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The outcome of Hematopoietic Stem Cell Transplant in a Patient Suffering from Congenital Dyserythropoietic Anemia Type II in India

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Abstract

Objectives: To study clinical, biochemical, and molecular details of the patient suffering from Congenital Dyserythropoietic Anemia type II (CDAII), treated with a hematopoietic stem cell transplant.

Methods: The 5-year-old patient having severe transfusion-dependent anemia was diagnosed with Congenital Dyserythropoietic Anemia type II (CDAII) based on light microscopic observation of bone marrow aspirate, biochemical tests, and molecular tests using Sanger sequencing. He underwent a Hematopoietic Stem Cell Transplant (HSCT) to treat CDA II. His clinical, biochemical, molecular, and mRNA transcript levels are studied before and after HSCT to understand the effect of HSCT.

Results: The patient had transfusion-dependent severe anemia, pallor, weakness, and icterus. A molecular study identified c.1385 A>G, p.Tyr462Cys homozygous mutation in the *SEC23B* gene causing CDAII. This is the most common type of congenital dyserythropoietic anemia. mRNA transcript levels of *SEC23B* gene were significantly decreased in this patient who received Hematopoietic Stem Cell Transplant from HLA-identical siblings. On follow-up at 18 months after HSCT, he did not require transfusion, other clinical features are normal and mRNA transcript levels are the same as that of healthy controls.

Conclusion: This study highlights the outcome of hematopoietic stem cell transplant on clinical, biochemical, molecular, and SEC23B gene expression levels in a patient suffering from congenital dyserythropoietic anemia type II in India.

Keywords: Congenital Dyserythropoietic Anemia (CDA); Hematopoietic Stem Cell Transplant; *SEC23B* gene expression; Iron overload

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Introduction

Congenital Dyserythropoietic Anemia (CDA) is a group of rare, hereditary disorders characterized by ineffective erythropoiesis, anemia, and iron overload. CDA patients usually show anemia of variable degrees, icterus, hepatosplenomegaly, and distinctive bone marrow features [1]. There are three main types of CDA – CDA I, CDA II, and CDA III. Other rare forms of CDA are also known. The most common type among these is CDA type II. Patients suffering from CDAII show anemia, icterus, pallor, and hepatosplenomegaly. The requirement for blood transfusion is variable. Some severe cases require regular blood transfusions while others require infrequent blood transfusions. The inheritance pattern of CDAII is autosomal recessive and is caused due to mutations in the *SEC23B* gene [2]. It has been reported that mRNA expression levels of *SEC23B* are reduced in CDAII [3]. CDA II is diagnosed mainly based on microscopic observation of bone marrow morphological features, and molecular analysis. According to the literature, the Mean Channel Fluorescence (MCF) in the Eosin-5'-Maleimide (EMA) test used for diagnosis of red cell membrane protein defect is also low or in the grey area for CDAII. This is because of the hypoglycosylation of band 3 protein in the red cell membrane of CDAII patients. The differentiation between hereditary spherocytosis and CDAII can thus be made based on the CD44 antibody binding test [4,5].

The disease is commonly managed with regular blood transfusion and iron chelation. In some cases, the number of transfusions has been reduced using splenectomy [6]. Currently, Hematopoietic

Stem Cell Transplant (HSCT) is known to be the only curative option for CDA [7,8]. Some reports suggest the efficacy of HSCT, but the data from the literature is very rare. There are very few reports of CDAII in the Indian population. Here, we report a case of CDAII having severe anemia, requiring regular blood transfusion, diagnosed with bone marrow analysis, biochemical and molecular tests, and managed with a hematopoietic stem cell transplant. On follow-up, we also evaluated the effect of hematopoietic stem cell transplant on mRNA transcript levels of the *SEC23B* gene in this patient.

