RESEARCH PAPER

Hematopoietic Stem Cell Transplantation for Primary Immunodeficiency Disorders: Experience from a Referral Center in India

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Objective: To share experience of over 15 years in hematopoietic stem cell transplantation in children with primary immunodeficiency disorders.

Design: Medical record review.

Setting: A referral center for pediatric hemato-oncological disorders.

Participants: Children (<18 y) diagnosed to have primary immune deficiencies who underwent hematopoietic stem cell transplantation between 2002 and August 2017.

Main outcome measures: Disease-free survival, morbidity and mortality.

Results: 85 primary immunodeficiency disorder transplants were performed with engraftment noted in 80 (94%) transplants and an overall survival of 67%. The conditioning regimen was individualized based on the underlying immune defect. Mixed chimerism was noted in 20% children with 56% (9/16) remaining disease-free. Graft *versus* host disease was noted in 33 (39.2%) children with most seen in children with chronic granulomatous disease. Severe combined immune deficiency transplants were mainly complicated by infections. Immune cytopenias complicated Wiskott Aldrich syndrome and Hemophagocytic lymphohistiocytosis transplants. 29.4% (25/85) children underwent haploidentical transplant in our cohort with a survival of 70% in this group. Infectious complications were the most common cause of death.

Conclusion: Primary immunodeficiency disorders are curable in India when transplanted in centers with experienced and trained pediatric transplant physicians and intensivists.

Keywords: *Immunity, Management, Outcome, Prognosis, Stem cell therapy.*

Primary immunodeficiency disorders (PID) are inherited disorders with impaired and dysregulated immunity characterised by recurrent infections, failure to thrive and a propensity for malignancy, especially lymphoma. Hematopoietic stem cell transplantation (HSCT) is a curative option available with intact survival post-HSCT. HSCT in PID can be challenging due to associated co-morbidities and underlying immune dysregulation.

PID are common in India due to a high incidence of consanguineous marriages. There is a paucity of data from India with recent studies reporting an incidence of more than one per million [1,2]. The lack of early diagnosis, awareness and late referral for HSCT are likely contributory factors to the hitherto poor outcome in these children [3].

We present the spectrum of PIDs including those undergoing HSCT at our centre over 15 years and the factors that influence morbidity and mortality.

METHODS

In this retrospective study in the Department of pediatric haematology, oncology and blood and marrow transplantation unit, we reviewed records of children less than 18 years of age diagnosed to have PIDs and who underwent HSCT from 2002 to August 2017. PID was confirmed by gene mutation analysis or laboratory studies, including T and B markers by flow cytometry, Immunoglobulin profile including IgG, IgA, IgM and IgE, nitroblue tetrazolium chloride test or dihydrorhodamine assay, flow cytometry for CD11b/18 and CD107a/Perforin levels. The ten warning signs for PID as proposed by the Jeffry Modell foundation were applied as a screening tool for initiating workup.

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We analyzed the number of children within each disorder, graft characteristics including the source of stem cells and donor, conditioning regimen, rates of

engraftment, post-transplant complications noted in general and those unique to each of the disorders, graft *versus* host disease, mortality and overall survival. Written informed consent was obtained from all parents for the procedure, and they were educated regarding the process of HSCT. The study was approved by the Institutional ethics review board.

In the matched related donor (MRD) transplants, peripheral blood stem cells were used as the source of stem cells in 21 (25%) and bone marrow in 17 (20%) children. In the matched unrelated donor (MUD) cohort, peripheral blood stem cells were used in 8 (9.5%) and umbilical cord blood units in 14 (16%) children. Haploidentical stem cell grafts were used in 25 (29%) children.

Among the children undergoing haploidentical stem cell transplants, techniques of T cell depletion included CD34 selection and Campath in the bag in 1 child each (4% each), CD 3/19 selection in 2 transplants (8%), TCR alpha/beta depletion in 6 transplants (24%) and post-transplant cyclophosphamide (PTCy) in 15 (60%) children.

The conditioning regimen was myeloablative in children with Wiskott Aldrich Syndrome (WAS), Hyper IgM syndrome and Chronic Granulomatous Disease (CGD) with fludarabine with busulphan or melphalan. Anti-thymocyte globulin was added to all MUD transplants. All children with Severe Combined Immune Deficiency (SCID), Hemophagocytic Lympho-histiocytosis (HLH), Common Variable Immune Deficiency (CVID), and Leucocyte Adhesion Defect (LAD) were treated with a reduced intensity conditioning using fludarabine and treosulfan [5,6]. Busulphan and cyclophosphamide were used only in the initial three patients before fludarabine became available. Among the children with SCID, 20% (6/29) were transplanted without conditioning.

