

## Enzymatic Changes in Serum of Benign and Malignant Brain Tumors Patients

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(XO) (ALP) (CK) (LDH)  
(5'-NT) - (CDA) (ADA)  
(DNase)

5'-NT CDA XO ALP CK-MB CK LDH)

(DNase

(ADA)

### Abstract

The present study is concerned with the investigation of the activity of some enzymes as lactate dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP), xanthine oxidase (XO), adenosine deaminase (ADA), cytidine deaminase (CDA), 5'-nucleotidase (5'-NT), deoxyribonuclease (DNase), in serum of benign and malignant brain tumor patients. Blood samples 93 from brain tumors patients, and 65 blood samples from healthy subjects as control group are collected for the present study. The results obtained indicated a significant increase in the activities of LDH, CK, CK-MB, ALP, XO, CDA, 5'-NT and DNase in serum of benign and malignant brain tumor patients compared with control group. A decrease in the activity of ADA was observed in malignant patients only.

### Introduction

In general, cancer is a condition in which there is an uncontrolled cellular mechanism, causing abnormal cellular structures, functions, and growth leading eventually to the destruction of tissues and organs. This abnormal growth produces a mass of cells called a tumor (1).

(\*) part of Ph. D. Thesis.

Cancer arises because of alterations in deoxyribonucleic acid (DNA) that result in unrestrained cellular proliferation. Most of these alterations involve actual sequence changes in the DNA (i.e. mutation). They may arise as a consequence of random replication errors, exposure to carcinogens (e.g. radiation), or faulty DNA repair process (2).

A brain tumor is a mass of abnormal cells that is growing in or around the brain. It develops when abnormal cells multiply for unknown reasons. They are generally named after the type of cell they developed from, benign and malignant terms are used to describe these tumors. (3).

Brain tumors are usually caused by a change in genetic structure. These changes in genetic structure may be inherited, or caused by environmental factors, or both. (4).

The enzymes which were believed to change their activities in the present of tumor cancer and are taken into consideration in this study as follows: Lactate Dehydrogenase (LDH), Creatine Kinase (CK), Alkaline Phosphatase (ALP), Xanthine Oxidase (XO), Adenosine Deaminase (ADA), Cytidine Deaminase (CDA), 5'-Nucleotidase (5'-NT), Deoxyribonucleases (DNase) and Proteins.

### **Materials & Methods**

Patients were enrolled in the present study to the neurosurgery unit in Ibn-Sina Hospital in Nineveh Governorate. Samples of 93 patients diagnosed clinically and radiologically as having brain tumor were included in this study 47 males and 46 females ranging in their age between 15-70 years, were collected during the period May-2004 to July-2005.

Depending on histopathological examination, 41 patients were diagnosed as having malignant brain tumor, while 52 patients were diagnosed as benign brain tumor. Sixty-five age matched healthy individuals were included in this study 45, males and 20 females as a control group.

### **Blood Samples**

Ten milliliters of venous blood was taken from each patient before the operation and left for 15 minutes at a room temperature for coagulation, serum then were separated by centrifugation at 3000 xg for 10 minutes. Serum was divided in aliquot and kept frozen at -20 °C for the enzymatic activities assays and other biochemical tests (5).

### **Methods**

Lactate dehydrogenase (LDH) was assayed using manufactured kit by Syrbio. (5).

Creatine kinase (CK) was assayed using manufactured kit by Randox, (5).

Creatine kinase isoenzyme (CK-MB) was determined using manufactured kit by DIALAB company. (6).

Alkaline phosphatase (ALP) activity was assayed using manufactured kit by BioMerieux (7).

Xanthine oxidase (XO) activity (the oxidase form) was determined by the method of (8).

Adenosine deaminase (ADA) was determined according to Guisti method (9).

Cytidine deaminase (CDA) activity was assayed by a spectrophotometric assay according to (10).

5'-Nucleotidase (5'-NT) activity was measured by following Wood and William's method (11).

Acid and alkaline Deoxyribonuclease (DNase) Activities were determined by a modified method of Kunitz (12).

Serum total protein was determined by Biuret method using kit manufactured by Syrbio (13).

### **Results and Discussion**

The results in table (1) showed that there is a significant increase ( $P < 0.001$ ) in LDH activity in serum of patients with benign and malignant brain tumors compared with the control group. No significant effect had been found on LDH activity in serum of benign and malignant brain tumor groups. Table (2) showed the results of LDH activity in males and females with benign and malignant brain tumors which indicated that there is no significant differences in LDH activity between these groups.

