Original Article

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Genetics Section

Differential Expression of SLC25A38 Gene in Patients of Acute Lymphoblastic Leukaemia

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ABSTRACT

Introduction: The SLC25A38 gene produces protein that belongs to the mitochondrial solute carrier family, SLC25. It is implicated in apoptotic pathways, which regulate intrinsic caspase-dependent apoptosis.

Aim: To determine level of expression of SLC25A38 gene in patients of Acute Lymphoblastic Leukaemia (ALL).

Materials and Methods: A cross-sectional study was done in the Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, from April 2019 to March 2020. The study included 30 leukaemia patients out of which 25 were adult males and five were adult females and 10 healthy volunteers were included as the control group. Level of expression of SLC25A38 gene normalised to Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH) gene relative to normal healthy volunteers among the ALL patients was measured using quantitative real time polymerase chain reaction. All data collected were analysed using Statistical Package for the Social Sciences (SPSS) version 16.0 software.

Results: An average of 5.3 fold expression of SLC25A38 gene normalised to GAPDH relative to normal healthy volunteer among the ALL patients was seen. The fold of expression was determined by $2^{-\Delta\Delta Ct}$ method. There was a positive correlation between blast cell abundance and level of SLC25A38 gene expression (Pearson's r=0.408, p=0.025). The expression level was found to be associated with the proportion of blast cells in the bone marrow.

Conclusion: High expression of SLC25A38 gene 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: Gene expression, Peripheral blood mononuclear cell, Quantitative real-time polymerase chain reaction

INTRODUCTION

The Acute Lymphoblastic Leukaemia (ALL) is a disease characterised by recurrent genetic alteration which causes blockage of proliferation of precursor B and T cells leading to abnormal cell proliferation and survival [1]. This haematological malignancy causes accumulation of abnormal lymphocytes in bone marrow and peripheral cells. There is extramedullary leucocytosis of various organ such as lymph nodes, central nervous system and in thymus as mediastinal mass [2]. ALL has bimodal age presentation. It accounts for 15-25% of all adult acute leukaemias. Prognosis in older individual is poor and overall survival rate is 33-35% in middle aged adults and about 30% in adults over sixty years of age [3-5]. Five year survival rate of ALL is about 90% in children [6,7] and 75-85% in adults [8].

Due to advancing molecular and cytogenetic methods, genetic alterations have been seen in more than 80% cases of ALL. These abnormalities influence the prognostic and therapeutic approach used for treatment of ALL [9]. With the advent of advance molecular technology, it is found that these genetic aberration include chromosomal translocation, mutations and aneuploidies in the gene responsible for cell cycle regulation of lymphocytes [10].

The SLC25 is a member of Solute Carrier (SLC) family of membrane transport protein. The SLC25 gene is a nuclear gene which encodes mitochondrial carrier protein, localised in inner mitochondrial membrane and functions as transporter of various nucleotide, co-factor and inorganic ions [11]. This protein has six alpha helical membrane spanning domain [12]. They are synthesised in cytosol and translocated to mitochondrial inner membrane where it acts as a transporter between mitochondria and cytosol. The SLC5A38 belongs to SLC25 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 inner mitochondrial membrane, so, it is required in erythropoiesis [13-15]. This gene is abundantly expressed in liver in embryonic period which suggests its role in haematopoiesis [16]. It is also demonstrated that SLC25A38 is highly expressed in erythroid cells [17]. Though there are very few studies linking the SLC25A38 gene to ALL, an earlier study showed the SLC25A38 protein was over expressed in ALL patients and cancer cell lines [18]. However, the underlying mechanism is largely unknown. This study investigates whether there is a similar expression of SLC25A38 gene in ALL patients.

Objectives

- To measure fold of expression of SLC25A38 gene in patients of ALL relative to healthy volunteers.
- To find a correlation between blast cells abundance in ALL with level of expression of SLC25A38 gene.