In transplants where TCR alpha/beta depletion was used, conditioning included fludarabine/treosulfan/thiotepa/anti-thymocyte globulin. Conditioning in transplants with PTCy included fludarabine/treosulphan with or without single dose 2 gray total body irradiation (TBI) in conditions with a robust underlying immune system to facilitate enhanced myeloablation and immunosuppression. Fludarabine/melphalan was an alternative in children less than three years of age where radiotherapy cannot be used.

RESULTS

A total of 85 PID transplants were performed at our centre during the study period (***Table* I**). Engraftment was noted in 80 (94%) children by 16-21 days post-HSCT, with primary graft failure seen in 5 (5.8%) children. All five children underwent a haploidentical transplant, each with Hyper IgM syndrome, CGD, MSMD and WAS. Two children with WAS died of sepsis, while the other three children had autologous reconstitution and are awaiting second HSCT.

Among the 80 children who achieved engraftment, complete chimerism with a durable graft was noted in 64 (80%) children; mixed chimerism was seen in 20%. SCID was the underlying diagnosis in 8 out of 16 children, of which three underwent a second transplant; four are doing well with mixed chimerism and remain infection free, and one child died of refractory cytomegalovirus reactivation. One child each with LAD and IL10 R deficiency died with loss of graft while one child with WAS underwent a second HSCT. The other children with mixed chimerism including two with WAS and one each with IPEX syndrome, HLH, CGD and Hyper-IgE syndrome are clinically well with no infections. Therefore, 56% (9/16) children with mixed chimerism remained disease-free with a chimerism of 70% to 85%. Donor lymphocyte infusion was performed in seven children which resulted in stabilization of chimerism.

Graft *versus* host disease (GvHD) was noted in 33 out of 85 children (39.2%) with maximum GvHD rates in CGD (7/10, 70%). Children with HLH had GvHD rates of 57% (8/14) while WAS had rates 42% (6/14). GvHD rates were 16% (5/30) in SCID. Each of the children with Hyper IgM syndrome, Hyper IgE syndrome and IKZF mutation had grade 2-3 skin and gut GvHD.

GvHD was the cause of death in 12% children (4/33); one child each with SCID and WAS and two children with CGD. GvHD was managed with steroids including methylprednisolone and prednisolone. Second line immunosuppressants namely Etanercept, Tocilizumab, and Rituximab were required in 15/33 (45%) children.

TABLE I PRIMARY IMMUNODEFICIENCY DISORDERS IN THE STUDY POPULATION (*N*=85)

Disorder	**Number (%)*
Severe combined immunedeficiency	29 (34)
Wiskott Aldrich syndrome	15 (17)
Hemophagocytic lymphohistiocytosis	14 (16)
Chronic granulomatous disease	10 (11)
Hyper-IgM syndrome	5 (5.8)
Leucocyte adhesion defect	2 (2.3)
Common variable immunedeficiency	2 (2.3)
Mendelian susceptibility to mycobacterial diseases	2 (2.3)
IL10R deficiency	2 (2.3)

**One patient each with Hyper-IgE syndrome, IPEX syndrome, IKZF mutation, and X-linked agammaglobulinemia.*

WHAT IS ALREADY KNOWN?

- Hematopoietic stem cell transplantation (HSCT) is a curative option available for primary immunodeficiency disorders.

WHAT THIS STUDY ADD?

- Expierience of HSCT, including haploidentical stem cell transplantation, from a tertiary-call center with 67% overall survival rate.

The overall survival rate in our cohort was 67% (57/85) with mortality rates of 32% (***Table* II**). Seven children died before engraftment, of which four children with SCID died due to sepsis. Among children with SCID, five died of sepsis, four of whom died before engraftment, two children who underwent haplo SCT died of unexplained encephalopathy, two died of refractory cytomegalovirus (CMV) disease, and one died of GvHD. One child who received busulphan as conditioning died due to fibroelastosis of the lung four years post-HSCT. One child with bare lymphocyte syndrome died of disseminated invasive aspergillosis. Among children with WAS, causes of death included GvHD, refractory CMV, acute respiratory distress syndrome (ARDS) and pulmonary haemorrhage.

