

# Clinical significance of thymidine kinase in Egyptian children with acute lymphoblastic leukemia

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## Abstract

**Background:** Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, representing one-third of pediatric cancers. Thymidine kinase-1 (TK-1) is expressed in proliferating cells so elevated TK-1 indicates active tumor growth. **Objective:** To study the clinical significance of TK-1 in children with ALL. **Patients and Methods:** This study was carried out on 40 children with newly diagnosed ALL who were admitted to Oncology Unit, Pediatric department, Tanta University (26 males and 14 females) with their ages ranged from 4 to 10 years and 30 healthy children of matched age and sex as a control group. For all patients the following were done: Complete blood picture, bone marrow examination, immunophenotyping and TK-1 serum levels. **Results:** Mean TK-1 level was significantly higher in patients at diagnosis than controls and significantly higher in patients with unfavorable outcome than patients with favorable outcome. Mean TK-1 level was significantly higher in patients in relapse than patients in remission and controls. No significant differences in mean TK-1 level between patients in remission and controls. There were statistically significant differences in disease free survival and overall survival between patients with favorable and unfavorable outcome. **Conclusion:** From this study we concluded that TK is a helpful marker in diagnosis and follow-up of patients with ALL. **Recommendations:** Thymidine kinase-1 should be routinely assessed at diagnosis and during follow-up in ALL patients for better diagnostic and prognostic assessment and should be taken in consideration in designing future therapeutic strategies based on patients-specific risk factors.

**Key words:** Acute lymphoblastic leukemia- childhood malignancies, thymidine kinase I, Risk stratification of ALL

## Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, representing one third of pediatric cancers.<sup>[1]</sup> With the advent of aggressive multimodality therapy, it has become curable disease in over than 80% of patients however, the treatment of ALL results in a significant morbidity and mortality.<sup>[2]</sup> The use of risk-adapted treatment protocols has improved cure rates while limiting the toxicity of therapy.<sup>[3]</sup>

Thymidine kinase (TK) is involved in nucleic acid synthesis and is very low in nonproliferating cells, but increases dramatically at late G1 to late S-phase/early G2 phase during the cell-cycle in proliferating cells and tumor cells. This makes TK-1 an interesting marker for cell proliferation and tumor growth.<sup>[4]</sup>

Thymidine kinase is present in human cells in two major forms, TK-1 and TK-2. During the G1/S transition of normal cells, TK-1 levels increase by 10-20 folds. TK-1 levels remain elevated in the cell until M phase, at which time TK-1 is rapidly degraded. The rate of degradation appears to change in a cell-cycle-dependent manner, resulting in the increased observed levels of TK-1 activity. Cancer cells are known to have lost cell-cycle control of TK-1, which leads to increased levels of TK-1 in these cells and could possibly explain the elevations found in serum. TK-2, the other major TK isozyme, is of mitochondrial origin and its levels are independent of cell-cycle and remain constant in cancer cells and normal cells as well as sera.<sup>[5]</sup>

## Aim of the work

The aim of this work was to study the clinical significance of TK-1 in children with ALL.

## Patients and Methods

This study was done after approval from research Ethical Committee of Tanta University Hospital and informed written

parental consent from parents of included children and was carried out on forty children (26 males and 14 females) with newly diagnosed ALL who were admitted to Oncology Unit, Pediatric Department, Tanta University in the period between December 2011 and May 2014 with their mean age of  $8.15 \pm 1.18$  years (range 2-10 years) and thirty healthy children of matched age and sex; their main age was  $7.75 \pm 1.30$  years (range 2-9 years) and included 23 males and 7 females.

Diagnosis and classification of ALL were made according to French–American–British criteria and immunophenotype analyses. The immunophenotyping was Pre-B (CD19, CD22, CD10-), common ALL (CD19, CD22, CD10), and T-ALL (CD3, CD5, CD7). The clinical data from all patients were obtained, including age at diagnosis, sex, presence of purpura, hepatosplenomegaly, lymphadenopathy, manifestations of central nervous system and testicular infiltration.