Material and Methods

Patient

A 5-year-old male child born of consanguineous marriage complaining of weakness and lethargy, having transfusion-dependent anemia was referred to ICMR- National Institute of Immunohematology, Mumbai, India for a hemolytic anemia workup. Routine hematological studies were carried out by standard protocols. Complete Blood Count (CBC), absolute reticulocyte count, and corrected reticulocyte count were taken. Morphological changes in blood cells were observed using light microscopy. Hemoglobinopathies were ruled out using hemoglobin variant analysis. To rule out red cell membrane protein defect, Eosin-5'-Maleimide Assay (EMA) was performed according to the protocol described earlier using BD FACSAria™ Fusion Flow Cytometer [9]. Red cell enzyme (pyruvate kinase, glucose-6-phosphate dehydrogenase, glucose phosphate isomerase) studies were conducted by the methods recommended by the International Committee for Standardization in Hematology [10]. Serum ferritin level, Lactate Dehydrogenase (LDH) level, liver function test, and renal function test were performed. Bone marrow aspiration and light microscopy to study bone marrow morphology were performed [11]. Allogeneic hematopoietic stem cell transplantation was performed as per the standard protocol. He received Hematopoietic Stem Cell Transplant from an HLA-identical sibling's 15-year-old elder healthy sister, in whom CDA II was ruled out using genetic analysis.

CD44 antibody binding test

The patient showed low Mean Channel Fluorescence (MCF) in the EMA test. Therefore, for the differential diagnosis of hereditary spherocytosis and CDAII, a CD44 antibody binding test was performed. The protocol used was: Whole blood (10 µL) was diluted with Phosphate Buffer Saline (PBS). 30 µL of diluted blood sample and titrated volume of PE- anti-CD44 antibody (BD Biosciences) was incubated in the dark at room temperature for 30 min. To remove unbound antibodies, RBCs were washed with PBS and resuspended in 300 µL, and analyzed on BD FACSAria™ Fusion Flow Cytometer. Results were expressed in Mean Channel Fluorescence (MCF) values as described earlier [4,5].

Molecular analysis of the *SEC23B* gene

The genomic DNA of the patient and parents was isolated using the Flexigene DNA extraction kit. 20 exons of *SEC23B* gene, flanking splice junctions, and 5'- and 3'-untranslated regions were amplified with polymerase chain reactions. The PCR conditions were as follows: 95°C for 5 min; 35 cycles of 94°C for 20 sec, 58°C for 20 sec and 72°C for 20 sec; and 72°C for 5 min. Amplified DNA was purified and sequenced using ABI Prism 3730xl Genetic Analyzer. CHROMAS software v2.6.6 was used for the analysis of sequences.

Real-Time PCR and gene expression study

mRNA was isolated from the peripheral blood sample using the TRIZOL-based standard method. cDNA was made from mRNA using a cDNA synthesis kit (Applied Biosystems) as per the manufacturer's protocol. The PCR conditions for cDNA synthesis were as follows: 25°C for 10 min, 40 cycles of 37°C for 1 h, 37°C for 1 h, and 85°C for 5 min. Quantitative Real-Time PCR (qRT- PCR) was performed according to the manufacturer's protocol. The reactions were run on an Applied Biosystems StepOne™ Real-Time PCR system. The protocol used was as follows: 2 min at 95°C, 10 sec at 95°C, and 20 sec at 60°C for 40 cycles. The stability of a control gene - Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) was determined for the normalization of RT-PCR products. We performed this assay in triplicate. The analysis of data for studying relative mRNA expression of the *SEC23B* gene was carried out using the $2^{-\Delta\Delta Ct}$ method [12].

Results

Clinical details

Before the hematopoietic stem cell transplant, the patient required regular blood transfusions and had recurrent jaundice, pallor, and icterus. The patient's serum ferritin was 2546 ng/mL. Peripheral blood smear showed hypochromic microcytes, anisopoikilocytosis, and few spherocytes. He showed indirect hyperbilirubinemia. Activities of 3 common red blood cell enzymes – Pyruvate Kinase (PK), Glucose-6-Phosphate Dehydrogenase (G6PD), and Glucose Phosphate Isomerase (GPI) were normal. Low Mean Channel Fluorescence (MCF) was observed in Eosin-5'-Maleimide test. Thus, an anti-CD44 antibody binding test was used to rule out CDAII [4,5] (Table 1). The patient showed significantly raised CD44 antibody binding. Bone marrow smear showed marked erythroid hyperplasia and mild dyserythropoiesis. Few binucleate and multinucleate forms (~9%) and occasional karyorrhectic forms.

