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# Evaluating the expression of *LKB1*, *SHMT1*, and *GLDC* genes in Acute Lymphoblastic Leukemia patients

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**Abstract**

**Background:** Epigenetic changes in cancer cells have an immense effect on tumorigenesis. As a tumor suppressor and an epigenetic regulator, liver kinase B1 (LKB1) reduces gene methylation by downregulating metabolic pathways such as the serine-glycine pathway. This study seeks to examine the gene expression levels of serine hydroxymethyltransferase 1 (SHMT1) and glycine decarboxylase (GLDC), as two serine-glycine pathway regulatory genes, along with LKB1 in acute lymphoblastic leukemia (ALL) patients.

**Materials and Methods:** In this analytical study, qRT-PCR was used to evaluate the gene expression levels of LKB1, SHMT1, and GLDC in 50 ALL patients with an average age of 11.64 ± 10.6 years. The patients were compared to 10 healthy controls. Subsequently, the correlation between the gene expression levels and the patients' demographic data was investigated.

**Results:** No significant difference was found between the ALL patients and the control individuals in terms of LKB1 and GLDC gene expressions, but SHMT1 was significantly overexpressed in the ALL patients ($p = 0.003$). Moreover, there was a significant association between GLDC and the other SHMT1 ($p = 0.020$) and LKB1 ($p = 0.047$). No significant connection was also found between the age ($pL = 0.304$, $pS = 0.305$, $pG = 0.899$), gender ($pL = 0.475$, $pS = 0.299$, $pG = 0.388$), and blast percentage ($pL = 0.335$, $pS = 0.148$, $pG = 0.459$) of the patients and the genes.

**Conclusion:** The increased expression of SHMT1 suggests the oncogenic role of this gene. Thus, the present study offers a novel diagnostic marker in ALL patients.

**Keywords:** Glycine dehydrogenase , Precursor cell lymphoblastic, leukemia-Lymphoma, STK11 protein

## Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous lymphoid leukemia caused by the malignant proliferation of lymphoid progenitors. It has diverse morphological, immunological, and genetic characteristics as well as symptoms such as fever, gum bleeding, bone pain, and distributed lymphadenopathy (1, 2). Various mechanisms are essential for ALL development, and it is known that genetic and epigenetic abnormalities such as noncoding RNA regulation, histone modifications, and DNA methylation are the basis for the etiology of ALL (3, 4).

An epigenetic change commonly observed in ALL is DNA hypermethylation, which results in leukemogenesis through the silencing of tumor suppressor genes (3).

Liver Kinase B1 (LKB1), a serine-threonine kinase and tumor suppressor gene, has a pivotal role in the regulation of various biological functions such as cell growth, survival, polarity, and metabolism (5). It is already known that metabolic changes are often tightly related to epigenetic alteration (6). Serine/glycine biosynthesis is a pathway involved in cancer survival, whose overactivation

promotes tumorigenesis and gives a single-carbon unit for one-carbon metabolism. This process supplies substrates for methylation reactions (7). Interestingly, LKB1 serves as the major regulator of a network that links metabolism to epigenetic alterations. This agent hinders hypermethylation through the restraining of serine metabolism and the SGOC (serine-glycine-one-carbon) network, which can contribute to producing SAM, as a common methylation substrate in epigenetic processes (8). Therefore, LKB1 downregulation induces abnormal methylation, which may lead to genomic instability, oncogene upregulation, or inactivation of tumor suppressor genes, and consequently tumor onset and development (9).

As a principal enzyme in the SGOC metabolism, serine hydroxymethyltransferase (SHMT) takes part in cancer metabolic reprogramming. It facilitates the generation of 5,10-methylentetrahydrofolate by transmitting a one-carbon unit to tetrahydrofolate while catalyzing the reversible conversion of serine into glycine (10, 11). Humans have two SHMT isoforms, namely SHMT1 and SHMT2, which encode cytoplasmic and mitochondrial isozymes, respectively (12). Located on chromosome 17 (17q11.2), SHMT1 provides one-carbon units for methionine, thymidylate, and purine, thereby triggering DNA synthesis and gene methylation (13). Thus, overexpressed SHMT1 can contribute to hypermethylation and ultimately tumorigenesis (9, 13).

