

5'-Nucleotidase activity in sera from Iraqi children with leukemia.

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Summary

Background: 5'-Nucleotidase (5'-NT) is an established plasma membrane marker in many mammalian cells where the enzyme presents as an ectoenzyme and has its active site facing the external medium. The leukemias are the most common form of childhood cancer. They account for about one third of new cases of cancer diagnosed each year. The acute lymphocytic leukemias make up about 76% of cases, with a peak in incidence around the age of 4 yr. Serum 5'-NT activity varies between different children with leukemia. Activities of this enzyme were determined in sera from 25 cases of normal children, 21 children with ALL, 5 children with NHL, 4 children with ANL, and one case with CML.

Results: The results of this study show about 70.9 % different levels of serum 5'NT in 22/31 Iraqi children with leukemia compared with healthy controls. Different level of serum 5'NT were found in 15/21 patients with ALL (71.4%), 4/5 children with NHL (80%) and 3/4 children with ANL (75%). 14.2% of children with ALL have a lower 5'NT activity compared with healthy controls, about 57.1% of patients with ALL have an increased value of serum 5'NT activity and 6/21 children have normal value of serum 5'NT activity (28.5%)

Key word: Serum, 5'- NT and Leukemia.

Introduction

The leukemias are the most common form of children cancer. They account for about one third of new cases of cancer diagnosed each year. The acute lymphocytic leukemias (ALL) make up about 76% of cases, with a peak in incidence around the age of 4 years. Acute nonlymphocytic leukemia (ANL) accounts for about another 20%, with incidence increasing with age into late adulthood. Chronic myelogenous leukemia (CML) and other leukemias difficult to classify account for the remainder. Chronic lymphocytic leukemia (CLL) is essentially never seen in childhood^(1,2,3)

The acute lymphocytic leukemia (ALL) of childhood was the first form of disseminated cancer to respond

completely to chemotherapy. It is, therefore an important model on which concepts of chemotherapy in other malignancies have been developed⁽⁴⁾

The general clinical features of the leukemias are similar since all involve a severe disruption of bone marrow function. Specific clinical and laboratory features differ, however, and there are considerable differences in the responses to therapy and prognosis^(1,5).

A number of cellular functions, such as cellular adhesiveness, contact inhibition of growth and movement, and antigenicity are regulated by the cell plasma membrane. Transformed or malignant cells, aberrant in these biologic characteristics, differ from

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their normal counterparts with respect to structure and composition of their plasma membranes. Changes in the cell surface and the serum during malignant transformation have been established. The content and composition of glycoproteins and glycolipids are affected^(6, 7, 8).

5'-Nucleotidase (5'-NT) is an established plasma membrane marker in many mammalian cells where the enzyme present as an ectoenzyme and has its active site facing the external medium. Its function is the extracellular dephosphorylation of nucleotides, to which cells are generally impermeable, into nucleosides and the transport of these nucleosides through the cells membrane^(9,10).

The aim of the present investigation is to compare the 5'-NT activity in sera from Iraqi children with leukemia and normal children. In the search for a battery of biomarkers useful for diagnostic of this disease as differences may be helpful for this purpose.

Patients and methods:

Children with leukemia were patients of the clinic of pediatric oncohaematology, medical city, Baghdad. ALL children were within the active stage of disease and 1-7 years old.

5'-NT was measured in sera from 25 healthy children 31 children with leukemia (21 cases with acute lymphoblastic leukemia (ALL), five with non-hodgkin's lymphoma (NHL) four with acute nonlymphocytic leukemia (ANLL) and one case with chronic myelocytic leukemia (CML).

Bloods were obtained by venipuncture, and allowed to clot in room temperature. After centrifugation at 1500×g for 15 minutes, the sera were removed from the clots and placed immediately in plastic tubes for storage at -20°C until needed.

5'-NT activity was measured by the method of Wood and Williams (1981)⁽¹¹⁾. Table (1) shows the protocol standard and reagent blank tubes were prepared with each batch. Serum and barbiturate /HCL buffer (0.04 M, pH 7.5 at 37°C) were mixed and incubated at 37°C for 5 minutes, for temperature equilibration. Substrate (10mM of 5'-AMP) was added to start the reaction, which was allowed to proceed for exactly 30 minutes. Nickel chloride (0.1 M) is then added, followed rapidly by SDS (1% conc.), stannous chloride/hydrazine sulfate solution and acid molybdate reagent. This stops the enzyme reaction and develops the color. The contents of the test tubes were well mixed and left for 15 minutes before measuring the absorbance (A) at 618 nm vs. the reagent blank. Degradation of 5'-AMP in the presence of Nickel chloride represents the activity of nonspecific phosphatases which were subtracted from the total activity to obtain true activity of 5'-Nucleotidase.

Table 1: Protocol for 5'NT assay

	Test	control	standard (Vol. mL)	blank
Buffer	0.4	0.4	-	-
NiCl ₂	-	0.05	-	-
Serum	0.05	0.05	-	-
Mix and incubate for 5 minuet at 37°C				
Substrate	0.05	0.05	-	-
Mix and incubate for exactly 30 min. at 37°C				
NiCl ₂	0.05	-	0.05	0.05
Standard	-	-	0.5	-
D.W.	-	-	-	0.5
SDS	3.5	3.5	3.5	3.5
SnCl ₂ /Hydrazine Sulfate	2.0	2.0	2.0	2.0
Acid Molybdate	2.0	2.0	2.0	2.0
Mix well, leave for 15 min and measure absorbance at 618 nm vs. the reagent blank.				

