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STUDY THE EFFECT OF ACUTE LYMPHOBLASTIC LEUKEMIA ON ACID DNASE ACTIVITY IN CHILDREN

DR. WESEN A.MEHDI * AND ALAA SH. ABDULBARI

Department of chemistry, College of sciences for women, university of Baghdad, Iraq

ABSTRACT

Acute lymphoblastic leukemia (ALL) represents the malignant proliferation of lymphoid cells blocked at early stages of differentiation and is the most common malignancy in children. In patients with ALL, also numerous bone marrow stem cells grow into a type of white blood cell titled lymphocytes. These abnormal lymphocytes are not able to contest infection very well.aboratory investigations including serum total protein, serum acid DNase, Blood samples were collected from 60 patients diagnosed to Acute lymphoblastic leukemia (ALL) after one month treatment with induction therapy. Age and sex matched 30 healthy persons selected as control. Activities and Specific Activities of Serum acid DNase showed A significant increase in patients group when compared to control group($p<0.001$).In Conclusions Acid DNase Activity may be a useful indicator of response to chemotherapy.

KEYWORDS: **Acute lymphoblastic leukemia, serum acid DNase, Gel Electrophoresis.**

DR. WESEN A.MEHDI
Department of chemistry, College of sciences for women, university of Baghdad, Iraq

INTRODUCTION

Leukaemia, a haemopoietic cells' neoplasm, is bone marrow malignancies often characterised by abnormal increase in white blood cells. Leukaemia constitutes the twelfth most common class of neoplastic disease, and the eleventh most common cause of cancer-related death[1].The four main types, acute myeloid leukaemia, chronic myeloid leu-kaemia, acute lymphocytic leukaemia and chronic lymphocytic leukaemia, according to the type of white blood cell that is affected [2- 3]. Childhood acute leukemia is the most common cancer in children[4].accounting for approximately one-third of all childhood neoplasms in developed countries[5-6].The etiology of this hematologic malignancy might be explained by a combination of genetic susceptibility and environmental exposure during early development in fetal life and infancy[7]. Deoxyribonucleases are a large group of enzymes characterizedby considerable structural and functional diversity.In eukaryotic cells they are involved in a range of cellular functions, including DNA repair, recombinationand genome degradation[8].The two main types of DNase found in humans are known as DNase I and DNase II[9]. DNase II (acid DNaseEC 3.1.22.1) is a mammalian endonucleasethat catalyzes the hydrolysis of the phosphodiester bonds of dsDNA to yield 3-phosphoryl oligonucleotidesunder acidic conditions in the absence ofdivalent metal ions. Activity and mRNA expression of DNase II are found in most tissues[10].

MATERIALS AND METHODS

This study was conducted on a cohort of 60 children with Acute Lymphoblastic Leukemia and 30 healthy children to be used as control. These patients were hospitalized at Research Institute for educational laboratories in the city of Medicine of the Ministry of Health. Five milliliter of blood sample were collected and centrifuged at [3000 xg] for 5 min.The resultant serum were separated and stored at [-20] C until used. Serum total protein was estimated by Lowery et al. method[11].AcidDNase activity was determined in serum by a method of Kunitz[12]with some modification, where [0.05] ml of serum added to a mixture consist of 0.75 ml of substrate and 0.75 ml of Tris-HCL buffer.The rate of increase in the absorbency of the sample solution was recorded at 260 nm and 25°C after 1.5 min.

Kinetic Parameters (Km and V max)

- ***Effect of substrate concentration***

Acid DNase enzymatic reaction was carried out under optimum reaction condition using different concentrations calf thymus DNA as a substrate [10,20,30,40,50,60,70,80,90,100 mM], The relationship between each substrate concentration and the enzyme activity was plotted in order to determine the optimum substrate concentration for each enzyme activity. Then the values of Km and Vmax for Acid DNase toward calf thymus DNA were determined using the Lineweaver–Burk plot [the relationship between 1/V versus 1/[S]].

- ***Effect of the pH***

The enzymatic reaction solution was carried out using buffers with different pH [4 ,4.5, 5, 5.5 , 6.0 ,6.5]for Acid DNase. The pH optimum was estimated by plotting the relationship between the enzyme activities versus the pH values.