MATERIALS AND METHODS

This was a cross-sectional study done in Department of Biochemistry in Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, from April 2019 to March 2020. The patients included in the study were new case diagnosed with ALL by cytological examination, peripheral blood and bone marrow aspirates analysis. Out of 40 patients, 30 patients with ALL and 10 healthy controls were included in the study to compare the gene expression. Patients were taken from Out Patient Department (OPD) of State Cancer Institute IGIMS. This study was approved by Institutional Review Board Research Ethics Committee of IGIMS, Patna, Bihar, India, letter no 809/IEC/IGIMS/2019. It has been performed in accordance with the ethical standards laid down in the1964 Declaration of Helsinki. After taking written consent from all the participants 5 mL blood was taken in an Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainer tube.

Inclusion criteria:

- Newly diagnosed cases of acute lymphatic leukaemia
- Patient age between 18-65 years
- No other co-existing malignancy

Exclusion criteria:

- Patients with sideroblastic anaemia
- Patients with any other form of anaemia
- Patients with any erythroid malignancy

Peripheral Blood Mononuclear Cell (PBMC) Separation

About 2.5 mL of FICOLL Plaque was taken in a 15 mL conical tube. A 5 mL blood was mixed with 5 mL Phosphate Buffered Saline (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 centrifuge, pelleted and washed with PBS twice. For control, blood of 10 healthy volunteers were pooled and then processed.

Ribonucleic Acid (RNA) Isolation from PBMC

About 5 to 10 µg Ribonucleic Acid (RNA) was extracted from the pelleted PBMC using Qiagen RNeasy kit after counting the number of cells using haemocytometer. 1x106 PBM Cells was shredded with Qiashredder column and then processed according to the protocol with spin column for RNA isolation. A 10 µL of DNase treatment with 70 µL RDD buffer was done on the lysate before loading it into the spin column. The RNA was eluted in 50 µL of elution buffer. A 260/280 ratio of the extracted RNA was measured by Nano drop spectrophotometer and was found to be around 2. Average concentration of all extracted RNA was around 100 ng/µL. The 260/230 ratio were on an average greater than 2.

Complementary DNA (cDNA) Synthesis

All the reaction components were kept on ice. They were mixed, and then centrifuged to collect the contents at the bottom of the tube before adding to a 0.2 mL tube. To the tube, 4 µL qScript cDNA Super Mix (5X), Quantabio, USA (final concentration 1X); 300 ng total RNA and RNase/DNase-free water was added to adjust total volume of reaction to 20 µL. After sealing, each reaction was vortexed 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 five minutes at 25°C then 30 minutes at 42°C and finally five minutes at 85°C. Thereafter, the reaction mix was held at 4°C until use.

Polymerase Chain Reaction (PCR) Amplification

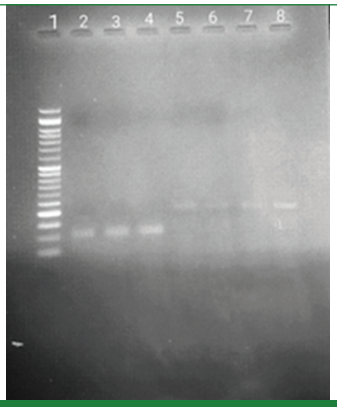
Amplification of SLC25A38 and Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH) were carried out in different well but in the same run. Each run was carried out in triplicate and variation greater than 0.99 Ct was discarded. Amplification protocol of both the genes was the same as follows:

The SLc25A38 and GAPDH gene were amplified using forward and reverse primer [Table/Fig-1].

Primers		Sequence
SLC25A38	Forward primer	5′-AAGACGCGCTATGAGAGTGG-3′
	Reverse primer	5′-GTAGTCCATAGTCCATGAGGCAC-3′
GAPDH	Forward primer	5’-ACAACTTTGGTATCGTGGAAGG-3’
	Reverse primer	5′-GCCATCACGCCACAGTTTC-3′

[Table/Fig-1]: Forward and reverse primer set for SLC25A38 and GAPDH gene.

The qPCR reactions were run in a total volume of 25 µL with 5 µL cDNA using 12.5 µL 2X SYBR Green master mix (Applied Biosystems, Thermofisher, USA), 1 µL of forward and reverse primer (10 µM) each and rest nuclease free water. The amplification was performed under following conditions; initial denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing at 59°C for one minute. Amplification efficiency of GAPDH as a normalising gene was evaluated and it turn out to be 99.3%. The amplified product was also analysed on 2% agarose gel which were 197 bp and 101 bp for SLC25A38 and GAPDH, respectively [Table/Fig-2].



