Iraqi Journal of Cancer and Medical Genetics

Clinical importance of Thymidine kinase-1 in children with acute lymphoblastic leukemia as a prognostic tumor marker

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is a complex heterogeneous disorder characterized by the proliferation of immature lymphoid cells in the bone marrow, peripheral blood, and other organs. It is the most common form of cancer and predominant subtype of leukemia in children.

Thymidine kinase-1 (TK-1) is a key enzyme in DNA precursor synthesis.Presence of this marker in the cell is an indicator of active cell proliferation, so elevated TK-1 indicates active tumor growth.

Aims of study 1- To evaluate the level of Tk_1 in newly diagnosed pediatric ALL.

2-To correlate the level of TK_1 marker with hematological and clinical parameters.

3-To evaluate the patient's level of TK-1 in serum after induction of chemotherapy.

Patients and methods: This prospective cross sectional study was conducted on 35 pediatric patients with newly diagnosed untreated ALL and 20 non leukemic individuals as a control. Diagnosis was based on morphology ,cytochemistry of the peripheral blood and/or bone marrow aspirate smears by expert hematologist with flow cytometric immunophenotyping test.The patient's peripheral blood and bone marrow samples were re-evaluated morphologically at day 28 from the start of chemotherapy for assessment of complete remission (CR) achievement and for measuring serum Thymidine kinase-1 by ELISA

Results: In this study,the mean TK-1 level was significantly higher in patients at diagnosis than controls.The mean TK-1 level was significantly higher in patients in failure to induction than patients in remission and controls.No significant differences in mean TK-1 level between patients in remission and controls.TK-1 showed a significant positive correlation with total WBC and blast counts and the only significant association was that patients with LAP had significantly higher TK-1 level than those without LAP.

Conclusion:

1-TK-1levels seem to be a very good parameter during follow up, because of:
acceptable sensitivity, low cost and elimination the need of requirements for screening B.M samples.

2-TK-1levels were positively correlated with WBC count and blast%

Key words: *Thymidine kinase-1, lymphoblastic leukemia.*

Introduction:

Acute lymphoblastic leukemia (ALL) is a malignant disorder resulting from expansion of immature lymphoid cells that originates in a single B- or Tlymphocyte progenitor from multistep somatic mutations in a single lymphoid progenitor cell at one of any stages of normal lymphoid development (1).It's the most common pediatric malignancy.Current standards for ALL diagnosis integrate the study of cell morphology,immunophenotype & genetic/cytogenetic as detailed in theWHO classification of lymphoid neoplasm (2)Flowcytometric Immunophenotyping of childhood ALL plays an important role not only in the diagnosis and classification of B and T cell lineages, but also in predicting the outcome (3).

Thymidine kinase (TK) is involved in nucleic acid synthesis and is very low in nonproliferating cells, but increases dramati-

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cally 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 (4)Thymidine kinase is present in human cells in two major forms, TK-1 and TK-2 (6). 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, Its activity has been shown to be correlated with the proliferative activity of tumor cells, Therefore, it may be useful for the early detection of tumor cell division and proliferation (5). 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(6)

Aims of study:

1.To evaluate the level ofTK-1 in serum of the newly diagnosed ALL.

2.To correlate the level of TK-1marker with hematological and clinical parameters.

3.To evaluate patient TK-1 level post induction o.5

Patients and methods:

This prospective cross sectional study include 35 pediatric patients with newly diagnosed ALL as cases and 20 non leukemic individuals as control.From each patient included in this study,3 ml of peripheral blood sample was collected totally,2 ml from this 3 ml and at least 1 ml of bone marrow aspirate were both collected in two ethylene diamine tetra-acytic acid (EDTA) tubes, and examined for blood and bone marrow smears after staining with Leishman stain and special cytochemical stains including Sudan Black B and Periodic Acid Schiff stains.At least, 1 ml from the 3 ml of the aspirated anticoagulated peripheral blood sample or marrow was sent to Flow cytometry unit of the Medical City for confirmation of the diagnosis and sub typing of ALL patients into B- or T- lineage ALL.All patients were re-evaluated for achievement of complete remission(CR)at the day28 of induction phase by morphological evaluation of blood and bone marrow smears, under the supervision of an expert hematopathologist. Two ml of venous blood samples were taken from pediatric patients before chemotherapy and at the day 28 after induction phase, and from control group by vein puncture under aseptic technique collected in a serum separator tube and samples allowed to clot for30 minutes before centrifugation, aliquots stored at -80C 3 months then used for measuring serum TK-1 level by enzyme linked immunosorbent assay(ELISA)

Inclusion criteria

1. All patients were newly diagnosed with ALL(B-&T-ALL).
2. The patients were sequentially collected in relation to sex and age.
3. Age of the patients was less than 15 years old.

Exclusion criteria

1. ALL (L3 subtype)
2. Patients who received steroid or chemotherapy.
3. Patients with other neoplastic diseases.

Results:

The Clinical Characteristics of the Newly diagnosed Patients in this study are listed in table(1)The vast majority of patients were anemic with Leucocytosis and thrombocytopenia.Serum Concentration of TK-1 was much higher than controls with a highly significant difference p value<0.001as shown in (Figure1).TK-1 showed a significant positive correlation with total WBC (p=0.047) and blast% (p=0.020) as shown in table(2). The only significant clinical association was that patients with LAP had significantly higher TK-1 level than those without LAP (p value=0.049). Although male patients, had higher TK-1 than female patients, (p value=0.2) as shown in Table(3). The sensitivity and specificity of the test at cut off values of TK-1= 540.22 pg/ml were 0.80 and 1.0 respectively.Post induction TK-1 Level revealed that failed to induction patients had higher TK-1 level (p value<0.001) than cured patients and controls. Interestingly, there was no significant difference between remission group and controls (p value=0.941)as shown in Figure(2).