- ***Effect of the Temperature***

AcidDNase enzymatic reaction was carried out under optimum reaction condition using different temperatures [15 ,20,25,30,35,40,45] . The optimum temperature was estimated by plotting the relationship between the enzyme activities versus the temperature values.

- ***Partial purification of acidDNase by gel filtration chromatography***

Gel filtration chromatography was used to separate serum acidDNase different forms following Murai K's method [13], Sephadex G-75 column (65 × 1.6) cm with bed volume of (120.6) cm3 was used for the separation step. The packing of the column was checked using blue dextran. where the void volume was determined and found to be equal to 45 ml.The sample of serum 2 ml containing approximately 40 mg/ ml

protein was applied into the column. The elution was carried out with the eluent buffer at a flow rate of 20 ml/ hours. Fractions of 5 ml were collected and the presence of protein in these fractions was followed by the measurement of the absorbance at 280 nm. The acidDNase activities and protein concentration were measured in each fraction .

- ***DNA-polyacrylamidegel electrophoresis***

Polyacrylamide gel 7.5% containing 40 µg/ ml DNA was prepared by mixing 7.5 ml of distilled water, 33 ml of Stock buffer (Tris-glycine 0.15 M) pH 8.9, 22.2 ml of Acrylamide solution. The mixture was de-gassed for 15 minutes, then 3.2 ml of Ammonium persulfate solution and 0.1 ml of N,N,N,N,tetramethylenediamine (TEMED) were added to the mixture solution .The mixture was gently mixed and loaded in the gel plates.The gel was allowed to polymerize for about 40 minutes . Pre electrophoresis was carried out at 50 mA and 15 v/cm for 30 min, then of 10 µl of the samples were applied into the wells in the gel . Electrophoresis was continued at 40 mA and 15 v/cm for 3 hours or until the Bromophenol Blue dye reached the gel margin. All statistical analyses in the present study were achieved using SPSS version 15.0 for Windows [Statistical Package for Social Science, Inc]. Expressive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was calculated by Student T-Test. The probability $P< 0.05$ = significant, $P> 0.05$ = non-significant. Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the differences between variables of differentiated groups.

RESULTS

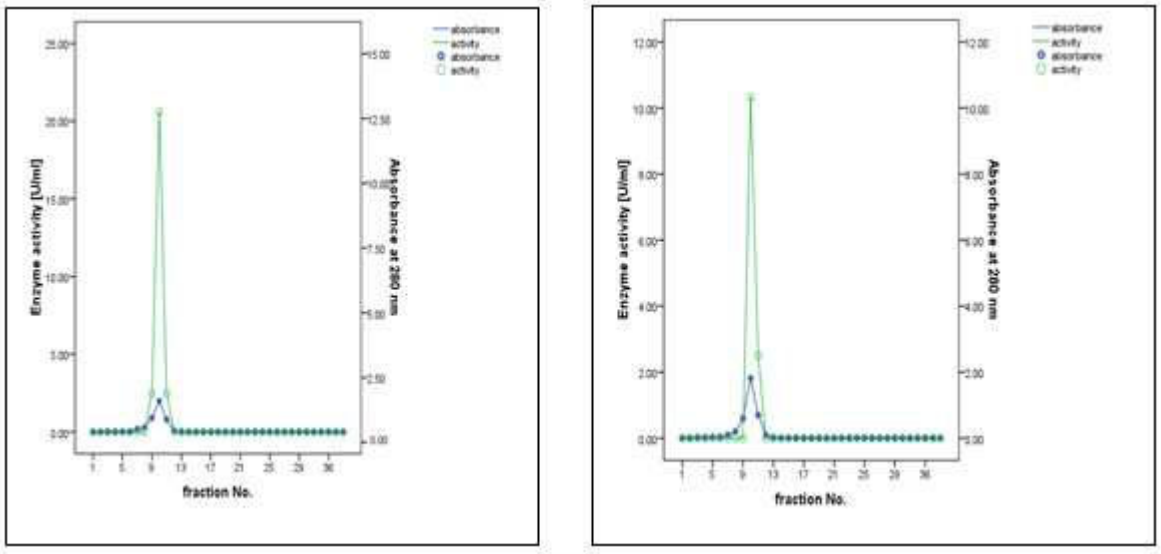
A total of 60 of child with ALL after one month treatment with induction therapy were included in the present study.All patients were matched for age 1-16 years[50% (1-8 year),50%(9-16 year)] and sex was divided into[31 male, 51.67%] and [29 female,48.33%]. The results in table (1) showed a significant increase in both acidDNase activity and specific activity in serum patients group when compared to control group($P<0.001$).