An increased amount of LDH activity in the serum of benign and malignant brain tumor patients might be a sign of tissue damage and cell death, their LDH is released and finds its way into the blood circulation (14).

Also, the results in table (1) showed that there was a significant increase ( $P < 0.001$ ) in CK activity, in serum of benign and malignant cases respectively in comparison with controls. The CK activity was higher in malignant cases in comparison with benign, but this increase was not significant. Table (2) showed that the activity of CK was significantly higher in males compared with females in benign and malignant brain tumor patients. The results also indicated higher activity in malignant male cases in comparison with benign and to a lesser value between females.

The causes of the increment could be due to the presence of enzyme in much higher concentration inside the cell. This enzyme would

be released into the systemic circulation as the result of tumor necrosis which would lead to the changes in the membrane permeability of the cancer cells (15, 16).

The higher activity of CK in males might be due to increase incidence of detectable prostate cancer and the treatment of patients which might include testosterone which increased the activity of this enzyme (17).

On the other hand, the results in table (1) showed that isoenzyme of CK (CK-MB) activity was significantly increased ( $P<0.001$ ) in serum of benign and malignant tumors when compared with control group. Table (2) also indicated that there was no significant differences between males and females in respect to benign and malignant brain tumor patients.

Serum CK-MB activity was increased in patients with brain tumors, because the activity was affected by the accumulation of CK-MB in the serum and its clearance from the serum. The accumulation was affected by the amount of tissue necrosis and probably the tissue destruction (18).

The results in table (1) showed that there was a significant increase ( $P<0.01$ ) in ALP activity in serum of benign and malignant brain tumor patients compared with control group. Within the patients group there was no significant difference between benign and malignant cases. Table (2) showed the results of ALP activity in males and females with benign and malignant brain tumor, which was noted that there was no significant difference in the enzyme activity between them.

This could be explained by the fact that the presence of the enzyme in high concentration inside the cell, and the changes in the membrane permeability of the tumor cells would lead to the release of the enzyme into the systemic circulation (5).

Table (1): The enzymes activity and total protein level in blood serum of patients with benign and malignant brain tumors

Parameters	Mean $\pm$ SE				
	Control	Benign Tumors	No. of folds	Malignant Tumors	No. of folds
LDH (U/L)	92.20 $\pm$ 5.32	380.99 $\pm$ 31.35***	4.1	391.63 $\pm$ 44.51***	4.2
CK (U/L)	97.14 $\pm$ 7.72	129.57 $\pm$ 12.88***	1.3	148.06 $\pm$ 13.41***	1.5
CK-MB (U/L)	5.08 $\pm$ 0.35	11.05 $\pm$ 1.04***	2.2	12.83 $\pm$ 1.13***	2.5
ALP (U/L)	58.68 $\pm$ 2.44	74.49 $\pm$ 5.09**	1.3	75.36 $\pm$ 9.75**	1.3
XO (U/L)	7.70 $\pm$ 0.46	9.47 $\pm$ 0.79*	1.2	11.46 $\pm$ 0.8***	1.5
ADA (U/L)	1.75 $\pm$ 0.07	1.70 $\pm$ 0.14	1.0 <sup>(-)</sup>	0.47 $\pm$ 0.03*** ###	3.7 <sup>(-)</sup>
CDA (U/L)	21.61 $\pm$ 0.66	43.01 $\pm$ 3.86***	2.0	58.77 $\pm$ 2.17*** ##	2.7
5'-NT (U/L)	12.50 $\pm$ 0.54	16.98 $\pm$ 0.70*	1.4	22.10 $\pm$ 1.55*** ##	1.8
Acid DNase (U/L)	20.89 $\pm$ 1.43	32.09 $\pm$ 5.14**	1.5	45.57 $\pm$ 7.83***	2.2
Alkaline DNase (U/L)	3.67 $\pm$ 0.29	10.52 $\pm$ 1.11***	2.9	10.84 $\pm$ 1.68***	3.0
Total protein (g/dL)	7.24 $\pm$ 0.55	4.89 $\pm$ 0.12***	1.5 <sup>(-)</sup>	4.99 $\pm$ 0.18***	1.5 <sup>(-)</sup>

\*\*\* Significant difference between control at ( $P \leq 0.001$ )

\*\* Significant difference between control at ( $P \leq 0.01$ )

\* Significant difference between control at ( $P \leq 0.05$ )

## Significant difference between benign at ( $P \leq 0.001$ )

# Significant difference between benign at ( $P \leq 0.01$ )

(-) decreased in the number of folds

A significant increase was found in XO activity in serum of benign and malignant brain tumor patients in comparison with control group as shown in table (1). The activity was higher in malignant cases in comparison to benign, but the difference was not significant. Table (2) also showed that there was a significant increase in females with benign and malignant brain tumors, while the activity of XO in males was also increased but not significant when compared with control group.