Allogeneic hematopoietic stem cell transplantation was planned for the patient. The donor was an HLA-matched, 15-year-old elder sister, in whom CDA II was ruled out using genetic analysis. The transplant was successful and the patient was followed up since then. After 1.5 years of transplant, hematological, biochemical, genetic analysis, and gene expression study was performed for the patient. After 18 months of a bone marrow transplant, a peripheral blood smear was normocytic and normochromic. Serum ferritin was 1215 ng/mL. The hematological and other biochemical parameters before and after bone marrow transplant are normal (Table 1). It is suggested

Table 1: Hematological, biochemical and molecular details of patient before and after bone marrow transplant.

	Pre-HSCT	Post-HSCT (1.5 years)
WBCs (× 10^3/uL)	10.8	7.0
RBCs (× 10^6/uL)	1.89	4.63
Hemoglobin (g/dL)	4.3	13.5
HCT (%)	13.8	36.9
MCV (fL)	73	85.7
MCH (pg)	22.8	29.2
MCHC (g/dL)	31.2	34.1
PLT (× 10^3/uL)	263	175
RDW (%)	29.7	12.7
EMA (MFI) (NR: 65000-95000)	61757.46	77060.76
CD44 Binding	Raised	Normal
Sanger Sequencing	c. 1385 A>G Homozygous	c. 1385 A>G Heterozygous
Amino acid Change	p.Tyr462Cys (Homo)	p.Tyr462Cys (Hetero)

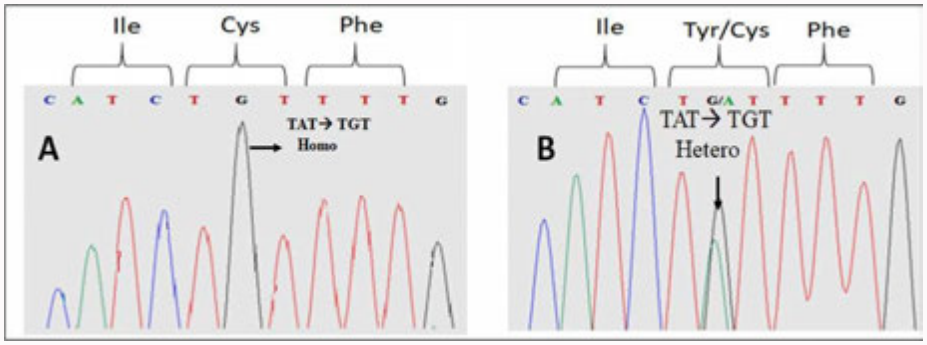

Figure 1: Molecular characterization of exon 12 of SEC23B gene using Sanger sequencing (A) Sanger Sequencing before hematopoietic stem cell transplant of CDA II patient showing c. 1385A→G Homozygous mutation (B) Sanger Sequencing after hematopoietic stem cell transplant showing c. 1385A→G (p.Tyr462Cys) Heterozygous mutation.

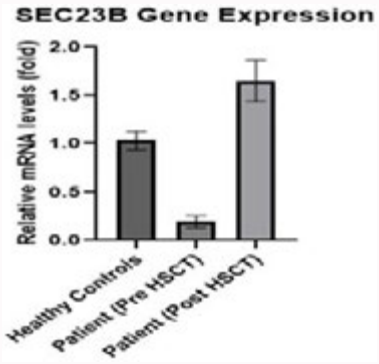

Figure 2: Relative mRNA expression levels of SEC23B gene in the patient before HSCT and after HSCT compared with healthy control.

that allogeneic HSCT may improve the prognosis of the patient.