Table 1

Activities and Specific Activities of Sera AcidDNaseof ALL group and control Group.

Characteristic	Patients group[n=60]	Control group [n=30]	P Value
Activities*10^3 [U / L] (mean value ±SD)	97.82±25.10	34.58 ±8.29	<0.001
S. Protein[g/dl]	6.29 ± 1.07	7.00 ± 0.66	<0.01
Specific Activities [U/mg] (mean value ±SD)	1.63±0.52	0.50±0.24	<0.001

Partial purification of serum acid DNase from patients and control samplewere performed by using sephadex G-75 column (1.6 x 65) cm as a matrix. The void volume (Vo) of the column was determined by using blue dextran . The results in figure (1) show the elution profile of the Acid DNase from two groups by gel filtration chromatography.



[1] [2]

Figure 1

Gel filtration profile of acid DNase from serum of [1]patients group,[2] control group. (a) Absorbance at 280 nm, and (b) Acid DNase activity. Gel filtration of acid DNase from serum sample was carried out using sephadex G-75 column (1.6 x 65) cm, flow rate was 20 ml/h, fraction volume was 5 ml, and void volume was 45 ml.

The results obtained from filtration chromatography of control and patientsamples are summarized in table (2).Acid DNase was purified 37.77 fold with a yield of 51.03% for patient sample and purified 50.33 fold with a yield of 62.51 % for control sample.

Table 2

Partial purification of acid DNase in sera of Acute lymphoblastic leukemia using gel filtration chromatography.

Group		Volume [ml]	Activity [U/ ml]	Total activity [U]	Protein [mg/ ml]	S.A [U/mg]	Yield %	Fold of purification
ALL Patients	Crude	2	101.27	202.54	66.50	1.52	100	1
	isolated form	5	20.67	103.35	0.36	57.42	51.03	37.77
Control	Crude	2	41.33	82.66	72.01	0.57	100	1
	isolated form	5	10.33	51.67	0.36	28.69	62.51	50.33

The effect of pH and heat sensitivity of the acid DNase was determined at various pH and temperatures measured its activity as showed in figure (2)

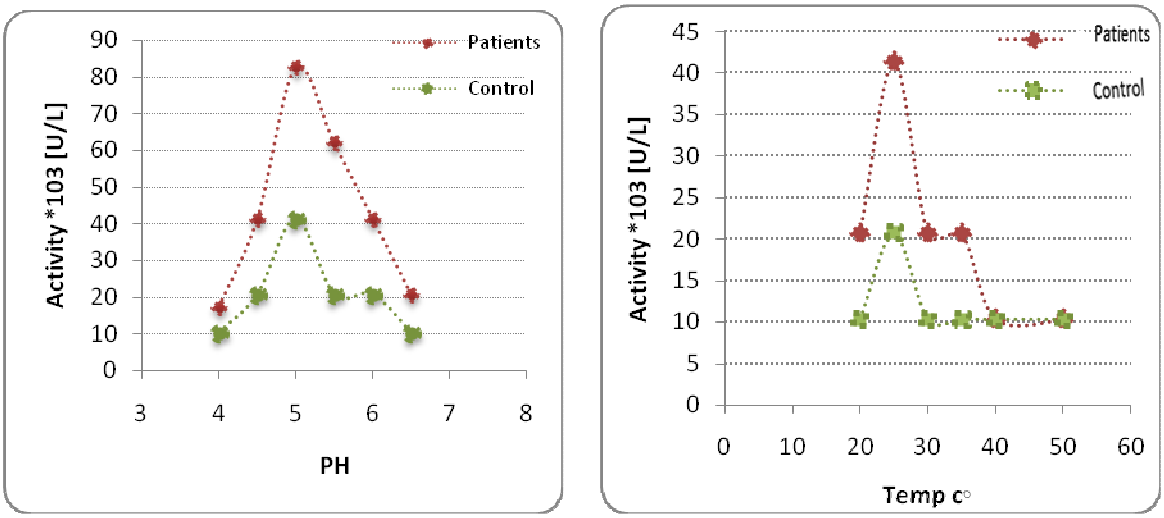


Figure 2
The effect of pH and temperature of the enzymatic activity of Acid DNase in serum of control and patients group

