Differential Expression of *SLC25A38* Gene in Patients of Acute Lymphoblastic Leukemia

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ABSTRACT

SLC25A38 gene produces a protein that belongs to the mitochondrial solute carrier family, SLC25. It is implicated in apoptotic pathways, which regulate intrinsic caspase-dependent apoptosis. The present study evaluates the expression of the *SLC25A38* gene in acute lymphoblastic leukemia (ALL) patients. Among 30 leukemia patients, 25 were adult males and 5 were adult females. An average of 5.3-fold high expression of *SLC25A38* gene among the ALL patients that was normalized to GAPDH relative to normal healthy volunteer was observed. There was a direct positive correlation between blast cell abundance and level of expression ($r = 0.408$, $p = 0.025$). The expression level was found to be associated with the proportion of blast cells in the bone marrow. The present results show that expression of *SLC25A38* is a common feature in ALL and may be a novel biomarker for prognosis and diagnosis, as well as a potential therapeutic target for ALL.

Keywords: Acute lymphoblastic leukemia, Gene expression, Peripheral blood mononucleotide, Solute carrier 25A38.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a disease characterized by recurrent genetic alteration which causes blockage of the proliferation of precursor B and T cells leading to abnormal cell proliferation and survival.¹ This hematological malignancy causes the accumulation of abnormal lymphocytes in the bone marrow and peripheral cells. There is extramedullary leukocytosis of various organs such as lymph nodes, central nervous system, and in the thymus as a mediastinal mass.² Acute lymphoblastic leukemia has bimodal age presentation. It accounts for 15–25% of all adult acute leukemia. Prognosis in older individuals is poor and the overall survival rate is 33–35% in middle-aged adults and about 30% in adults over 60 years of age.^{3–5} Five-year survival rate of ALL is about 90%^{6,7} in children and 75–85% in adults.⁸

Because of advancing molecular and cytogenetic methods in >than 80% of cases of ALL, genetic alteration has been seen. These abnormalities influence the prognostic and therapeutic approach used for the treatment of ALL.⁹ With the advent of advanced molecular technology, it is found that these genetic aberrations include chromosomal translocation, mutations, and aneuploidies in the gene responsible for cell cycle regulation of lymphocytes.¹⁰

SLC 25 is a member of the solute carrier (SLC) family of membrane transport proteins. *SLC25* gene is a nuclear gene that encodes mitochondrial carrier protein, localized in the inner mitochondrial membrane, and functions as a transporter of various nucleotide, cofactor, and inorganic ions.¹¹ This protein has six alpha-helical membrane-spanning domains.¹² They are synthesized in the cytosol and translocated to the mitochondrial inner membrane where it acts as a transporter between mitochondria and cytosol.¹¹ *SLC 5A38* belongs to *SLC 25* gene superfamily which is located on chromosome number 3p22.¹¹

A mitochondrial transporter protein is coded by this gene which functions as a glycine/5-aminolevulinic acid (ALA) transporter across the inner mitochondrial membrane. So, it is required in erythropoiesis.^{13–15} This gene is abundantly expressed in the liver in an embryonic period which suggests its role in hematopoiesis.¹⁶ It

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is also demonstrated that *SLC25A38* is highly expressed in erythroid cells.¹⁷

Therefore, we hypothesized that the *SLC25A38* protein may be significant in the pathological changes associated with leukemia. To further clarify the significance of *SLC25A38* protein expression in ALL, samples were collected from patients with different types of lymphoblastic leukemia, and the abnormal expression of the *SLC25A38* gene was observed.

MATERIALS AND METHODS

Patients and Samples

The patients included in the study were newly case diagnosed with ALL by cytological examination, peripheral blood, and bone marrow aspirates analysis. A total of 30 patients with ALL and 10 healthy control were included in the study. After taking written consent from all the participants, 5 mL blood was taken in an EDTA test tube.

PBMC Separation

2.5 mL of FICOLL Plaque was taken in a 15 mL conical tube 0.5 mL blood was mixed with 5 mL PBS to make a 1:1 mixture. The

blood-PBS mixture was layered gently over the FICOLL Plaque and was centrifuged for 30 minutes at 400 *g* with brakes off. The layer in-between the upper and lower layer was carefully drawn with the help of a pipette. The extracted clear layer was centrifuged, pelleted, and washed with PBS twice. For control, blood of 10 healthy volunteers was pooled and then processed.