[Table/Fig-2]: The amplified product of GAPDH 101 bp and SLC25A38 197 bp lane 2,3,4 and 5,6,7,8, respectively.

Gene Expression Analysis

The SLC25A38 gene expression in ALL patients normalised to GAPDH relative to normal healthy control was analysed. A $2^{-\Delta\Delta Ct}$ method was used for analysis of gene expression as our amplification efficiency was close to 100% [19].

STATISTICAL ANALYSIS

All data collected were analysed using Statistical Package for the Social Sciences (SPSS) version 16.0 software, (SPSS, Inc., Chicago, IL, USA). Pearson correlation coefficients were calculated between blast cell abundance and level of SLC25A38 gene expression. A two tailed p-value of <0.05 was considered as significant.

RESULTS

Total 30 patients of ALL were enrolled in our study. Mean age of patients was 26 years with male to female ratio of 5:1. Ten healthy volunteers were enrolled as the comparison group. Their age ranged from 20 to 55 years, with a median value of 26 years. There were 7 (70%) males and 3 (30%) females in the control group. Lymphadenopathy was present 19 (63.3%) of patients. Pallor was present in all 30 cases [Table/Fig-3].

Parameters		Number of ALL patients	Percentage (%)
Sex	Male	25	83.33
	Female	5	16.67
Lymphadenopathy	Present	19	63.33
	Absent	11	36.67
Splenomegaly	Present	22	73.33
	Absent	8	26.67
Hepatomegaly	Present	23	76.67
	Absent	7	23.33
Fever	Present	25	83.33
	Absent	5	16.67
Pallor	Present	30	100.0

[Table/Fig-3]: Summary of clinical presentation of the ALL patients. (N=30)

Haematological findings such as White Blood Cell (WBC) count, haemoglobin, platelets, peripheral blood blast cell percentage and bone marrow blast cell percent, range and median value have been listed in [Table/Fig-4].

Parameters	Median (Range)
WBCs 1×10^3/µL	23.1 (2.7-97.7)
Haemoglobin g/dL	8.7 (4.1-11.4)
Platelets 1×10^3/µL	110 (45-230)
Peripheral blood blasts cell (%)	37 (22-60)
Bone marrow blasts cell (%)	66 (45-87)

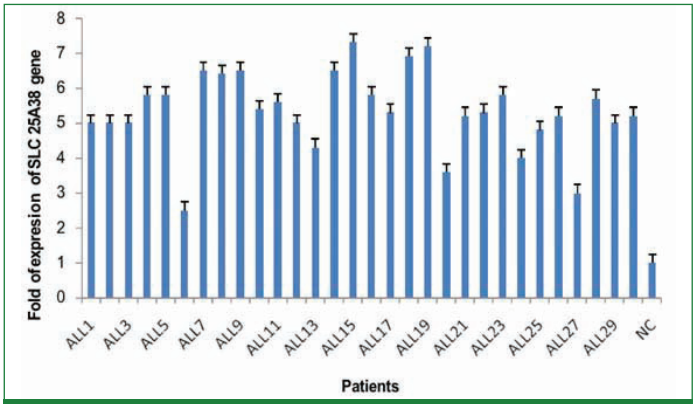
[Table/Fig-4]: Summary of laboratory data of the ALL patients at diagnosis.
WBC: White blood cells

An average of 5.3-fold expression of SLC25A38 gene normalised to GAPDH relative to normal healthy volunteer among the ALL patients was found [Table/Fig-5].

Groups	SLC25A38 Avg Ct	GAPDH Avg Ct	ΔCt	ΔΔCt	Normalised expression relative to control $2^{-\Delta\Delta Ct}$
ALL patients	27±0.12	22.7±0.07	4.3±0.19	2.4±0.19	5.3
Control	30.1±0.21	23.4±0.16	6.7±0.2	0.00±0.2	1