The figure shows that the enzyme was stable at 25ºC, but lost its activity over 30ºC . And the enzyme has the highest effectiveenzymatic at 5pH but lost its activity in high pH (alkaline pH).As it is obvious from figure(3) that DNase activities is inhibited by a high concentration of its substrate..At relatively higher concentrations of substrate, the initial rate of reaction decreases rapidly with increase in substrate concentration. The products of the reaction are also inhibitory.Figure (3) showed acid DNase obey to Michaelis–Menten kinetics. Figure (4) explain the Lineweaver–Burk plot of DNase from control and patient group . These plots were used for determination of Km and Vmax values of the enzyme and the results are shown in table(3)

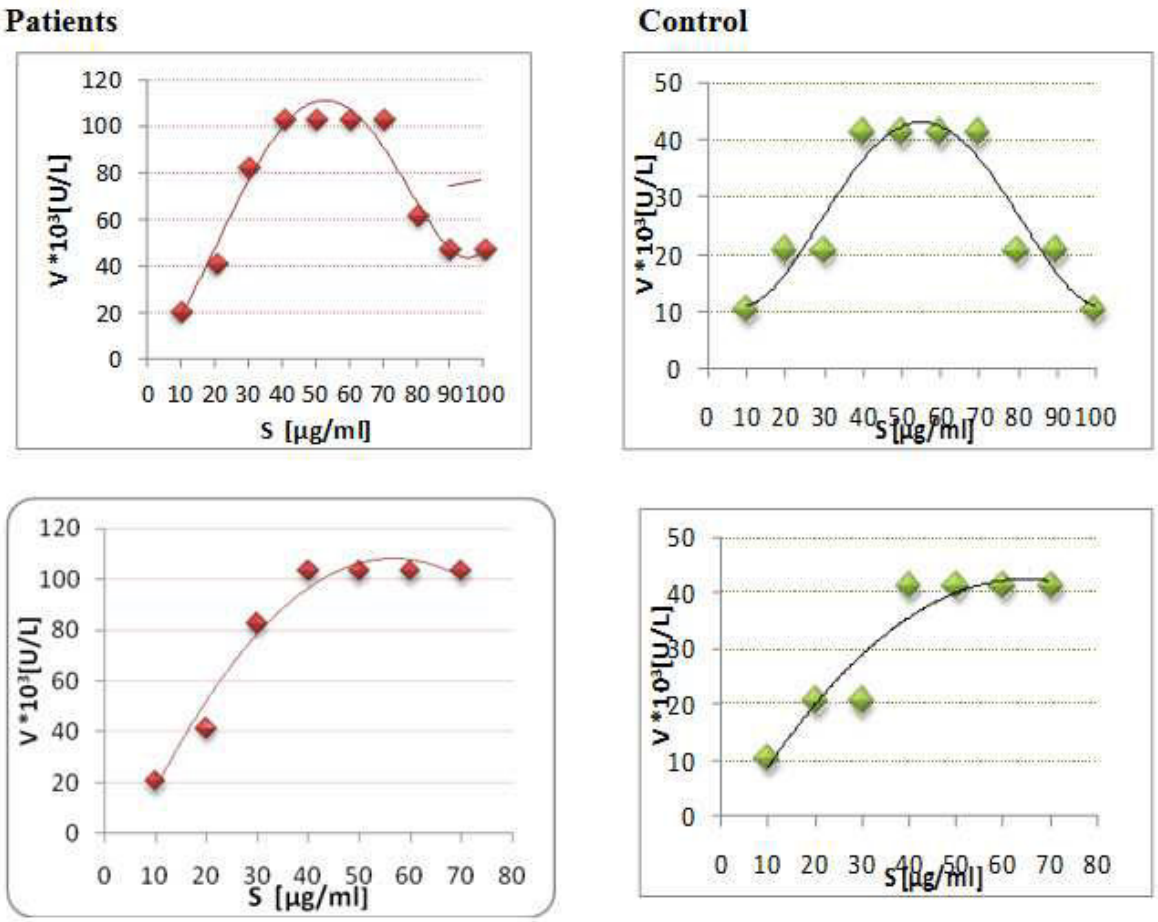


Figure 3
The effect of substrate concentration of the enzymatic activity of Acid DNase in serum of control and patients group.

