complexes from ammonia liberated from adenosine and quantitated spectrophotometrically at 630 nm [14].One unit of ADA is defined as the

amount of enzyme required to release one micromole ammonia per minute from adenosine at standard assay conditions. The activity of ADA is expressed in units / liter [15].

AMP deaminase Activity Assay: For the detection of adenosine monophosphate deaminase activity in serum the same method used for ADA activity except changing the buffer and substrate (5`- adenosine monophosphate) used.

5`-Nucleotidase (5`-NT) Activity Assay: The enzyme activity was monitored spectrophotometrically by measuring phosphate liberated at 680 nm according to the Wood and Williams's method [16].

Xanthine oxidase (XO) Activity Assay: The enzyme activity was monitored spectrophotometrically, according to the Ackermann and Brill method [17] by measuring uric acid formation at 293 nm with saturated concentration of xanthine (20 µM) as the substrate (if not mentioned otherwise for a specific experiment) in 1 ml of 50 mM phosphate buffer, pH 7.5, at 25°C.

Xanthine dehydrogenase (XDH) Activity Assay: The enzyme activity was measured the blue insoluble formazan formation at (530-580) nm with using tetrazolium salt (yellow)according to the Fried *et al* method [18].

Total Protein Estimation: The protein content of all samples was determined by modified Lowery method [19] using bovine serum albumin as standard protein for all samples.

Separation of Enzymes by Conventional-PAGE: The separation of enzymes was conducted by using conventional-PAGE lab gel electrophoresis by using modification of Lammeli method [20]. Gel were stained for ADA, AMP deaminase [21] and 5-Nucleotidase [22] activities.

Statistical Analysis [23]: The data of the study subjected to statistical analysis is expressed as mean ± SD. Statistical comparisms were performed by Students t-test. Student's t-test was used to estimate differences between the groups and differences were considered significant when the probability was high significant at ($p<0.001$).

RESULTS:

The specific activity of ADA,AMP deaminase ,5`-NT and XO were evaluated in sera of patients with prostate cancer compared with that of control group.

The specific activity of sera ADA of patients with prostate cancer show a significant decrease ($p<0.001$) (312.4% of that of the control) when compared with that of control, figure (1) and table (1).

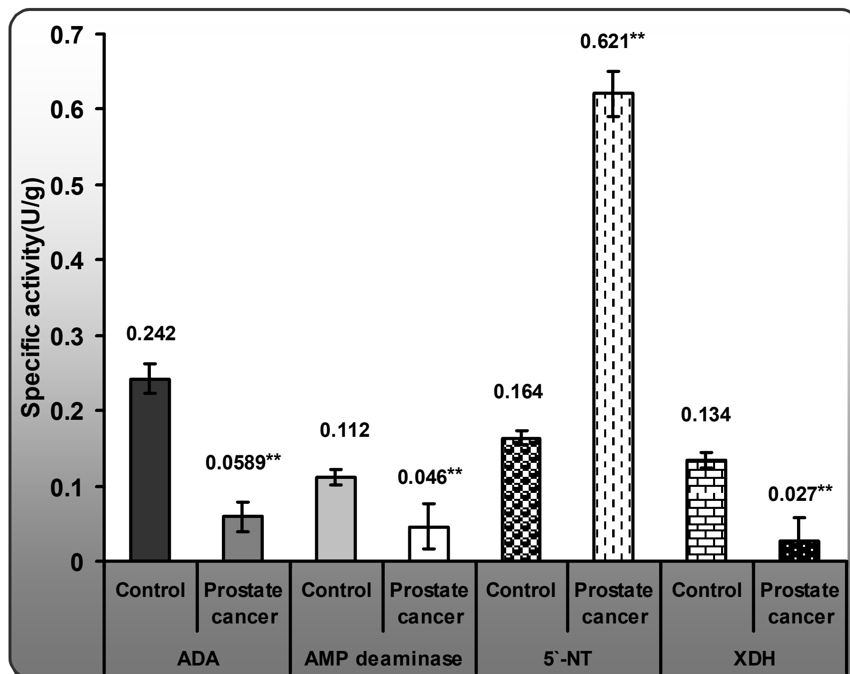


Figure (1): Mean Value (±)SD of ADA, AMP deaminase, 5`-NT and XDH Specific Activities in Control and Prostate Cancer Patients.