The higher activity of XO in serum of patients with brain tumors was due to the function of the enzyme which catalyzed the breakdown of nucleotides to form uric acid, which contributes to the antioxidants capacity of the blood (19). The XO is most recognized for its role as the rate-limiting enzyme in nucleic acids degradation through which all

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purines are channeled for terminal oxidation. The enzyme served as a source of oxygen-derived free radicals which induced both cellular injury and edema as well as changes in vascular permeability. This occurred during the damaging cell membranes by reacting with membrane fatty acids which lead to release of the enzyme into the blood (20).

Table (2): The enzymes activity and total protein level in blood serum of males and females patients with benign and malignant brain tumors

Parameters	Sex	Mean $\pm$ SE.		
		Control	Benign Tumors	Malignant Tumors
LDH (U/L)	Males	92.26 $\pm$ 6.65	371.47 $\pm$ 65.39***	384.19 $\pm$ 45.10***
	Females	92.06 $\pm$ 8.63	385.79 $\pm$ 44.63***	398.43 $\pm$ 60.09***
CK (U/L)	Males	96.50 $\pm$ 6.04	147.24 $\pm$ 21.45***	178.21 $\pm$ 23.97***
	Females	99.58 $\pm$ 9.73	110.80 $\pm$ 12.77 # #	119.27 $\pm$ 10.01 # #
CK-MB (U/L)	Males	5.40 $\pm$ 0.42	10.33 $\pm$ 1.33***	10.72 $\pm$ 0.68***
	Females	4.60 $\pm$ 0.60	11.76 $\pm$ 1.68***	13.72 $\pm$ 1.52***
ALP (U/L)	Males	59.28 $\pm$ 2.60	69.94 $\pm$ 5.44	70.95 $\pm$ 6.55
	Females	57.33 $\pm$ 5.48	78.75 $\pm$ 8.49*	79.77 $\pm$ 18.87*
XO (U/L)	Males	7.72 $\pm$ 0.60	8.11 $\pm$ 1.03	10.27 $\pm$ 1.21
	Females	7.64 $\pm$ 0.67	10.53 $\pm$ 1.11*	12.56 $\pm$ 1.01***
ADA (U/L)	Males	1.78 $\pm$ 0.08	1.52 $\pm$ 0.20	0.54 $\pm$ 0.06***
	Females	1.57 $\pm$ 0.10	1.79 $\pm$ 0.19	0.39 $\pm$ 0.02***
CDA (U/L)	Males	21.60 $\pm$ 0.83	38.89 $\pm$ 10.62***	56.19 $\pm$ 5.86***
	Females	21.65 $\pm$ 1.02	44.77 $\pm$ 3.75***	60.48 $\pm$ 0.72***
5'-NT (U/L)	Males	12.36 $\pm$ 0.68	17.31 $\pm$ 1.16*	26.35 $\pm$ 3.58***
	Females	12.81 $\pm$ 0.89	16.83 $\pm$ 0.92*	20.40 $\pm$ 1.10***
Acid DNase (U/L)	Males	19.89 $\pm$ 1.75	26.16 $\pm$ 5.73	33.98 $\pm$ 11.15*
	Females	23.69 $\pm$ 2.20	39.75 $\pm$ 7.39*	57.16 $\pm$ 6.90***
Alkaline DNase (U/L)	Males	3.49 $\pm$ 0.33	9.11 $\pm$ 0.57***	8.26 $\pm$ 0.97***
	Females	4.09 $\pm$ 0.61	11.71 $\pm$ 1.96***	14.46 $\pm$ 3.34***
Total protein (g/dL)	Males	7.28 $\pm$ 0.06	4.84 $\pm$ 0.13***	4.98 $\pm$ 0.24***
	Females	7.14 $\pm$ 0.07	4.94 $\pm$ 0.19***	5.00 $\pm$ 0.28***

\*\*\* Significant difference between control at ( $p \leq 0.001$ )

\*\* Significant difference between control at ( $p \leq 0.01$ )

\* Significant difference between control at ( $p \leq 0.05$ )

# # Significant difference between males at ( $P < 0.01$ ).

The results in table (1) showed that there was no significant difference in serum ADA activity between control and benign tumor groups, while an obvious significant decrease ( $P<0.001$ ) was found in malignant tumor. The result also indicated that there was a significant decrease ( $P<0.001$ ) of ADA activity in serum of malignant group when compared with benign brain tumor patients. Table (2) also showed the result of ADA activity in males and females with benign and malignant tumors and it has been found that there was no significant difference between these groups.