Table(1) Hematological characteristics of the newly diagnosed patients

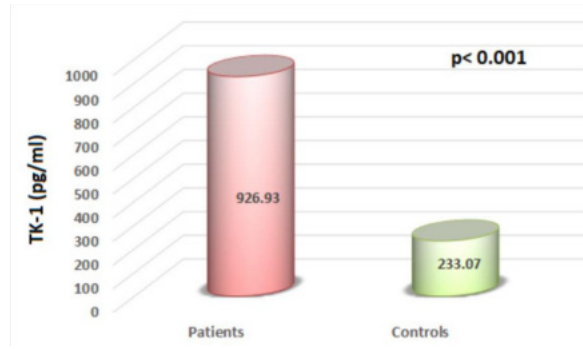
Variables	Frequency	Percentage
Hemoglobin (g/dL) <7 7-11 >7	 4 29 2	 11.43% 82.86% 5.71%
White blood cells x109 /L ≤50 >50	 29 6	 82.86% 17.14%
Platelet x109 /L ≤100 >100	 27 8	 77.14% 22.86%
Immunophenotyping B-ALL T-ALL	 30 5	 85.71% 14.29%

Table (2) correlation between protein TK-1 with other variables in ALL

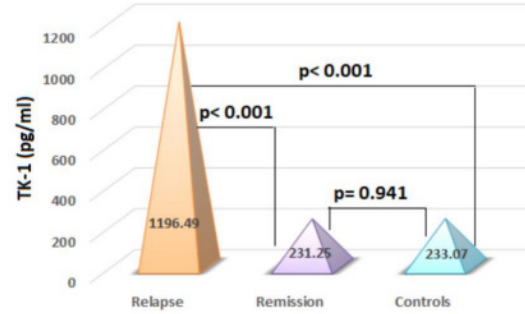
Variables	P-value
Age	0.295
Total leukocyte count	0.047
Platelet	0.692
Hemoglobin	0.582
Blast	0.020

Table(3)Association of TK-1 with clinical features of ALL patients

Variables	Pre induction TK-1(pg/ml)
Anemia Yes(n=21) No(n=14)	 882.25±401.18 940.57±424.57
P-value	0.690
Fever Yes(n=27) No(n=8)	
Fever Yes(n=27) No(n=8)	 909.77±409.65 891,45±463.58
P-value	0.915
Bone pain Yes(n=7) No(n=28)	 1131.36±141.91 849.13±443.16
P-value	0.109
HSM Yes(n=26) No(n=9)	 870.76±422.15 1006.18±418.3
P-value	0.407
Bruising Yes(n=7) No(n=28)	 846.79±436.17 920.29±417.13
P-value	0.682
LAP Yes(n=13) No(n=22)	 1082.17±365.42 801.23±415.31
P-value	0.049
Subtype B-ALL(n=30) T-ALL(n=5)	 855±412.18 910.22±411.21
P-value	0.642



Figure(1) Mean serum concentration of TK-1 in ALL patients and controls (pre-induction)



Figure(2) Mean serum concentration of TK-1 in remission(n=29), failure to induction (n=4) and control groups (post induction).

Discussion:

The Tk-1in newly diagnosed patients were significantly higher than control group with out significant differences between B-ALL&T-ALL,this was in the line of O Neil et al study. (6), cancer cells are known to have lost cellcycle control of TK-1, which leads to increased levels of TK-1 in these cells and could possibly explain the increment found in serum.

There was no significant difference between B and T-ALL as regard outcome, these results in agreement with Raetz E A and Teachey D.T et al (7),Who had found that with recent advances in treatment, including refinement in corticosteroid, asparaginase and CNS-directed therapy, outcomes for T-ALL have improved significantly and now approach those observed in B-lineage disease. The therapy needed to achieve cure is intensive, with risks for acute and late toxicities.But not in the line with Teachey DT et al study (8) who concluded that patients with B-ALL have better outcome than that with T-ALL after appropriate intensive therapy.There were significantly lower Tk-1 values in remission than relapse,this result com-

parable with Votalva et al study (9) ,who concluded that Tk-1 is very good parameter during follow up,because of the ability to recognize the relapsed cases as early as 1month before the occurrence of clinical signs and symptoms and the elimination of requirements for B.M samples screening .

TK-1 was markedly higher in males than females. (10) and there was a strong positive correlation with some un favorable prognostic parameters(Leucocytosis,Blast count and LAP),these results were comparable with Konoplev SN (99) and Hagar AA studies (11)

(who concluded that TK-1 was significantly higher in un favorable than favorable prognosis in pediatric patients with ALL) this is related to the proliferative activity of neoplastic cells as the presence of TK-1 in these cells is associated with DNA synthesis and cell proliferation,other study explained that patients with higher TK-1expression showed significantly increased lymphatic/vascular permeation and lymph node involvement with high stromal invasions.(12)

Conclusion:

1-TK-1levels seem to be a very good parameter during follow up, because of acceptable sensitivity ,low cost and elimination the need of requirements for screening of B.M samples.

3-TK-1 levels were positively correlated with WBC count and blast%

TK-1 Recommendations:

1. TK-1must be assessed routinely in newly diagnosed ALL and during followup for better assessment.
2. It should be taken in consideration in designing future therapy according to patient-risk factors.
3. Further studies with large numbers of patients and longer time of follow-up to determine the relation between TK-1 and LAP.

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