Results:

Individual values of 5'-NT activity in healthy children and children with leukemia are shown in (table2), and a description of the patients from whom sera were obtained are presented in (table3). The activity of 5'-nucleotidase in sera from 25 healthy controls ranged from 2.5 to 11.9 U/L,

children with leukemia had different values of 5'-NT activity compared with healthy children (70.9%). Highest present of 5'-NT activity compared with healthy children was observed in sera from children with non-Hodgkins lymphoma (80%), then in sera from children with acute nonlymphocytic leukemia (75%) and finally in sera from children with acute lymphoblastic leukemia (71.4%). Among the children with ALL the highest differences from the healthy children were found in patients with 5'-NT activity higher than 11.9 U/L (57.1%), (table 4). The serum enzyme activity of these patients with blood cancer was measured in 31 children with leukemia, treated similarly in the clinic of pediatric oncohaematology.

Discussion:

A statistically significant decrease in ecto-5'-nucleotidase activities was observed in peripheral blood lymphocytes and in B and T populations from patients with chronic lymphocytic leukemia, and transiently in the lymphocytes from patients with infectious mononucleosis^(12, 13) Schwartz and Bondansky⁽¹⁴⁾ determined serum 5'-nucleotidase in control groups and in patients with various types of neoplastic disease and found that the activity of this enzyme was elevated in the serum of cancer patients with hepatobiliary disease. Another retrospective study of the changes in level of biological parameters has been carried out in 13 children who underwent one or more bone marrow transplantations in cases of malignancy, all patients developed liver injury characterized by an early and very elevated 5'-NT activity (sometime more than 40 times the

upper references limit). 5'-NT seems to be the best marker for the detection and follow-up of liver disease in this patient group.⁽¹⁵⁾

Gutensohn and Thiel⁽¹⁶⁾ determined ecto-5'-nucleotidase activity in 191 patients (71 children and 120 adults) with acute leukemia and found that elevated values for 5'-NT were registered in common acute lymphoblastic leukemia (ALL), but blast cells of T-cell ALL (T-ALL) and common ALL antigen-negative non-T-ALL had low enzyme activity comparable with the values of acute non-lymphocytic leukemia. Statistical evaluation showed that an interaction between immunologic subtype of the blast cells and their 5'-NT activity had prognostic significance for remission duration. In addition to the independent factor, initial age, this interaction was also prognostic for survival.

The results of the present study show about 70.9% different levels of serum 5'-NT in 22/31 Iraqi children with leukemia compared with healthy controls (table 2,3). Different levels of serum 5'-NT were found in 15/21 patients with ALL (71.4%), 4/5 children with NHL (80 %) and 3/4 children with ANL (75%). 14.2% of children with ALL have a lower 5'-NT activity compared with healthy controls about 57.1% of patients with ALL have an increased value of serum 5'-NT activity and 6/21 children have normal value of serum 5'-NT activity (28.5%), (table 4).

Further studies of this enzyme, with more cases such as purification and characterization, are being done to determine whether 5'-nucleotidase may be a useful marker of leukemia.

Table 2: 5'-Nucleotidase activity in sera from Iraqi children with leukemia and normal children.

5'NTactivity U/L				
ANL	ALL	CML	NHL	Normal Children
49.7	2.25	9.7	15.0	8.7
25.0	16.9		28.7	11.9
1.5	1.8		13.3	9.4
6.5	11.5		1.25	4.02
	12.0		10.2	7.7
	1.0			8.9
	25.0			4.5
	40.0			2.5
	55.0			4.2
	10.4			8.7
	11.7			6.1
	66.0			4.0
	22.5			8.1
	62.1			4.5
	16.9			5.0
	28.2			7.9
	17.2			3.9
	20.3			8.2
	5.3			4.2
	2.5			3.0
	7.8			10.5
				9.1
				8.7
				9.4
				7.0

Table 3: Comparison of the 5'-Nucleotidase activity in sera from healthy children and children with leukemia.

Group	Age range (yr)	No. of cases	5'-NT activity range (U/L)	Difference from control (%)
Normal children (control)	3-6	25	2.5-11.9	
Children with leukemia:	3-7	31	1.0-66.0	70.9 *
ALL	4-7	21	1.0-66.0	71.4
NHL	4-6	5	1.25-28.7	90.0
ANL	4 & 5	4	1.5-49.7	75.0
CML	4	1	9.7	With the N.R

*No. of cases= different from control group /Total no. of cases ×100.

Table 4: Comparison of the 5'-NT activity in sera from healthy children and children with ALL.

Group	Age range (yr.)	No. of cases	5'-NT activity range (U/L)	Difference from control (%)
Control	3-6	25	2.5-11.9	
Children with ALL:	4-7	21	1.0-66.0	
	5-7	3	1.0-2.25	14.2 less than 2.5 U/L
	4-6	12	12.0-66.0	57.1 More than 11.9 U/L
	4-6	6	2.5-11.7	28.5 with N.R.

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فعالية 5'-Nucleotidase في مصول الاطفال المصابين بابيضاض الدم
والاطفال الاصحاء في العراق.

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ملخص:

تمت دراسة فعالية الإنزيم 5'-Nucleotidase في مصول الأطفال المصابين بابيضاض الدم مقارنة بالأطفال الأصحاء حيث تم دراسة 31 حالة منها 21 حالة لمرضى الابيضاض اللمفاوي الحاد (ALL) و5 حالات لمرضى اللمفاوية (NHL) و4 حالات لمرضى ابيضاض الدم اللامفاوي الحاد (ANL) وحالة واحدة لابيضاض الدم النقوي المزمن (CML) مقارنة بـ 25 نموذج للأطفال الاصحاء. أظهرت النتائج تغير في فعالية الإنزيم في مصول الاطفال المصابين بنسبة %70.9 مقارنة بمصول الأطفال الاصحاء.