As a crucial glycine cleavage system enzyme located on chromosome 9 (9p24.1), glycine decarboxylase (GLDC) is frequently overexpressed and plays key roles in many human cancers (14, 15). This enzyme catalyzes the conversion of glycine to 5,10-methylenetetrahydrofolate, CO2 and NH3 so as to fuel one-carbon metabolism (a pathway providing methylation substrates) (16). So, like SHMT1, GLDC upregulation leads to cell growth and tumor progression via genome hypermethylation (9).

Given the importance of epigenetic changes in the occurrence of ALL and due to the role of LKB1 in epigenetics and gene methylation as well as the increased expression of some metabolic enzymes involved in methylation in various cancers, the present study compares the levels of LKB1, SHMT1, and GLDC gene expression in ALL patients and normal controls.

## Materials and Methods

### 2.1. Study population

Bone marrow specimens were collected from 50 newly diagnosed ALL patients and 10 healthy controls at Taleghani Hospital, Tehran, Iran (17). The patients were diagnosed by flow cytometry and molecular techniques and classified into early pre-B, pre-B, B, and T-ALL. All the participants provided their informed written consent. The ethical code of this analytical study is IR.IAU.PS.REC.1399.053.

### 2.2. RNA extraction and cDNA synthesis

The total RNA was extracted using Trizol reagent (Invitrogen, USA). The quality and quantity of the isolated RNA were evaluated with a NanoDrop spectrophotometer (Thermo Scientific, USA); the OD 260/280 nm ratio was 1.8-2). Subsequently, cDNA was produced according to a synthesis kit (Thermo Scientific, USA).

### 2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

The qRT-PCR was carried out in a total reaction volume of 20 µl, comprising 10 µl of real-time PCR Master Mix (Kawsar Biotech, Iran), 1 µl of forward and reverse primers (10 pmol), 2µl of template target cDNA, and 7 µl of distilled water. During the first step of the qRT-PCR process, the initial holding was set at 95°C for 10

minutes. A thermal cycling program for 40 cycles was run under certain conditions including denaturation (95°C for 15s), annealing (60°C for each of the three target genes for 15s), and extension (72°C for 15s). This was done in the ABI Step One Plus system (USA). For each target gene, a standard curve was plotted with five dilutions of the cDNA samples (0.0001, 0.001, 0.01, 0.1, and 1.0). The assessments were duplicated using a negative control. Table I displays the sequences of the primers used for the qRT-PCR.

### 2.4. Data analysis

The statistical analyses were conducted with the GraphPad Prism and SPSS (version 26) software programs. The relative expression levels of *LKB1*, *SHMT1* and *GLDC* were calculated using the Livak formula (Fold change: $2^{-\Delta\Delta Ct}$), normalized with the ABL gene as the housekeeping gene, and then compared between the patients and the control group. To compare the gene expression to other variables, including gender, age and blast percentage, $2^{-\Delta Ct}$ was calculated for each participant, and an appropriate analysis was performed. The significance level was set at $p < 0.05$.

## Results

### 3.1. Demographic data of the patients

In this study, the samples were obtained from 50 newly diagnosed ALL patients (25 males and 25 females with the mean age was 11.64 years) and 10 controls (5 males and 5 females with an average age of 31.9 years). The patients had an average of 77.88% blast and were categorized as early pre-B ALL (22 out of 50), pre-B ALL (21/50), B ALL (2/50), and T ALL (5/50). More detailed demographic information is provided in Table II.

### 3.2. *LKB1*, *GLDC* and *SHMT1* expression levels in ALL patients and healthy controls

The expression levels of *LKB1*, *GLDC* and *SHMT1* genes were compared between the the patients and the healthy controls using the *ABL* gene as a reference gene and the $2^{-\Delta\Delta Ct}$ formula. No significant differences were found between the two groups in terms of the expression levels of *LKB1* ($p = 0.663$) and *GLDC* ($p = 0.905$). Conversely, *SHMT1* had a significant upregulation in the patients as compared to the controls (Fold change: 3.60, $p = 0.003$) (Fig. 1).