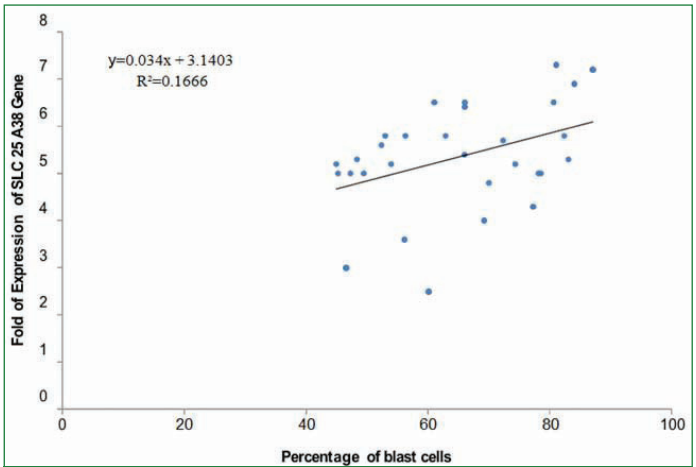
[Table/Fig-5]: Showing the average Ct value of SLC25A38 gene and GAPDH gene.

Level of expression of SLC25A38 gene normalised to GAPDH relative to healthy control is shown for all 30 cases of ALL. In all cases SLC25A38 gene was over expressed [Table/Fig-6].



[Table/Fig-6]: Expression of SLC25A38 gene normalised to GAPDH relative to healthy control.

With increasing blast cells, the expression of SLC25A38 gene increases. There was a weak positive correlation between blast cell abundance and level of expression (Pearson's r=0.408, p=0.025) [Table/Fig-7].



[Table/Fig-7]: Scatter plot showing relationship of SLC25A38 gene with blast cells abundance in ALL patients.

DISCUSSION

The SLC25A38, a solute carrier protein of mitochondria whose role has not been elucidated, is found to be over expressed in ALL. In the present study, gene for SLC25A38 was 5.3 times over expressed normalised to GAPDH relative to healthy volunteers. Chen H et al., found high expression of SLC25A38 protein in patients of ALL. Authors also found high expression of this gene in four different cell line of ALL. Additionally, he also found that it was over expressed both in adults (15/32, 46.9%) and paediatric patients (7/23, 30.4%) [18]. In our study, authors also found positive correlation between blast cell abundance and level of expression. A possible reason for this may be due to the fact that a mitochondrial transporter protein is coded by this gene which functions as a glycine/5-ALA transporter across inner mitochondrial membrane. So, it is required in erythropoiesis [13-15]. This gene is abundantly expressed in liver in embryonic period which suggests its role in haematopoiesis [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 needs to be investigated. In ALL there are various genetic alterations resulting into uncontrolled clonal proliferation of lyphoblast [20-23].

The outcome of ALL has now been improved due to combinational chemotherapy, but some subtypes have 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 a number of studies have been done on sideroblastic anaemia and SLC25A38 but very few studies have been conducted on lymphoid cancer [18]. An unusual overexpression in all is indicating an underlying mechanism that may be connected to proliferation of lymphoblastic tumour cell. 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 this study, it can be hypothesised that over expression of SLC25A38 gene is connected with ALL.

Limitation(s)

Though this study found that over expression of SLC25A38 is indeed seen in ALL patients, the underlying mechanism could not be established. As the sample size is small, further studies are required to establish the absolute association of the gene expression with ALL and other lymphoid lineage malignancies.

CONCLUSION(S)

High expression of SLC25A38 gene was observed in patients of ALL. Also there was a relatively higher abundance of blast in ALL patients that was positively correlated with the level of gene expression. So, determination of this gene expression is paving a promising way for gene targeting treatment of ALL. The SLC25A38 gene expression level can be used in diagnosis and prognosis of ALL. There are very few studies reporting the association of SLC25A38 gene with ALL and more studies are needed to further enhance the understanding of this gene involvement in ALL.