The cause of death among children with HLH is varied. Two children who were less than six months of age died of diffuse alveolar haemorrhage and ARDS. One child died of immune hemolysis. Two children died of relapse of central nervous system disease.

Both deaths among those with CGD were due to GvHD. Late graft rejection with a resultant recurrence of disease was the cause of death in the baby with IL10R deficiency. The child with LAD died due to loss of graft with refractory CMV.

TABLE II DISEASE-SPECIFIC OVERALL SURVIVAL RATES

Disorder	*Survival*
Severe combined immunodeficiency	16/29 (55%)
Wiskott Aldrich syndrome	9/16 (60%)
Hemophagocytic lymphohistiocytosis	9/14 (64%)
Chronic granulomatous disease	8/10 (80%)
Hyper-IgM syndrome	5/5 (100%)
Leucocyte adhesion defect	1/2 (50%)
Common variable immunedeficiency	2/2 (100%)
Mendelian susceptibility to mycobacterial diseases	2/2 (100%)
IL10R deficiency	1/2 (50%)
Hyper-IgE syndrome	1/1 (100%)
IPEX syndrome	1/1 (100%)
IKZF mutation	1/1 (100%)
X-linked agammaglobulinemia	1/1 (100%)

DISCUSSION

In this series from India about HSCT in PIDs, survival rate was 67%. The Australian and New Zealand Children's Haematology Oncology group had published an overall survival of 72% in their cohort in 2013 [4]. Recent data on HSCT in adults with PIDs reported an overall survival at 3 years of 85.2% [5]. Conditioning regimens need to be chosen based on the genotype of an individual child. The pre-engraftment phase is critical in babies with SCID due to maximum mortality risk secondary to bacterial sepsis. Wiskott-Aldrich syndrome poses unique challenges due to immune dysregulation and these children need to be monitored for late immune cytopenias. GvHD is a predominant problem in children with CGD. In children with primary HLH and less than six months of age, acute pulmonary haemorrhage is a risk factor affecting mortality. In all these children, CMV viral load needs to be monitored and treated early. Whole exome sequencing further aids with treatment modifications and adds to the spectrum of PIDs being transplanted including IL10R deficiency, IKZF mutation, and MSMD. Haploidentical SCT in PID present a ray of hope for a cure in children with no compatible matched family or unrelated donor with the advantage of ease of donor availability and cost [6]. Post-transplant cyclophosphamide is cost effective and well tolerated. Supportive care with trained pediatric intensivists is a major determinant of outcome. Advances in conditioning chemotherapy with increasing use of reduced intensity conditioning and treosulfan based protocols have decreased regimen-related toxicity and improved overall survival [7,8]. Stable mixed chimerism is found to be acceptable in PIDs [4,9].

The concept 'one size fits all' does not apply to PID transplants. Each one of the PIDs has a unique underlying pathophysiology involving immune dysregulation and treatment needs to be tailored accordingly [10,11]. SCID has a varied spectrum of phenotypic and genotypic characteristics. Based on the gene mutation and the percentage of T cells, B cells and NK cells, conditioning

is modified. T- B+ NK- SCID can be transplanted before six months of age without conditioning [12]. The presence of NK cells requires reduced-intensity conditioning given the increased risk of rejection and chemotoxicity [13].

Early diagnosis and referral for HSCT is the most important determinant of outcome. Referral may not be delayed for complete stabilisation of these children as it may be difficult with resultant loss of optimal time for HSCT. The use of the Jeffry Modell chart with the ten warning signs of PIDs in all pediatric clinics could help raise awareness and aid in early recognition. The use of simple newborn screening (NBS) techniques will aid in early diagnosis. The absence of thymus on chest *X*-ray and an absolute lymphocyte count of less than 2000 are indications for further workup. SCID has been included in NBS through an assay to detect T cell receptor excision circles (TRECs) which are biomarkers for T cell development [14].

Gene therapy is evolving into an alternative for cure for children with PIDs with no potential donor for HSCT. In particular, trials have been conducted with encouraging results in X-linked SCID, adenosine deaminase deficiency, chronic granulomatous disease and Wiskott Aldrich syndrome. Further research is aimed at improving viral vectors so as to achieve transgene transfer with reduced mutagenesis [15].

The future of PID in India lies on the pillar of shared care between pediatricians and hematologists and transplant physicians. The first step towards improving outcome in these children in India lies with pediatricians who are the key to early recognition and institution of appropriate care including parent counselling for a curative option.

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