### 3.3. Correlations among the *LKB1*, *GLDC*, and *SHMT1* expression levels

An analysis of the correlations among the genes showed a positive and significant association between *LKB1* and *GLDC* ($p = 0.047$, Fig. 2A) as well as *GLDC* and *SHMT1* ($p = 0.020$, Fig. 2B). However, the correlation between *LKB1* and *SHMT1* was not statistically significant in the patients ($p = 0.433$, Fig. 2C).

### 3.4. Correlations of the *LKB1*, *GLDC*, and *SHMT1* expression levels and age and gender

The patients were categorized into the age groups of < 18 years (78.4%) and >18 years (21.6%). Using the Spearman correlation coefficient, the association between age and *LKB1*, *GLDC* and *SHMT1* gene expression levels was evaluated, but no significant correlation was found between the two parameters (Fig. 3). There was also no significant difference between gender and the expression levels of *LKB1*, *SHMT1* and *GLDC* ($p = 0.475$, 0.299 and 0.388, respectively).

### 3.5. Correlation of the *LKB1*, *GLDC* and *SHMT1* expression levels and the blast percentage

In this study, the blast percentage of the patients ranged from 25% to 98%. The relationship between the *LKB1*, *GLDC*,

and *SHMT1* expression levels and the blast percentage was examined using the Spearman correlation coefficient. As it emerged, there was a positive but non-significant correlation between these factors (p = 0.335, 0.459 and 0.148, respectively) (Fig. 4).

*Table I: Primer sequences used in qRT-PCR*

| gene | Forward (5' to 3') | Reverse (5' to 3') |
|---|---|---|
| **ABL** | TGGAGTAACACTCTAAGCATAA | GATGTAGTTGCTTGGGACCCA |
| **LKB1** | TCTACAACATCACCACGGGTC | TTCGTACTCAAGCATCCCTTTC |
| **SHMT1** | GAACACTGCCATGTGGTG | CTTTGCCCAGTCTTGGGATC |
| **GLDC** | AAACCAGGGAGCAACACATTCG | GCGCCATATTCGCCAAGAGG |

ABL: Abelson murine leukemia viral oncogene homolog
LKB1: Liver kinase B1
SHMT1: Serine hydroxymethyltransferase 1
GLDC: Glycine decarboxylase

*Table II: Demographic data of the ALL patients and healthy individuals*

| variables | patients | Healthy (controls) |
|---|---|---|
| **Age (year; mean ± SD)** | 11.64 ± 10.6 | 31.9 ± 28.83 |
| **Gender** | | |
| **Male (N)** | 25 | 5 |
| **Female (N)** | 25 | 5 |
| **Blast percentage (mean ± SD)** | 77.88 ± 22.39% | < 5% |
| **25-50% (N)** | 6 | None |
| **51-75% (N)** | 8 | None |
| **76-100% (N)** | 36 | None |
| **WHO classification** | | |
| **Early pre-B ALL (N)** | 22 | None |
| **Pre-B ALL (N)** | 21 | None |
| **B ALL (N)** | 2 | None |
| **T ALL (N)** | 5 | None |

