Original Article

Differential Expression of SLC25A38 Gene in Patients of Acute Lymphoblastic Leukaemia

PRITAM PRAKASH1, SANTOSH KUMAR2, SANJAY KUMAR3, SHRADDHA RAJ4, POONAM SINHA5

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 leukemia 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-ΔΔCt 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.
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 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].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of ALL patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>83.33</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>16.67</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>19</td>
<td>63.33</td>
</tr>
<tr>
<td>Absent</td>
<td>11</td>
<td>36.67</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>22</td>
<td>73.33</td>
</tr>
<tr>
<td>Absent</td>
<td>8</td>
<td>26.67</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>23</td>
<td>76.67</td>
</tr>
<tr>
<td>Absent</td>
<td>7</td>
<td>23.33</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>25</td>
<td>83.33</td>
</tr>
<tr>
<td>Absent</td>
<td>5</td>
<td>16.67</td>
</tr>
<tr>
<td>Pallor</td>
<td>Present</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Gene Expression Analysis
The SLC25A38 gene expression in ALL patients normalised to GAPDH relative to normal healthy control was analysed. A 2^-△△t method was used for analysis of gene expression as our amplification efficiency was close to 100% [19].

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].

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].
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 lymphoblast [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**