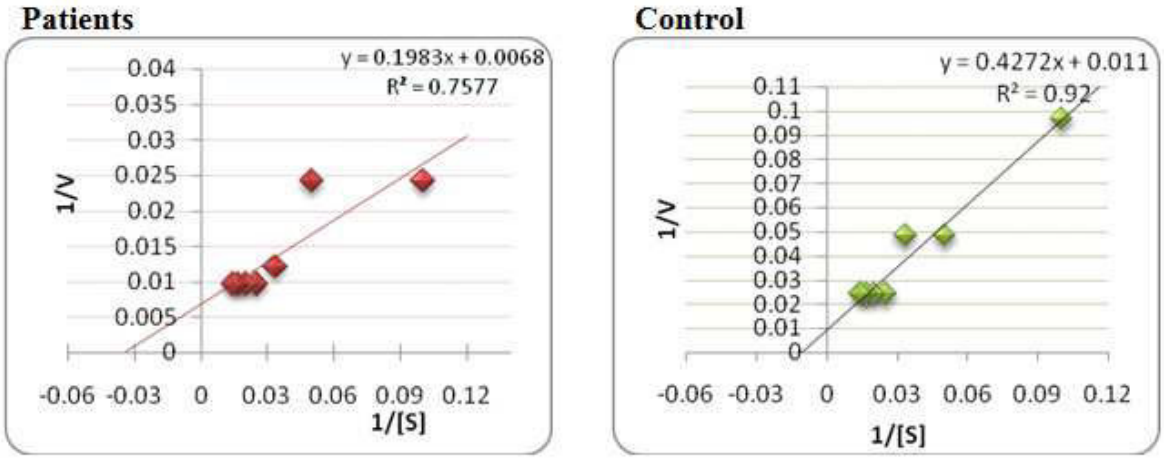


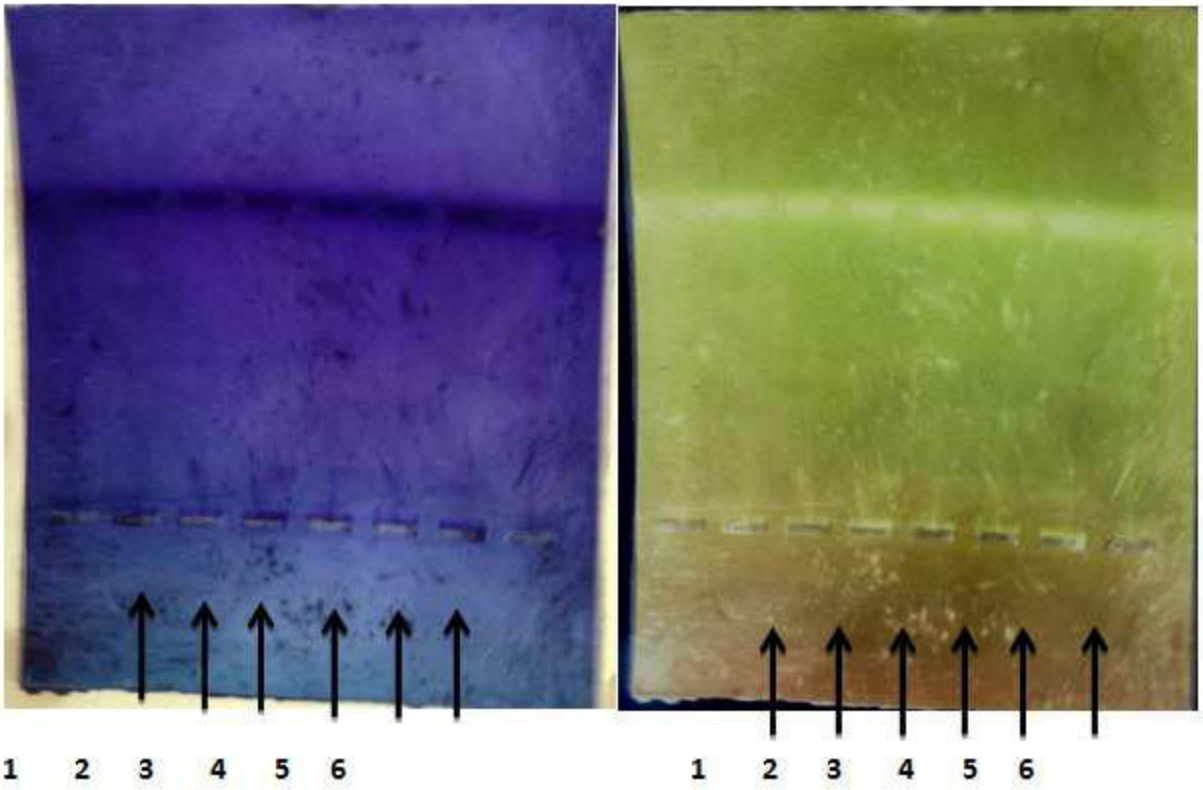
Figure 4
Determination of Km and Vmax for Acid DNase of control and patients group using Lineweaver-Burk plot.