N: Number
ALL: Acute lymphoblastic leukemia

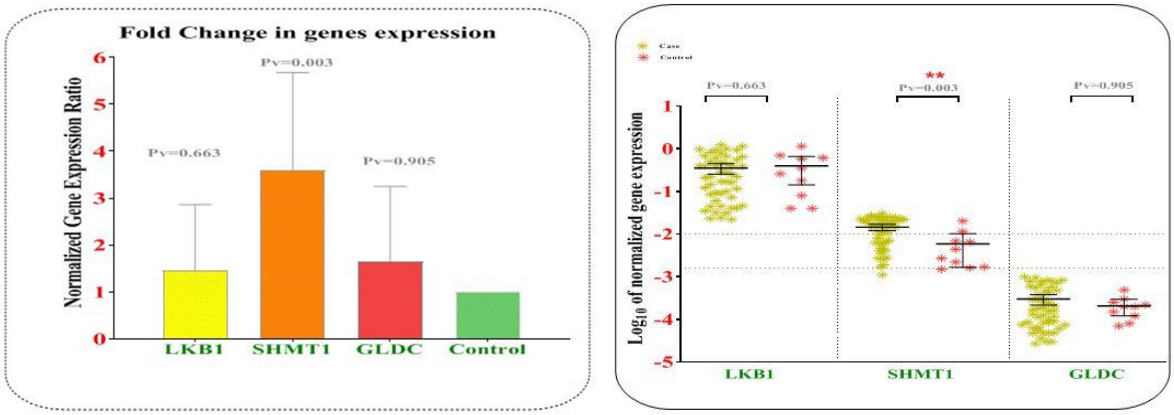


Figure 1. LKB1, GLDC, and SHMT1 expression levels in ALL patients and healthy controls. There were no significant differences in the expression levels of LKB1 (p = 0.663) and GLDC (p = 0.905) between the two groups. Conversely, SHMT1 demonstrated significant upregulation in the patients compared to the controls (Fold change: 3.60, p = 0.003).