## For all patients the following were done

Bone marrow (BM) examination with morphological, cytochemistry and immunophenotypic classification: 1 ml BM and 2 ml venous blood were collected from each patient under complete aseptic conditions in ethylenediaminetetraacetic acid tubes for complete blood count and BM morphologic, cytochemistry and immunophenotyping.<sup>[6]</sup> Follow-up of patients was carried out clinically and by blast count in BM on day 21 after induction chemotherapy which includes: Vincristine 1.5 mg/kg/m<sup>2</sup>/week IV (days 0, 7, 14, 21, 28, 35), doxorubicin 25 mg/m<sup>2</sup>/week IV infusion (days 0, 7, 14, 21, 28, 35), L-Asparaginase 6000 u/m<sup>2</sup> SC on alternate days for 10 doses, and prednisone 40 mg/m<sup>2</sup>/day for 6 weeks orally. On day 21, BM aspiration was done. In nonresponding cases, we add etoposide 100 mg/m<sup>2</sup>/dose IV (days 22, 25, 29), cyclophosphamide 750 mg/m<sup>2</sup>/dose IV infusion (days 22, 25, 29), aracytin 100/m<sup>2</sup>/dose IV (days 22, 25, 29), and high-dose methotrexate 5 g/m<sup>2</sup> over 4 h on day 28.<sup>[7]</sup>

## Measurement of serum thymidine kinase-1 level

Serum samples were measured using radio assay as well as quantitative sandwich enzyme immunoassay (enzyme-linked immunosorbent assay) employing TK-1-specific monoclonal antibody using kits manufactured by CUSABIO BIOTECH Co., Ltd.<sup>[8]</sup>

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**Definition of disease response and relapse**

Complete remission (CR) is defined as a cellularity of >20% with fewer than 5% blasts in the BM after induction chemotherapy.<sup>[9]</sup> Relapse is defined by the appearance of >50% lymphoblasts in a single BM aspirate or >25% lymphoblasts in the BM and 2% or more circulating lymphoblasts or progressive repopulation of lymphoblasts in excess of 5% culminating in >25% on two or more BM samples separated by 1 week or more or leukemic cell infiltration in extramedullary organs as gonads or lymphoblasts in cerebrospinal fluid with cell count >5 white blood cells/mm<sup>3</sup>.<sup>[10]</sup> Favorable prognosis is considered if the patient is maintained in CR during period of follow-up while unfavorable prognosis is considered if the patient died or relapsed during period of follow-up that lasted for 2 years.

**Statistical analysis**

Data were collected and analyzed using Statistical Package for Social Sciences for windows (version 12). All Data were expressed as in terms of mean values ± SD. Comparisons of parameters among groups were made using the paired *t*-test. Two-group comparisons were performed nonparametrically using the Mann–Whitney U-test. All statistical tests were two-tailed, and *P* < 0.05 was considered statistically significant.

**Results**

- The most common presenting clinical manifestations in studied patients were pallor, purpura and fever followed by hepatomegaly, splenomegaly and lymphadenopathy
- There were statistically significant differences between ALL patients and controls as regards platelets, red blood cells (RBCs) and white blood cells (WBCs) with significantly lower platelets and RBCs and significantly higher WBCs in patients than controls
- Mean serum TK-1 levels were significantly higher in ALL patients at time of diagnosis than control and were significantly higher in ALL patients with unfavorable outcome compared to ALL patients with favorable outcome [Table 1]
- Mean TK-1 levels were significantly higher in ALL patients in relapse than ALL patients in remission [Table 1]
- Mean TK-1 levels were significantly higher in ALL patients in relapse than control group [Table 1]
- No significant differences in mean TK-1 levels between ALL patients in remission and control group [Table 1]
- No significant differences in outcome between B-ALL and T-ALL including remission, relapse and death [Table 2]
- There were statistically significant differences in disease free survival (DFS) and overall survival (OAS) between ALL patients with favorable and unfavorable outcome [Figure 1].

**Discussion**

Acute lymphoblastic leukemia is a malignant disorder of lymphoid progenitor cells that proliferate and replace the normal hematopoietic cells of the BM resulting in marked reduction of normal blood cell production.<sup>[11]</sup>

Thymidine kinase is involved in nucleic acid synthesis and is very low in non-proliferating cells, but increases dramatically at late G1 to late S-phase/early G2 phase during the cell-cycle

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in proliferating cells and tumor cells. This makes TK an interesting marker for cell proliferation and tumor growth.<sup>[4]</sup>

This study aimed to study the diagnostic and prognostic value of serum TK-1 in 40 Egyptian children with ALL.

In this study, there were normocytic normochromic anemia, leukocytosis and thrombocytopenia in patients with ALL. This is in agreement with Biswas *et al.* 2009<sup>[12]</sup> who found the same results and explained this by direct result of diffuse and heavy BM and peripheral blood infiltration due to uncontrolled proliferation of lymphoblasts.