The deficiency of ADA led to severe immunodeficiency disease in which T-lymphocytes and B-lymphocytes do not develop properly. Also the deficiency of ADA is one of the first targets of human gene therapy trials (16).

Compared with control group, CDA activity was found to be significantly increased ( $P<0.001$ ) in serum of patients with benign and malignant brain tumors (Table 1), which represents a two and three fold increase. Also the results showed that there was a significant increase ( $P<0.01$ ) of CDA activity in serum of malignant group when compared with benign brain tumor patients. The research also showed that there was no significant difference in CDA activity between males and females with benign and malignant tumors as shown in table (2).

The increased activity of CDA in serum of brain tumor patients may be attributed to several possibilities. One of them serum factor such as autoantibodies, immune complexes or specific antigens that stimulate the release of CDA, or attributed to the damaged B or T cells which mainly increased in this disease (21).

In addition to the above suggestions, the release of CDA from cells in which plasma membrane was damaged by ROS might be due to oxidative stress associated with cancer and may reveal high CDA activity (22).

Table (1) showed a significant increase of 5'-NT activity in serum of benign ( $P<0.05$ ) and malignant ( $P<0.001$ ) brain tumor patients when compared with control group. Also there was a significant increase ( $P<0.01$ ) in malignant cases compared with benign. Table (2) showed the results of 5'-NT activity in serum of males and females with benign and malignant brain tumors which explain that there was no significant differences between these groups.

It has been suggested that increased 5'-NT activity in serum of benign and malignant brain tumor patients might be a physiological attempt of the cancer cell to provide more substrate needed by cancer cells to accelerate the salvage pathway activity (23). There are accumulating evidences that enzymes of purine metabolism might be biochemical markers of lymphoid malignancy, since one of the purine



enzyme is 5'-NT which play an important role in lymphocytes functions (21). The results shown in table (1) revealed a highly significant increase in the activity of serum acid DNase in patients with benign ( $P<0.01$ ) and malignant ( $P<0.001$ ) brain tumors in comparison with that of control group. The results in table (2) showed that there were no significant differences in activity between males and females acid DNase activity in both types of tumors. It was suggested that the high activity of acid DNase in serum of cancer patient might be a sign of tissue damage which will lead to the changes in cell membrane permeability and the enzyme released into the serum (24).

Acid DNase activity was observed on the periphery of spontaneously occurring tumor necrosis, at early stage of the induced tumor necrosis. The effect of acid DNase activity in malignant tumor cells was probably linked to natural enzyme inhibitors and its reversal to early stage of tumor necrosis (25).

The results presented in table (1) also revealed a highly significant increase ( $P<0.001$ ) in alkaline DNase activity in serum of patients with benign and malignant brain tumor when compared with control group. The results also indicated that there were no significant differences between males and females of brain tumor cancer, (Table 2).

The variation in serum alkaline DNase activity could be a simple, rapid and valid marker for monitoring cancer therapy and disease evolution (26). These variations in enzyme activity could have a prognostic value in early detection of recurrence of cancer (27).

Also it was suggested that serum alkaline DNase a known circulating tumor marker could be used for treatment monitoring of cancer patients (26). The activity of alkaline DNase in serum appeared to be useful in predicting treatment response in the long term follow up of patients. It has been suggested that the measurement of acid and alkaline DNase could be considered as malignant disease markers (24).

In the present study, serum total protein in patients with benign and malignant brain tumors were found to be significantly decreased ( $P<0.001$ ) when compared with control group as shown in table (1). Within the patients groups, there was no significant difference in the serum of benign and malignant brain tumors. In table (2) the results showed that there were no significant differences in total serum protein concentration between males and females in both types of tumors.

This decrease could be due to the decrease of albumin concentration in blood, or due to the deficiency of immunoglobulin or because of the deficiency of protein in the diet (28). Another suggestion that the decrease in total serum protein might be due to the involvement of albumin in the defense against oxidative stress associated with cancer (5).

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