Molecular analysis of the *SEC23B* gene

The molecular analysis showed missense, homozygous mutation -c.1385 A>G, p.Tyr462Cys in the exon 12 of the *SEC23B* gene. Parents showed heterozygous mutation -c.1385 A>G, p.Tyr462Cys (Figure 1). The prediction of the severity of mutation was performed using bioinformatic tools such as PolyPhen 2.0, SIFT, and PROVEAN. According to all these bioinformatics tools, the mutation is damaging and deleterious. The Sanger sequencing of exon 12 of *SEC23B* gene post allogeneic hematopoietic stem cell transplant showed heterozygous c.1385 A>G, p.Tyr462Cys mutation in the patient (Figure 1).

Real-Time PCR and gene expression analysis

For the evaluation of the consequence of mutations in the *SEC23B* gene, we studied mRNA transcript levels of the *SEC23B*in patient before and after HSCT and healthy individuals using quantitative Real-Time PCR (qRT-PCR). Before HSCT, the patient showed a significant reduction in the mRNA expression level of the *SEC23B* gene ($p<0.05$). Approximately 70% reduction was observed. The mRNA expression level of the *SEC23B* gene of the patient was almost equal to that of a healthy individual post-HSCT. There was no statistically significant difference in the mRNA transcript level in patients and healthy individuals ($p>0.05$) (Figure 2).

Discussion

CDAII is a rare hematological disorder. Due to difficulty in diagnosis, many cases remain undiagnosed despite having severe clinical features. The diagnosis of CDAII needs to be suspected in congenital anemia cases having features of hemolytic anemia and patients showing low mean channel fluorescence values in the EMA test [13]. The molecular analysis using Next Generation Sequencing and/or Sanger sequencing of the *SEC23B* gene is the confirmatory test for CDAII. The protein band 3 is hypoglycosylated in the red cell membrane of CDAII patients. Thus, the EMA test shows low MCF in these patients [4]. Therefore, differentiation of CDAII and HS is necessary using CD44 antibody binding test [4,5].

Clinical presentation in CDAII patients is variable. Some show severe anemia with splenomegaly, requiring regular blood transfusion and some show mild anemia with no transfusion requirement. Patients showing severe clinical features are managed mainly by supportive care by regular blood transfusions, iron chelation therapy, rarely splenectomy, and allogeneic HSCT. In many patients, throughout life iron accumulates steadily [14]. Currently, iron overload in these patients is managed by following guidelines used for the treatment of thalassemia major.

Hematopoietic Stem Cell Transplant (HSCT) using a fully matched sibling donor is currently considered to be a curative therapy for CDA [15]. According to the literature, there are few reports of CDA managed successfully with HSCT, and the outcome was good. There is one report from the Indian population showing the clinical benefits of HSCT in CDAII. The patient reported here required transfusion every month and had continuous weakness and fatigue. Parents were not ready for lifelong transfusions followed by iron chelation therapy. Splenectomy was suggested but they were not ready because of the risk of infection susceptibility. Thus, the only curative option was HSCT. His elder sister has no symptoms of CDAII and was checked with the molecular test of the *SEC23B* gene.

For the first time, we have studied the molecular, biochemical, and clinical benefits of HSCT for the management of CDAII with allogeneic hematopoietic stem cell transplant may improve the prognosis in patients. The changes in the expression of the *SEC23B* gene regarding mRNA transcript levels were first time studied before and after an HSCT in a patient suffering from severe CDAII.

Conclusion

There are no definitive treatment guidelines available for CDA because of its rarity. But, in severe cases where an HLA-matched donor is available, allogeneic HSCT should be considered. In this study, we have reported the outcome of HSCT in a CDAII patient

having severe clinical features. This is the first study reporting the mRNA expression level of the *SEC23B* gene before and after HSCT in the CDAII case in the Indian population. The mRNA expression level was significantly decreased suggesting the clinical severity and it was almost equal to that of healthy individuals after treatment with a hematopoietic stem cell transplant. Post HSCT, the patient is now normal, healthy, and completely transfusion independent.

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