REFERENCES

[1] Belver L, Ferrando A. The genetics and mechanisms of T cell acute lymphoblastic leukaemia. Nat Rev Cancer. 2016;16(8):494-507.
[2] Hales EC, Taub JW, Matherly LH. New insights into Notch1 regulation of thePI3K-AKT-mTOR1 signaling axis: Targeted therapy of γ-secretase inhibitor resistant T-cell acute lymphoblastic leukemia. Cellular Signalling. 2014;26(1):149-61.
[3] Faderl S, O'Brien S, Pui CH, Stock W, Wetzler M, Hoelzer D, et al. Adult acute lymphoblastic leukemia: Concepts and strategies. Cancer. 2010;116(5):1165-76.
[4] Gökbuget N, Kneba M, Raff T, Trautmann H, Bartram CR, Arnold R, et al. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. Blood. 2012;120(9):1868-76.
[5] Korfi K, Smith M, Swan J, Somervaille TC, Dhomen N, Marais R. BIM mediates synergistic killing of B-cell acute lymphoblastic leukemia cells by BCL-2 and MEK inhibitors. Cell Death Dis. 2016;7(4):e2177.
[6] Santiago R, Vairy S, Sinnett D, Krajinovic M, Bittencourt H. Novel therapy for childhood acute lymphoblastic leukemia. Expert Opin Pharmaco ther. 2017;18(11):1081-99.
[7] Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992-2004). Cancer. 2008;112(2):416-32.
[8] Smith MA, Seibel NL, Altekruse SF, Ries LA, Melbert DL, O'Leary M, et al.

Outcomes for children and adolescents with cancer: Challenges for the twenty-first century. J Clin Oncol. 2010;28(15):2625-34.
[9] Genescà E, Jordi R, Josep-Maria R. Acute lymphoblastic leukemia of T progenitors: From biology to clinics. Med Clin. 2015;144(5):223-29.
[10] Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. N Engl J Med. 2015;373(16):1541-52.
[11] Palmieri F, Monné M. Discoveries, metabolic roles and diseases of mitochondrial carriers: A review. Biochim Biophys Acta. 2016;1863(10):2362-78.
[12] Zhang Y, Zhang Y, Sun K, Meng Z, Chen L. The SLC transporter in nutrient and metabolic sensing, regulation, and drug development. J Mol Cell Biol. 2019;11(1):01-13.
[13] Guernsey DL, Jiang H, Campagna DR: Mutations in mitochondrial carrier family gene SLC25A38 cause non syndromic autosomal recessive congenital sideroblastic anemia. Nat Genet. 2009;41(6):651-53.
[14] Bergmann AK, Campagna DR, Mcloughlin EM. Systematic molecular genetic analysis of congenital sideroblastic anemia: Evidence for genetic heterogeneity and identification of novel mutations. Pediatr Blood Cancer. 2010;54(2):273-78.
[15] Kannengiesser C, Sanchez M, Sweeney M, Hetet G, Kerr B, Moran E, et al. Missense SLC25A38 variations play an important role in autosomal recessive inherited sideroblastic anemia. Haematologica. 2011;96(6):808-13.
[16] Zhang H, Zhang YW, Chen Y. Appoptosin is a novel pro-apoptotic protein and mediates cell death in neurodegeneration. J Neurosci. 2012;32(44):15565-76.
[17] Hediger MA, Romero MF, Peng JB. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Pflugers Arch. 2004;447(5):465-68.
[18] Chen H, Lu Q, Zhang Y, Zhang C, Zhang H, Xu H. Overexpression of SLC25A38 protein on acute lymphoblastic leukemia cells. Oncology Letters. 2014;7(5):1422-26.
[19] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta DeltaC(T)) Method. Methods. 2001;25(4):402-08.
[20] Ferrando AA, Look AT. Clinical implications of recurring chromosomal and associated molecular abnormalities in acute lymphoblastic leukemia. Semin Hematol. 2000;37(4):381-95.
[21] Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell. 2002;1(1):75-87.
[22] Soulier J, Clappier E, Cayuela JM, Regnault A, García-Peydró M, Dombret H, et al. HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood. 2005;106(1):274-86.
[23] Ferrando AA, Armstrong SA, Neuberg DS, Sallan SE, Silverman LB, Korsmeyer SJ, et al. Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: Dominance of HOX dysregulation. Blood. 2003;102(1):262-68.
[24] Budhu A, Ji J, Wang XW. The clinical potential of microRNAs. J Hematol Oncol. 2010;3:37.
[25] Fernando TR, Rodriguez-Malave NI, Rao DS. MicroRNAs in B cell development and malignancy. J Hematol Oncol. 2012;5:7.
[26] Whitehead KA, Langer R, Anderson DG. Knocking down barriers: Advances in siRNA delivery. Nat Rev Drug Discov. 2009;8(2):129-38.

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