

BRIEF  
COMMUNICATION

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## Evaluation of Wilms' Tumor (WT1) Gene Methylation in Acute Lymphocytic Leukemia Patients

Fatemeh Alimohammadi<sup>1</sup>, Shaban Alizadeh<sup>1</sup>, Sasan Ghaffari<sup>1,2</sup>, Fatemeh Nadali<sup>1</sup>,  
Mehdi Azad<sup>\*3</sup>

### A B S T R A C T

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1. Department of Hematology, Faculty of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Students Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Medical laboratory sciences, Faculty of Allied Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.

**\*Corresponding Authors:**

Mehdi Azad, PhD

Qazvin, Shahid Bahonar Boulevard, University of Medical Sciences, Faculty of Allied Medicine (Hematology), Department of Medical laboratory sciences.

Tel: (+98)2833359501

Fax: (+98)2833338034

Fax: 3419759811

Email: haematologicca@gmail.com

**Background:** Leukemia is the most common type of childhood cancer, with acute lymphoblastic leukemia being the most common subtype among children. Epigenetic factors, such as DNA methylation, are found to be important in leukemia. According to the National Cancer Institute, Wilms' tumor (WT1) is among the most important tumor antigens. This study examines WT1 gene methylation as a diagnostic or therapeutic method in patients afflicted by ALL.

**Methods:** 37 ALL patients were enrolled in this study before initiation of treatment. Twelve healthy subjects were used as the control group. After DNA extraction and conversion with bisulfite, WT1 gene methylation in samples was analyzed via the MSP technique.

**Results:** The WT1 gene promoter was not methylated in patients or the control group.

**Conclusion:** Due to the absence of WT1 gene promoter methylation in patients, as well as the healthy group, it is likely that methylation of the promoter of this gene is of no consequence in leukemia development, and subsequently cannot aid in its diagnosis.

**Keywords:** acute lymphoblastic leukemia, WT1 gene, DNA methylation, epigenetics

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**INTRODUCTION:**

**A**cute lymphoblastic leukemia (ALL) is the most prevalent leukemia among children. Two age groups are at high risk for the development of this disease. The first comprises patients between the ages of 2-4 years, with prevalence dropping as age progresses towards adolescence and adulthood, and the second peak is observed at the ages of 50 or 65 years<sup>1-2</sup>. New information indicates that cancer is caused by both genetic and epigenetic factors. Unlike genetic changes, which are permanent, epigenetic changes are reversible. In other words, they can be identified and targeted via therapeutic agents designed to alter aberrant epigenetic events. Epigenetic changes can be inherited through mitosis<sup>3</sup>. Current researches implicate epigenetic changes in ALL, and DNA methylation is the most common example of this in leukemia<sup>4, 5</sup>.

The WT1 gene, which translates to a transcription factor, has a critical role in organ development and tissue maintenance, and also acts as an epigenetic regulator. Depending on the type of tumor, the WT1 protein may act as a tumor suppressor or a survival factor<sup>6</sup>. WT1 expression not only increases in leukemic blast cells in patients with AML, ALL, MDS, and CML but is also overexpressed in a variety of solid tumors. However, the expression of WT1 in blood CD34+ progenitor cells and normal blood cells is low or absent<sup>7, 8</sup>. This fact draws attention to WT1 and its epigenetic changes as a possible therapeutic and/or diagnostic target.

In this study, WT1 gene promoter methylation in ALL patients was examined in order to assess the importance of this factor in the diagnosis or therapy of these patients.

**METHODS:**

**The Subjects:**

This was a case-control study undertaken in Shariati hospital of Tehran, Iran. Thirty-seven patients, who were selected before the beginning of treatment, entered our study after obtaining written informed consent. Nine T-ALL patients (24.32%), 5 adults and 4 children; 2 B-ALL patients (5.41%); and 26 Pre-B ALL patients (70.27%), 13 children and 13 adults, comprised the patient group. The mean age of adults and children was 33 and 7, respectively. Also, 12 healthy subjects were included in the control group. The age and gender of patients and controls were matched. Peripheral blood samples, flow cytometry, cytogenetic tests and bone marrow cells' morphology were used for including or excluding patients and controls.