The specific activity of AMP deaminase of patients with prostate cancer show a significant decrease (143.5 % of that of the control) in sera samples ($p<0.001$) when matched with that of control group, figure (1) and table (1).

In table (1) and figure (1) ,sera 5`-NT specific activity of prostate cancer group was found to increase significantly($p<0.001$) (3.79% folds of that of the control) when compared with that of control group.

The two activities of XO (the dehydrogenase and the oxidase activity) were evaluated in sera of prostate cancer.

The oxidase activity was measured according to Ackermann and Brill method [15].No detectable activities were measured in sera of control and prostate cancer patients.

The Xanthine dehydrogenase activity was measured according to Fried *et .al* method [16]**.**The results show a significant decrease ($p<0.001$) (396.3% of that of the control) in its specific activity in sera of prostate cancer when compared with that of control, table (1) and figure (1).

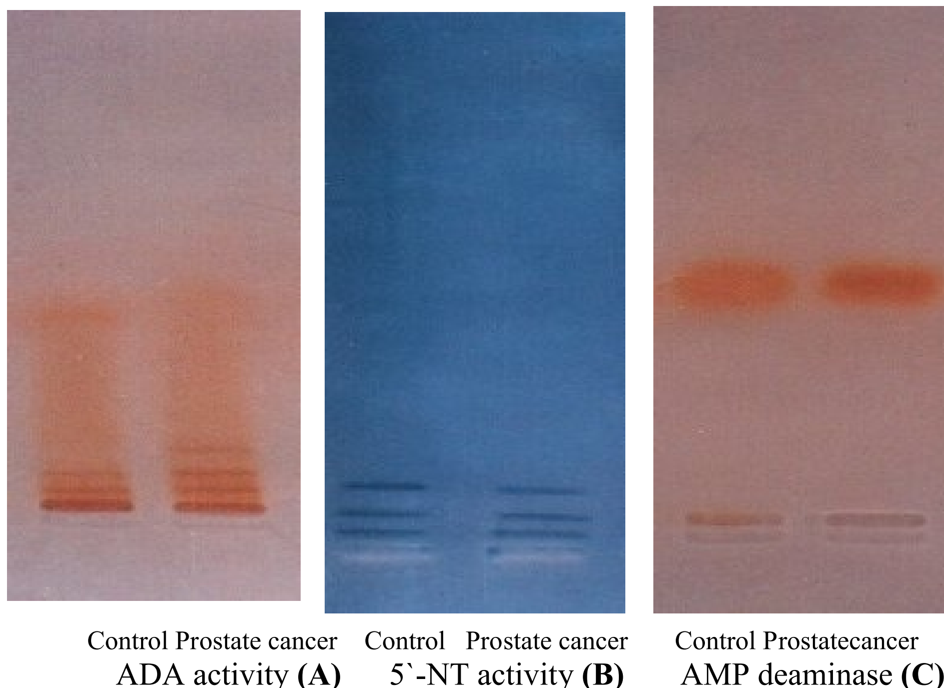
Table (1): Total Protein,Activity and Specific Activity of ADA,AMP deaminase,5`-NT , XO and Xanthine dehydrogenase Enzymes of Sera from Control and Patients with Prostate Cancer.(Mean value± SD, **= p<0.001).

Variables	Control n.=45				Prostate Patients n.=47			
	Age years	Total protein (G/L)	Activity (U/L)	Specific activity	Age years	Total protein (g/L)	Activity (U/L)	Specific Activity
Adenosine deaminase (ADA)	24-56	73.2 ±0.24	17.71 ±0.21	0.242±0.02	34-58	70.1±0.31	4.12**±0.31	0.058**±0.02
Deaminase (AMP)	24-56	73.2±0.24	8.19±0.23	0.112±0.01	34-58	70.1±0.31	3.22**±0.31	0.046**±0.03
5´-Nucleotidase (5´-NT)	24-56	73.2±0.24	12.0±0.22	0.164±0.01	34-58	70.1±0.31	43.5**±0.13	0.62**±0.03
Xanthine dehydrogenase	24-56	73.2±0.24	9.8±0.22	0.134±0.01	34-58	70.1±0.31	18.9**±0.32	0.02**±0.03

Electrophoresis on polyacrylamide gel was carried on sera samples of control and prostate cancer patients. The three enzymes (ADA,AMP deaminase and 5`-NT) were detected using their specific activities stains.

Figure(2, A) shows that sera ADA of prostate cancer patients was separated into three bands, while only two bands with ADA activity were detected in sera of control.