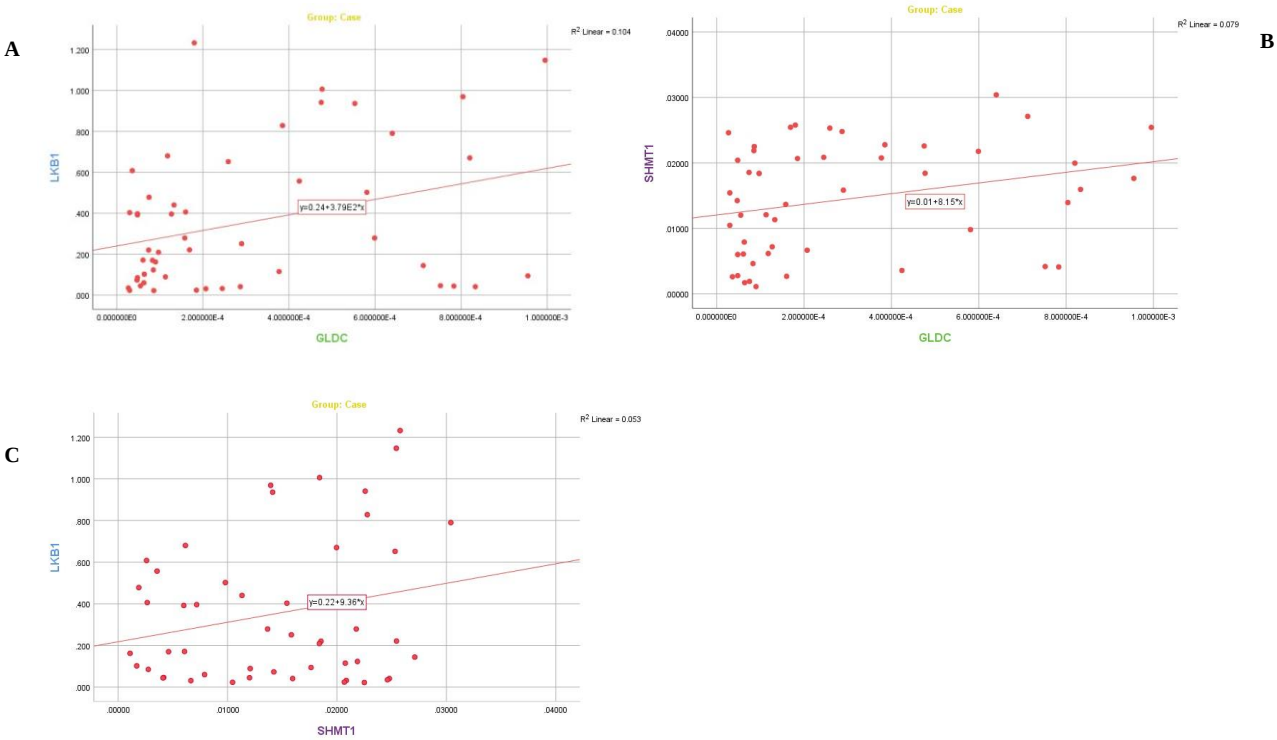


Figure 2. Spearman correlation test results revealed a correlation between the levels of GLDC with LKB1 and SHMT1 in the ALL patients. (A) The correlation analysis for LKB1 and GLDC indicated a positive and significant relationship (p = 0.047). (B) A positive and significant correlation existed between GLDC and SHMT1 (p = 0.020). (C) LKB1 and SHMT1 showed no statistically significant correlation in the patients (p = 0.433).

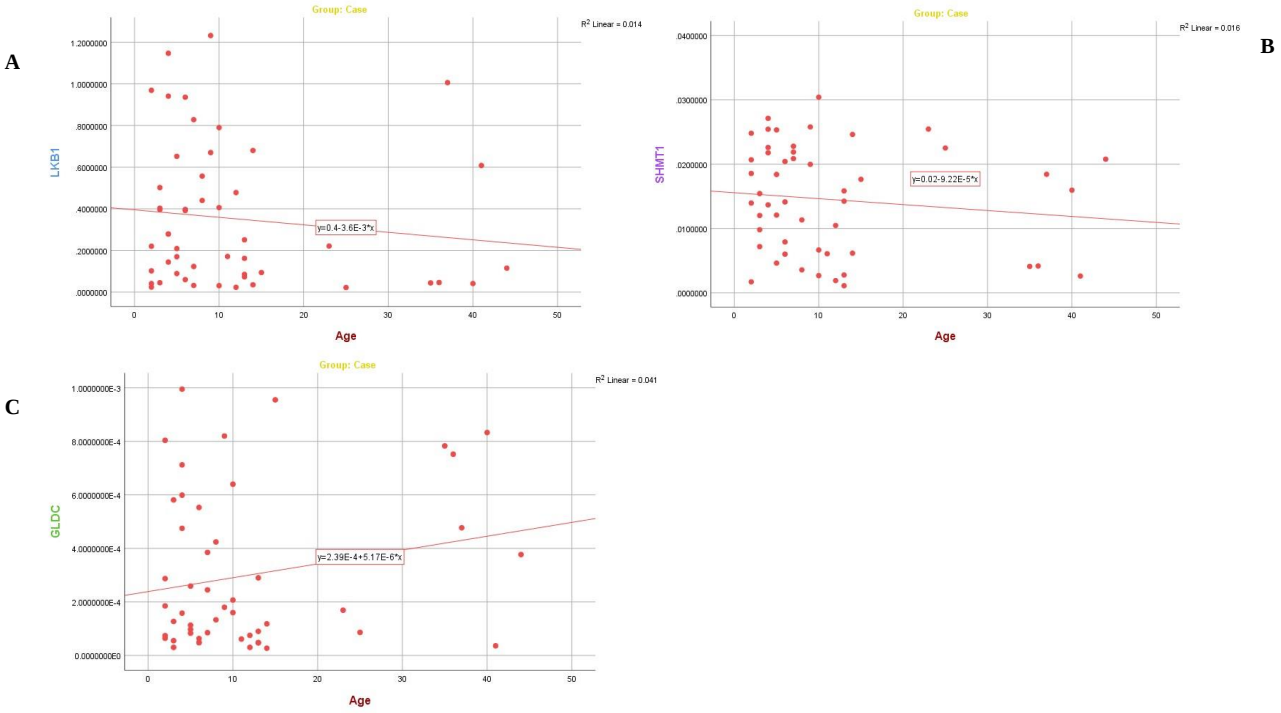


Figure 3. Correlation between LKB1, SHMT1 and GLDC expression levels and the age. LKB1 (A) and SHMT1 (B) expression levels were negatively correlated with the patients' age, but it was not statistically significant (p = 0.304 and 0.305, respectively). (C) There is a positive but non-significant correlation between age and GLDC expression (p = 0.899).