**DNA Extraction**

The DNA of patients' peripheral blood and bone marrow cells and that of healthy donors was extracted following protocol (GeneALL, Korea). DNA concentration and purity were ultimately measured using a Nanodrop device. Extracted DNA was treated with sodium bisulfite using the EpiTect Bisulfite Kit (Qiagen, USA).

**Methylation-specific PCR and gel electrophoresis**

After DNA extraction and bisulfite treatment, PCR (Eppendorf, Germany) was performed using two primer sets. One pair was used for methylated DNA (pairs M) and the other for unmethylated DNA (pairs U). Primer sequences were created using the Methprimer program (Table 1). MSP-amplified DNA was immediately separated via 2.5% agarose gel electrophoresis, and products

**Table 1. Primer names and sequences**

Primer Name	Primer sequence
<b><u>WT1-Methylated-Forward</u></b>	5'-TTTGGGTTAAGTTAGGCGTCGTCG-3'
<b><u>WT1- Methylated –Reverse</u></b>	5'-ACACTACTCCTCGTACGACTCCG-3'
<b><u>WT1-Unmethylated- Forward</u></b>	5'-TTTGGGTTAAGTTAGGTGTTGTTG-3'
<b><u>WT1- Unmethylated –Reverse</u></b>	5'-ACACTACTCCTCATACAACCTCCA-3'

were visualized in ultraviolet light.

**RESULTS:**

We found that the WT1 gene promoter of all patients and healthy subjects in this study were unmethylated. Pre-B ALL samples in adults and children failed to show any methylation patterns, and the same was also true for B-ALL and T-ALL samples. As evident by the results, no subjects in the control group showed any

signs of methylation pattern in the WT1 gene promoter (Figure 1). Therefore, the WT1 promoter is not methylated in either leukemic or healthy patients.

**DISCUSSION:**

ALL is a cancer of lymphoid cell lineage, which is mostly prevalent among children. Before the 1970s, the disease was fatal in children, but during the past four decades our knowledge of this disease has expanded,