Figure (2, B) shows that sera 5`-NT was separated in three bands in prostate cancer patients and control. Were as only one band with AMP deaminase activity was detected in sera of control and prostate cancer patients Figure (2, C).



Figure(2):Conventional-PAGE 7.5% profile of sera from control and prostate cancer. The gel was stained for ADA activity (A). The gel was stained for 5`-NT activity (B). The gel was stained for AMP deaminase activity(C)

DISCUSSION:

Defect of purine and pyrimidine metabolism are characterized by abnormal concentrations of purines, pyrimidines or their metabolites in the cells or body fluids due to the disturbance in the activities of the enzymes involved in this metabolism [24].

Major function of extrcellular enzyme appears to be termination of the physiological action of nucleotide that is released from the cells. Nucleotides represent the ubiquitous class of cell to cell signaling substances eliciting physiological response in every tissue. ATP, ADP exert their function via surface located receptors, which have a tissue distribution as broad as that of the ecto- nucleotidase. These receptor -mediated actions are involved in a large variety of physiological and pathophysiological function [25].

The results of this work indicate presence of a decrease in adenosine disaminase activity and specific activity (312.4% of that of the control) between prostate cancer and control. Studies on adenosine deaminase, the crucial enzyme of adenosine degradation, revealed that

its activity differ from tumor to tumor as well as, from tumor to peritumor normal tissue [26].This finding agree well with what of AL-Shammaree[27],she studied ADA activity in malignant cervix and uterine tumor, also agree with Zainb *et. al*.[28] their studies on the ADA level in sera of brain tumor.

It was established that poorly differentiated cancer, with elevated ADA activity, grew faster than well-differentiated ones with low ADA activity [29]. Interestingly this presumably is not entirely correlated with the rate of cell division. This may be due to the altered metabolism of cancer cells although tissue and cancer dependent variations are also possible. The reasons for these finding, remains unknown, but it can be speculated that the stage of the disease and the immune response of the host may have a negative or positive influence in the release of ADA from the tissue cells.

It has been suggested that elevated 5'-NT (3.79% folds of that of the control) might be a physiologic attempt of cancer cells to provide more substrate needed by them to accelerate the salvage pathway activity. Further more high ADA activity might also play part in the detoxification process of high amounts of toxic adenosine and deoxyadenosine substrate provided from accelerated purine metabolism in the cancerous tissue [30].

There are accumulating evidences that enzymes of purine metabolism may be biochemical markers of lymphoid malignancy, since some purine enzymes (5'-NT and ADA) play an important role in lymphocyte function [31]. Also using elevated 5'-NT activity, as a marker of liver metastases was reported [32, 33].

Decrease in the specific activities of XDH(396.3% of that of the control) were found in sera of prostate cancer patients, and no detectable activity of XO was found in sera of prostate cancer this may be due to the presence of high concentrations of XO antibody in the sera of humans as suggested by Bruder *et al.* [34].Our results were in agreement with many other studies [35-36] in that there were a significant decrease in XO activity.

Natsumeda *et.al* [35] suggested that the over all purine enzymatic pattern confers selective advantages to cancer cells by making them more efficient for retention and production of precursors for synthesis of purine and pyrimidine nucleotides and subsequently, for RNA and DNA biosynthesis.

CONCLUSIONS:

In this study, the result shown significantly decrease in ADA,AMP deaminase and XO activities, also significantly increase in 5`-NT activity of sera in prostate cancer patients in comparison with control.

The reduction of ADA activity could cause a state of immuno suppression, this is evident from the fact that sever combined immuno deficiency(SCID) patients have very few lymphocytes in their blood[37,38].

Increase the protein biosynthesis (increase in 5`-NT), are a known characteristic of uncontrolled proliferation of cancer cells [39].

The decrease in XO activity observed in cancer reflects the fact that the catabolic pathway is decreased in away to spare hypoxanthine and xanthine in order to permits its ready recycling to IMP.

The decreased in XO activity in cancer patients leads to a decreases in uric acid level, which is proposed to be a sacrificial antioxidant by binding metal ions in forms that will not stimulate radical reactions and also by scavenging (OH) and peroxy radicals [40].

In other words the decrease in uric acid level may cause a reduction in the antioxidant defense systems effectiveness.

I suggest that ADA,AMP deaminase,5`-NT and XO can be used as a marker in malignancy tumor and more study is need to detect the separation and purification of these enzymes.

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