In this study, 80% of patients have B-ALL and 20% were T-ALL. This was in agreement with Ahmed and Hassab 2008<sup>[13]</sup> who found that 83.3% of patients were B-ALL and 14.6% were T-ALL. An appreciable shift in the phenotyping toward higher incidence of precursor B lineage has apparently occurred.<sup>[14]</sup>

**Table 1: Comparison between serum thymidine kinase levels in ALL patients at different times during follow-up and control group**

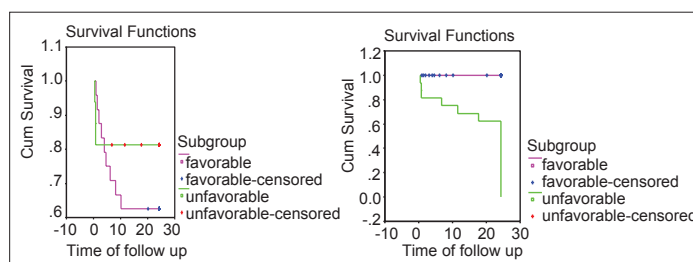
Thymidine kinase 1	Range (mean±SD)	t	P
Patients at diagnosis (n=40)	380-1900 (1091.75±457.34)	7.76	<0.001*
Controls (n=30)	77-313 (142.63±47.36)		
Favorable outcome** (n=24)	380-1280 (773.75±270.18)	14.03	<0.001*
Unfavorable outcome** (n=16)	1300-1900 (1568.75±165.56)		
Patients at remission (n=24)	89-340 (143.62±48.01)	2.53	0.18
Controls (n=30)	77-313 (142.63±47.36)		
Patients at relapse (n=4)	1400-1980 (1657.5±251.97)	13.15	<0.001*
Controls (n=30)	77-313 (142.63±47.36)		
Patients at relapse (n=4)	1400-1980 (1657.5±251.97)	14.03	<0.001*
Patients at remission (n=24)	89-340 (143.62±48.01)		

\*Significant, \*\*Favorable prognosis is considered if the patient is maintained in complete remission during period of follow-up while unfavorable prognosis is considered if the patient died or relapsed during period of follow-up. SD=Standard deviation, ALL=Acute lymphoblastic leukemia

**Table 2: Outcome of studied patients in relation to immunophenotyping**

Immunophenotyping (%)	Patients outcome (n=40)		
	Death (12 cases)	Remission (24 cases)	Relapse (4 cases)
B cell ALL (n=32) (80)	10 (25)	19 (47.5)	3 (7.5)
T cell ALL (n=8) (20)	2 (5)	5 (12.5)	1 (2.5)
Total number=40 (100)	12 (30)	24 (60)	4 (10)
χ <sup>2</sup>	0.840		
P	0.657		

ALL=Acute lymphoblastic leukemia



**Figure 1: Disease free survival and overall survival in favorable and unfavorable cases**

In the current study, TK-1 levels at diagnosis were significantly higher in patients than controls with no significant difference between B-ALL and T-ALL. This is in agreement with O'Neill *et al.*, 2007<sup>[5]</sup> who found the same results. Cancer cells are known to have lost cell-cycle control of TK-1, which leads to increased levels of TK-1 in these cells and could possibly explain the elevations found in serum.<sup>[5]</sup>

There were significantly lower TK-1 remission values than relapse values. This is in agreement with Votava *et al.* 2007<sup>[15]</sup> who studied TK-1 serum levels in 38 children with acute leukemia before the start of the treatment and at least twice during the follow-up and found extremely high-TK-1 serum levels at the time of diagnosis, much lower TK-1 levels in remission and considerably increased TK-1 levels again to pretreatment levels during relapse. They concluded that TK-1 is very good parameter during follow-up because of acceptable sensitivity, low cost, the ability of recognition of relapse as early as 1 month before the appearance of clinical signs and the elimination of requirement for screening of BM samples.<sup>[15]</sup>

Mean TK-1 levels were significantly higher in ALL patients with unfavorable outcome than ALL patients with favorable outcome with statistically significant differences in DFS and OAS between ALL patients with favorable and unfavorable outcome. This is in agreement with Konoplev *et al.* 2010<sup>[16]</sup> who studied serum TK-1 in patients with chronic lymphocytic leukemia (CLL) and concluded that high-serum TK-1 levels have prognostic significance, predict poorer OAS and may be useful in the risk assessment of patients with CLL, but not in agreement with Votava *et al.* 2007<sup>[15]</sup> who found no correlation between TK serum levels at diagnosis and prognosis in their study that included 38 patients with acute leukemia.

In our study there was no significant difference between T- ALL and B-ALL patients as regard outcome and this is not in agreement with Gaynon *et al.* 2010<sup>[17]</sup> who concluded that, with appropriately intensive therapy, children with T-cell ALL have an outcome approaching that of children with B-lineage ALL.

## Conclusion

From the current study we concluded that TK-1 is a helpful marker in diagnosis and follow-up of patients with ALL.

## Recommendations

Thymidine kinase-1 should be routinely assessed at diagnosis and during follow-up in ALL patients for better diagnostic and prognostic assessment and should be taken in consideration in designing future therapeutic strategies based on patients-specific risk factors.

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