RNA Isolation from PBMC

The RNA was extracted from the pelleted PBMC using a QiagenRNeasy kit after counting the number of cells using a hemocytometer. 1×10^6 PBMC cells were shredded with Qia shredder column and then processed according to the protocol with a spin column for RNA isolation. 10 μ L of DNase treatment with 70 μ L RDD buffer was done on the lysate before loading it into the spin column. A260/280 of the extracted RNA was measured on a Nanodrop spectrophotometer and was found to be around 2. The average concentration of all extracted RNA was around 100 ng/ μ L. A260/230 ratio was on average >2.

cDNA Synthesis

All the reaction mix components were kept on ice. Mix, and then briefly centrifuge to collect contents to the bottom of the tube before adding to a 0.2 mL tube. To the tube 4 μ L qScript DNA Super Mix (5 $\times$), Quantabio, USA (final concentration 1 $\times$); 300 ng total RNA and RNase/DNase-free water was added to adjust the total volume of reaction to 20 μ L. After sealing each reaction was vortex gently to mix contents and centrifuged briefly to collect components at the bottom of the reaction tube. The reaction mix was incubated in the thermocycler for 5 minutes at 25°C then 30 minutes at 42°C and finally 5 minutes at 85°C. Thereafter holding the reaction mix at 4°C until use.

PCR Amplification

Amplification of *SLC25A38* and GAPDH was carried out in different wells in the same run. Each run was carried out in triplicate and variation >0.99 Ct was discarded. The amplification protocol of both the genes was the same as follows.

SLC25A38 gene was amplified using forward primer 5'-AAGACGCGCTATGAGAGTGG-3', reverse primer 5'-GTAGTCCATAGTCCATGAGGCAC-3'. Similarly, the GAPDH gene was amplified using forward primer 5'-ACAACCTTGGTATCGTGGAAGG-3', reverse primer 5'-GCCATCAGCCACAGTTTC-3'.

The qPCR reactions were run in a total volume of 25 μ L with 5 μ L cDNA using 12.5 μ L, 2 $\times$ SYBR Green (Applied Biosystems, Thermofisher, USA) 1 μ L of forward and reverse primer (10 μ M) each and rest nuclease-free water. The amplification was performed under the following conditions; initial denaturation 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 59°C for 1 minute. The amplification efficiency of GAPDH as a normalizing gene was evaluated and turn out to be 99.3%. The amplified product was also analyzed on 2% agarose gel which was 197 and 101 bp for *SLC25A38* and GAPDH, respectively (Fig. 1).

Gene Expression Analysis

The *SLC25A38* gene expression in ALL patients normalized to GAPDH relative to normal healthy control was analyzed (Table 1). We used $2^{-\Delta\Delta C_t}$ method¹⁸ for analysis of gene expression as our amplification efficiency was close to 100%.

RESULTS

A total of 30 patients with ALL were enrolled in our study. The mean age of patients was 23 years with a male to female ratio of 2.5:1. Ten normal volunteers were enrolled as the control group. Their age ranged from 20–55 years, with a median value of 26. They were seven males (70%), three females (30%). Other clinical presentations and hematological findings have been listed in Tables 2 and 3.

We observed a mean of 5.3-fold expression of the *SLC25A38* gene normalized to GAPDH relative to normal healthy volunteers among the ALL patients (Table 1). Maximum expression up to sevenfold was observed in some subjects (Fig. 2).

There was a direct positive correlation between blast cell abundance and level of expression ($r = 0.408$, $p = 0.025$), with increasing blast cells the expression of the *SLC25A38* gene increases (Fig. 3).