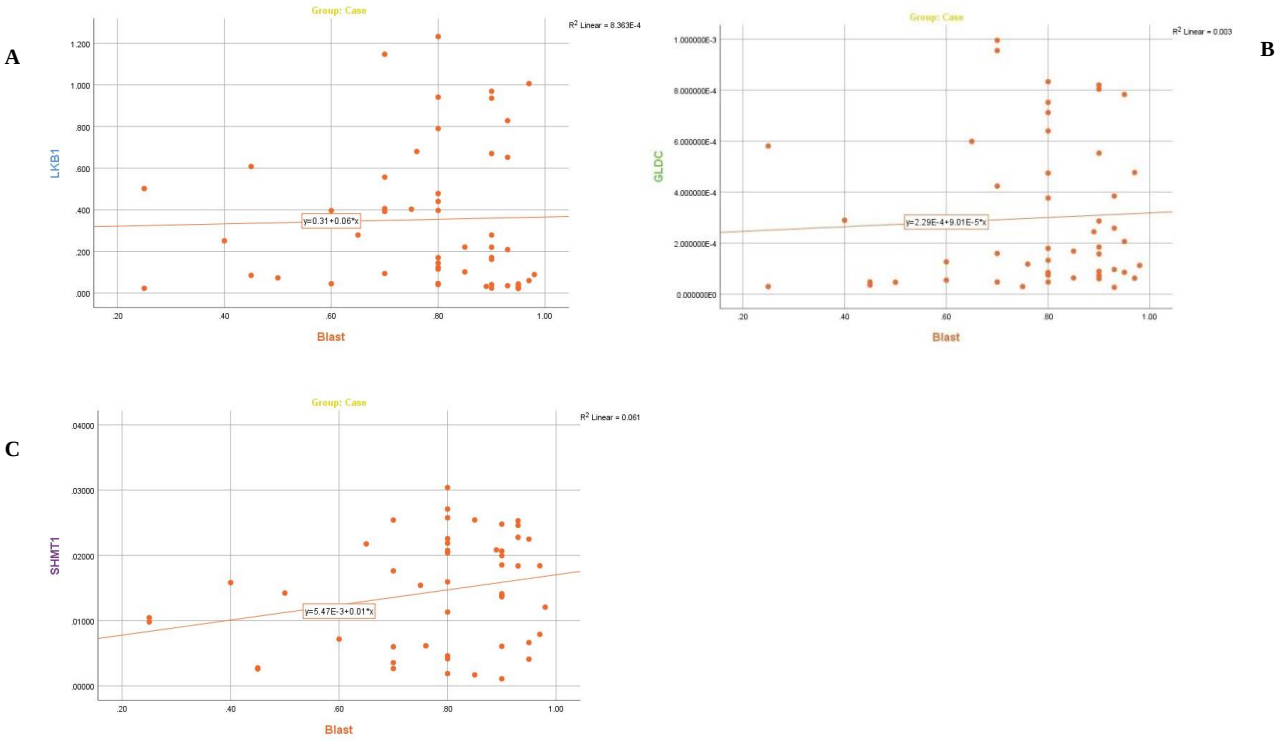


Figure 4. Correlation between LKB1 (A), GLDC (B), and SHMT1 (C) expression levels and blast percentage: There was a positive but non-significant correlation between blast percentage and LKB1, GLDC, and SHMT1 expression levels (p = 0.335, 0.459 and 0.148, respectively).

## Discussion

Genetic and epigenetic alterations have massive impacts on ALL progression. As a frequent epigenetic change in ALL, DNA hypermethylation can contribute to leukemia pathogenesis by inhibiting apoptosis or growth arrest (18). This epigenetic event can be regulated by LKB1, as the key regulator, and metabolic enzymes including SHMT1 and GLDC, as methylation substrate supporters. In the present study, the expression levels of LKB1, SHMT1 and GLDC genes were assessed in the ALL patients.