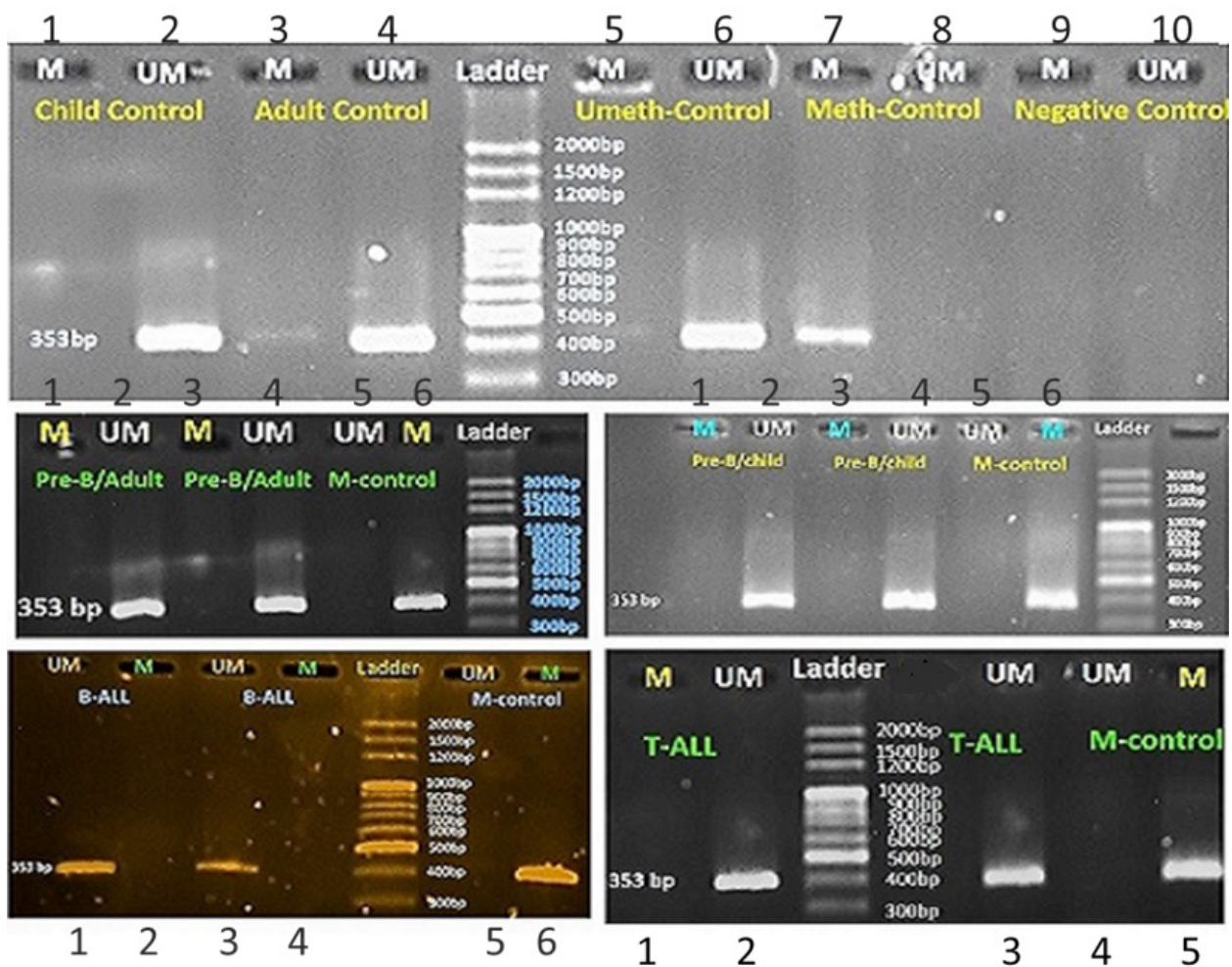


Figure 1. Gel electrophoresis of acute lymphoblastic leukemia (ALL) patients and controls' samples. All 37 patient samples, including B-ALL, T-ALL, and Pre-B ALL, had a 353-bp band under un-methylated, indicating an unmethylated WT1 promoter. Also, the control samples of the 12 healthy subjects were not methylated, meaning WT1 promoter is physiologically unmethylated. Meth-control (methylated primers), umeth-control (un-methylated primers), and negative control prove that the PCR reaction was not contaminated.

resulting in improved ALL treatment. More than 75% of children and 30-35% of adults affected by this disease can now be treated<sup>9</sup>. In the course of examination of the 37 patients and 12 healthy subjects in the control group, the WT1 gene promoter was found to be unmethylated. This meant that there had been no epigenetic change in the promoter region of the studied gene in patients compared to healthy controls. Thus, assessing the methylation of the WT1 gene promoter does not seem suitable for use in patient therapy or diagnosis. All the patients in this study were selected prior to beginning of treatment. In a study in 2009, Busse and colleagues stated that the prognostic significance of WT1 in AML and ALL is still not well known, but that WT1 can be considered as a therapeutic target in the majority of adult patients with ALL as well as AML<sup>10</sup>. According to a study by Loeb et al. in 2001 to investigate WT1 gene methylation in samples of breast cancer and normal tissue, WT1 was strongly expressed in cancer cells<sup>11</sup>. Due to the fact that we did not evaluate the expression of WT1 mRNA and its protein in this study, we cannot reach a conclusion regarding the correlation of unmethylated DNA and the expression level of the WT1 protein.

### CONCLUSION:

Based on our study, methylation of the WT1 gene promoter is potentially not suitable as a diagnostic factor in ALL patients.

### CONFLICTS OF INTEREST:

All the authors declare that they have no competing interests.

### ETHICAL APPROVAL:

The survey was approved by the Ethics Committee of Tehran University of Medical Sciences. The survey was preceded by the completion of written consent forms by all patients.

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