DISCUSSION

SLC25A38, a solute carrier protein of mitochondria whose role has not been elucidated, is found to be overexpressed in ALL. In the present study, gene for *SLC25A38* has 5.3 times overexpressed normalized to GAPDH relative to healthy volunteers. Chen et al. found a high expression of *SLC25A38* protein in patients of ALL. He also found high expression of this gene in four different cell lines of ALL. Additionally, he also found that it was overexpressed both in adults (15/32, 46.9%) and pediatric patients (7/23, 30.4%).¹⁹ In our study, we also found a positive correlation between blast cell

Fig. 1: The amplified product of GAPDH 101 bp and *SLC25A38* 197 bp lane 2, 3, 4 and 5, 6, 7, 8, respectively

Table 1: The average Ct value of *SLC25A38* gene and GAPDH gene

	<i>SLC25A38</i> Avg Ct	GAPDH Avg Ct	ΔC_t	$\Delta\Delta C_t$	Normalized expression relative to control $2^{-\Delta\Delta C_t}$
ALL patients	27 $\pm$ 0.12	22.7 $\pm$ 0.07	4.3 $\pm$ 0.19	-2.4 $\pm$ 0.19	5.3
Control	30.1 $\pm$ 0.21	23.4 $\pm$ 0.16	6.7 $\pm$ 0.2	0.00 $\pm$ 0.2	1.0

abundance and level of expression (Fig. 3). A possible reason for this may be because a mitochondrial transporter protein is coded by this gene which functions as a glycine/ALA transporter across the inner mitochondrial membrane. So, it is required in erythropoiesis.^{13–15} This gene is abundantly expressed in the liver in the embryonic period which suggests its role in hematopoiesis.¹⁶ It is also demonstrated that *SLC25A38* is highly expressed in erythroid cells.¹⁷ But such high expression in other cells of myeloid and lymphoid origin as indicated by the findings need to be investigated.

In ALL, various genetic alterations are resulting in uncontrolled clonal proliferation of lymphoblast.^{20–23} The outcome of ALL has now been improved due to combinational chemotherapy, but some subtypes have a poor prognosis and many of the patients are

resistant to chemotherapy and some of them experience relapse. Gene inhibitors and micro-RNA regulation give promising results in these situations.^{24–26} Although several studies have been done on sideroblastic anemia and *SLC25A38*, very few studies have been conducted on lymphoid or myeloid cancer. An unusual overexpression in ALL is indicating an underlying mechanism that may be connected to the proliferation of lymphoblastic tumor cells. Since the blast cell abundance was positively correlated to the expression level of protein, estimating the expression level of the gene may be of prognostic value. From our study, it can be hypothesized that overexpression of the *SLC25A38* gene is connected with ALL.

Table 2: Summary of clinical presentation of the 30 ALL patients

Parameter		Number of ALL patients	Percentage
Sex	Male	25	83.3
	Female	5	16.7
Lymphadenopathy	Present	19	63.3
	Absent	11	36.7
Splenomegaly	Present	22	73.3
	Absent	8	26.7
Hepatomegaly	Present	23	76.7
	Absent	7	23.3
Fever	Present	25	83.3
	Absent	5	16.7
Pallor	Present	30	100.0

Table 3: Summary of laboratory data of the 30 ALL patients at diagnosis

Parameters	Median (range)
WBCs $1 \times 10^3/\mu\text{L}$	23.1 (2.7–97.7)
Hemoglobin g/dL	8.7 (4.1–11.4)
Platelets 1×10^3	110 (45–230)
PB blasts%	37 (22–60)
BM blasts%	66 (45–87)

WBCs, white blood cell count; PB, peripheral blood; BM, bone marrow

CONCLUSION

Overexpression of the *SLC25A38* gene is observed in patients of ALL. So, the determination of this gene expression is paving a promising way for gene targeting treatment of ALL. As the sample size is small further studies are required to establish SLC gene expression analysis as a molecular target of ALL treatment and the role of this gene in the regulation of proliferation differentiation and apoptosis of leukemic cells.

Fig. 3: Correlation between the level of *SLC25A38* gene expression and blast cell abundance

Fig. 2: Expression of *SLC25A38* gene normalized to GAPDH relative to healthy control

ETHICAL PERMISSION

This study was approved by the Institutional Review Board Research Ethics Committee of IGIMS, Patna letter no 809/IEC/2019/IGIMS. It has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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