LKB1 is known as a tumor suppressor with various biological functions (19). In their study, Green et al. (20) demonstrated that LKB1 could play an inhibitory role in AML through the repression of mTOR-mediated mRNA translation. In another study, Ma et al. (21) found that LKB1 expression is low in gastric cancer tissues and its activation may suppress the proliferation of gastric cancer cells by inhibiting the Yap and B-catenin nuclear translocation. A meta-analysis by Lin et al. (22) revealed that lung cancer patients with low LKB1 expression had poor overall survival, suggesting that low expression of LKB1 can unfavorably affect prognosis in lung cancer patients. According to a study by Kou et al. (19), LKB1 exhibits a lower expression in thyroid cancer cell lines and tissues compared to the adjacent normal tissues and thyroid epithelial cells. Furthermore, it was shown that LKB1 overexpression can reverse tumor-favoring properties in thyroid cancer. No significant difference was found in the LKB1 gene expression between the participants, but it is clear that LKB1 has a potential role in tumor suppression, and its downregulation may adversely affect the prognosis and survival of cancer patients.

SHMT1 is a metabolic enzyme with a key role in tumorigenesis. A significant overexpression of SHMT1 was detected in the ALL samples. Consistently, Paone et al. (23) reported that SHMT1 was upregulated in lung cancer cell lines. According to their study, p53-mediated apoptosis and cell cycle arrest occurred after SHMT1 knockdown, suggesting the role of this enzyme in lung cancer. Another survey by Wang et al. (13) showed that SHMT1 polymorphism might increase the risk of non-Hodgkin lymphoma. Also, a previous study on AML samples indicated that SHMT1 gene expression in AML patients is significantly higher than that in healthy individuals (9). Taken together, these studies provide strong evidence for the oncogenic role of SHMT.

GLDC is another metabolic oncogene involved in methylation and tumorigenesis. This study showed a high but not significant expression of GLDC in the patients, which may be due to the evaluation of gene expression in a small population of patients. A study by Zhang et al. (16) demonstrated that GLDC is required for tumor-initiating cell proliferation, growth, cellular transformation, and tumorigenesis in non-small cell lung cancer, and its overexpression correlates with the survival of lung cancer patients. Alptekin et al. (24) reported that GLDC is vital for proliferation and tumorigenesis in MYCN-amplified neuroblastoma cell lines. They also showed that high GLDC expression in neuroblastoma patients is associated with poor prognosis and advanced stages of this disease. According to a study performed by Li et al. (25), both GLDC transcript and protein were elevated in p53-mutated B cell lymphoma tissues, and GLDC could induce p53-positive cells to proliferate. Therefore, the overexpression of GLDC may result in tumor formation and progression.

## Conclusion

In summary, this study demonstrated that the expression of LKB1, GLDC, and SHMT1 genes was altered in the bone marrow samples of ALL patients, but it had no significant correlation with age, gender and blast percentage of the patients. There was also no significant difference between the study and control groups in terms of LKB1 and GLDC gene expression levels, which may be due to the small size of the sample evaluated for gene expression. Adversely, SHMT1 induced a significant upregulation in the patients, suggesting that this molecule may serve as a potential oncogene in ALL.

Because of ethical issues and the reluctance of many individuals to donate bone marrow samples, we had to evaluate the gene expression level in a relatively small population. In case of a larger population, more significant results might have been obtained. The scope of this study was also too limited to investigate gene expression at the protein level. Therefore, for further research, it is recommended that LKB1, SHMT1 and GLDC gene expression be studied in a larger population of patients and that protein levels be examined as well.

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## Authors' contribution

P F: Conceptualization (supporting), data collection and analysis, and writing the original draft. F F: Methodology, review and editing. M N: Methodology, review and editing. M AF: Conceptualization (leading), methodology and supervision.

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## Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported in this paper.

## Ethical approval

This study was approved by the Ethics Committee of the Pharmacology Faculty at the Islamic Azad University of Medical Sciences of Tehran. The study is observable at https://ethics.research.ac.ir/ IR.IAU.PS.REC.1399.053.

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