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Thymidine Kinase: A Biomarker for Recently Diagnosed Acute Leukemia in Pediatric Patients According to the Cell Line Involved

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Background and Aims. Acute leukemia (AL) is a heterogeneous group of diseases characterized by a disorganized clone proliferation of hematopoietic cells. Thymidine kinase (TK) is a cell enzyme involved in DNA synthesis and is considered a cellular proliferation marker in some solid tumors.

Methods. A cross-sectional prospective and comparative study was performed in the Federico Gomez Children's Hospital in Mexico (HIMFG, in Spanish) in 125 samples of patients of the HIMFG with AL and 138 samples of children without leukemia. Serum TK levels were determined for both groups.

Results. Of the children with AL, 90 presented B-cell acute lymphoblastic leukemia (B-ALL); 13, T-cell acute lymphoblastic leukemia (T-ALL); and 22, acute myeloid leukemia (AML). A median (m) TK level of 23.7 IU (IQR 17−35.7) was observed in the group without AL and 91 IU (IQR 98−392) in the AL group. This difference was statistically significant ($p < 0.0001$). When analyzing TK levels according to the type of leukemia, the m was as follows: 68 IU (IQR 35−118) for B-ALL, 470 IU (IQR 88−750) for AML, and 1678 IU (IQR 288−2108) for T- ALL.

Conclusion. TK is an enzyme showing heterogeneous levels in B-ALL although it is significantly increased in 90% of patients with T-ALL and AML. © 2015 IMSS. Published by Elsevier Inc.

Key Words: Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Thymidine kinase 1 (TK1), Deoxythymidine triphosphate (dTTP), Reference values (RVs).

Introduction

Acute leukemia (AL) is characterized by an uncontrolled production and accumulation of hematopoietic precursor lymphoid or myeloid cells in the bone marrow (1). Thymidine kinase (TK) is a cellular enzyme from the deoxyribonucleotide kinase enzyme family that is involved in DNA synthesis and essential in the salvage pathway of pyrimidines (2). For a long time, TK was considered to function exclusively in the S-phase DNA replication process in the cell cycle. Nonetheless, further studies showed that TK deficiency in cell lines decreased deoxythymidine triphosphate (dTTP), and this was correlated with an increased damage of the DNA, thus inducing cell death and mutagenesis (3,4).

Biochemical studies show that there are four distinct human deoxyribonucleoside kinases with different subcellular locations. Deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1) are cytosolic enzymes, whereas deoxyguanosine kinase (dGK) and thymidine kinase 2 (TK2) are considered to be located in the mitochondria. cDNAs encoding TK1, dCK, and dGK have been cloned (5). The activity of TK1 is closely related to cell proliferation; thus, multiple mechanisms in the cell cycle regulate its mRNA and protein levels. mRNA and protein levels increase when

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the cells enter the S phase (6). TK1 is expressed during the late G1 phase with an ~20-fold increase in the S-phase, corresponding to the increase in DNA synthesis, decreases in the G2 phase, and develops during mitosis. The positive regulation during the S phase clearly correlates TK1 with cell proliferation (7,8). TK1 comprises 95% of the activity of this enzyme in most normal or pathological situations (9,10). When cell proliferation is absent, TK1 levels are low; nonetheless, in proliferating cells and tumor cells, these levels increase dramatically in the G1 phase, at the end of the late S phase, and at the beginning of the G2 phase of the cell cycle (11).

A correlation between the disease stage, repeated relapses, remissions and the serum TK1 level in patients with different types of cancer has been established (12). TK1 measurement in tumor tissue indicates the growth rate of the tumor cells, possibly revealing the prognosis of hematologic malignancies, brain tumors, colorectal cancer, prostate cancer, and breast cancer (13,14). Serum TK activity in patients with solid tumors may reveal the proliferative activity of the tumor (15−17).

Since the discovery of serum TK1, its levels have been studied in a variety of neoplasms including the diagnosis and classification of tumors and the characteristics of cell proliferation before treatment in several hematological malignancies. This marker has been used to assess the response to treatment and to monitor the remission or relapse of the disease. TK has been described as an unfavorable marker in patients with ALL and AML. Thus, its determination might benefit patient follow-up (12−18).

Considering the heterogeneity of AL, we proposed a cross-sectional prospective study to compare the levels of TK1 in patients with AL according to the cell line involved.

Materials and Methods

From October 2009−December 2011, patients who were newly diagnosed with AL and previously untreated were seen in a tertiary care hospital (HIMFG). Relatives signed informed consent forms at the start of treatment. All patients were submitted to the 17th article of the General Law of Health.

Characteristics of the Children

In total, 125 samples from patients diagnosed with AL and without previous treatment from the Federico Gomez Children's Hospital in Mexico (HIMFG, in Spanish) were included in the study. Additionally, 138 samples of school-age children seeking a general examination as part of the medical certificate for school and without clinical signs of the disease were included. A general medical examination was performed, and a blood sample was taken to analyze the complete blood count and the blood chemistry. Laboratory results were within the reference values (RVs).

Classification of ALs

In this study we considered, in the group of standard risk patients, >1 year old and <10 years old and white blood cells (WBC) < 50,000/μL. AL was diagnosed by bone marrow aspiration. The immunological phenotype was determined with flow cytometry to identify the cell line involved. Fluorophores and antigens from Becton Dickinson Biosciences were used. Leukemias were classified according to their lineage as follows: B-cell acute lymphoblastic leukemia (B-ALL), T-cell acute lymphoblastic leukemia (T-ALL), and acute myeloid leukemia (AML). Based on the WHO recommendations, the following markers were used: for T-ALL: CD3, CD5, and/or CD7; for the B-ALL cell antigen: CD10, CD19, CD20, and CD22; and for AML: CD 33, MPO, and CD13. We identified molecular mutations using Real Time PCR assays with the TIBMolBiol® probes to four different translocations: t(9;22), t(8;21), t(15;17) and Inv16.

TK Quantification

TK1 levels were quantified from the serum obtained by centrifuging 1 ml of blood at 3500 rpm using the LIAISON® equipment, in both the patients with AL and the children without leukemia. A two-step indirect immunoassay modified by chemiluminescence was used. The principle of the trial consisted of using an initial enzymatic reaction where TK1 converts AZT (3′-azido-3′-deoxythymidine) to AZTMP (3′-azido-3′-deoxythymidine monophosphate). The quantity of TK1 in the sample is determined by the amount of AZT converted to AZRMP. For the assay, 50 μl of the sample was incubated with 100 μl of assay buffer 1, 20 μl of assay buffer 2, and 20 μl of paramagnetic particles coated with anti-AZTMP polyclonal antibodies. The solid phase is coated with rabbit anti-goat IgG and later with goat anti-AZTMP polyclonal antibodies. The product is incubated for 40 min and with the further addition of 100 μl of a tracer, a conjugated AZTMP analogue with an isoluminol by-product. The AZTMP links to the solid phase on the first incubation. In the second incubation, the tracer by-product competes to attach with the AZTMP solution. After a 20-min incubation, the free material is eliminated. The light signal is measured in relative lighting units with a photomultiplier and is proportional to the concentration of TK1 in the sample.

Statistical Analysis

The distribution of serum TK was tested for normal distribution using skewness and kurtosis. Demographic data and serum TK values according to the groups of studies were analyzed using descriptive statistics; median and interquartile ranges (IQR) were used and the differences were determined with a Mann-Whitney U test. Kruskal−Wallis