Table 3
Km and Vmax values of partial purified Acid DNase from sera of control, patients group.

Groups	Km value [µg/ml]	V max value
Control	42.72	90.91
Patients	29.16	147.06

The result in table (5) showed that the Km values are decrease upon ALL In contrast in the V max increased with ALL. As our knowledge no previous studies have purified and determine kinetic study of acidDNase in ALL patients .Result in figure(5) showed a single band of acid DNase in both crude serum and purified fraction of patients and control .

Figure 5
DNA-PAGE 7.5% profile of crude serum and partial purified DNase. The gel was stained for Acid DNase activity.(from left to right).



1. *Crude serum patients 4. Crude serum control*
2. *Partial purified DNase patients 5. Partial purified DNase control*
3. *Partial purified DNase patients 6. Partial purified DNase control*

DISCUSSION

Deoxyribonucleases (DNases) produced by micro-organisms are extracellular endonucleases that cleave DNA, productive a high concentration of oligonucleotide[14,15]. Since DNase reactivation appeared to be linked to spontaneous or induced tumor necrosis and retrogression[16],Because endonuclease activation is one of the earliest changes

indicating irreversible commitment to cell death, it is generally believed to be interestedin the triggering of cell death rather than to be the result of it[17].Although DNA cleavage occurs in both self-driven and phagocytic phases, its mechanisms and resulting products are very different. Whereas a variety of executioner (cell-autonomous) nucleases participate in the initial phase of apoptotic cell disassembly, a single nuclease DNase II plays a fundamental role in the phagocytic phase of apoptosis, when cell corpses are taken away by professional phagocytes – macrophages, or neighboring cells [18-19].DNase II is present in lysosomes of phagocytizing cells and is fundamental for the final degradation of the engulfed DNA[20].It was conclusive evidence that DNaseII is a single source of DNA cleavage in engulfed and digested DNA and no other deoxyribonuclease is active during the phagocytic clearance[21].

Thus, it may be proposed that normal cells having lower DNase activity are more predisposed to malignant transformation due to their less active DNA repair mechanism[16].Several investigators noticed increased levels of serum nuclease in cancer patients[22-23]. Enzymes are vulnerable to various environmental factors. Their activity may be significantly diminished or destroyed by a variety of physical or chemical agents resulting in a loss of the functions performed by the enzymes[24]. As it is obvious from figure(3) that DNase activities is inhibited by a high concentration of its substrate. This come to an agreement well with what reported in the literature[25]. Result in figure(5) showed a single band of acid DNase in both crude serum and purified fraction of patients and control .These results are in agreement with the work of Love and Hewitt[26] In conclusion, The difference in serum acid DNase activity may be used as effective indication for monitoring acute lymphoblastic leukemia therapy .Correspondinglythe present result was suggested that serum acidDNase a known circulating tumor marker may be used for cure monitoring of ALL patients. The activity of AcidDNase in serum is useful in predicting treatment response in the long term follow up of patients. It has been suggested that the measurement of acid DNase could be considered as malignant disease markers .Furtherinclusive studies are needed if this situation is important to elucidate in the pathogenesis of the acute leukemia. Enzyme levels may be a useful indicator of response to chemotherapy,Testing this hypothesis will be the basis of the future work.

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