Hematopoietic Stem Cell Transplantation in Thalassemia Patients: a Jordanian Single Centre Experience

Maher Mustafa1, Mousa Qatawneh1, Mais Al Jazazi1, Omaiema Jarrah1, Ruba Al Hazaimeh1, Raida Oudat2, Moath Al Tarawneh1, Rami Al Majali1

ABSTRACT

Introduction: Beta thalassemia major is the commonest inherited hematological disorder worldwide which needs lifelong sufficient supportive management. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment available till now. Aim: To evaluate the outcome of children who underwent allogenic hematopoietic stem cell transplantation as a curative approach for Thalassemia Major, treated at Queen Rania Al-Abdullah children Hospital (QRCH). Methods: A retrospective review of the medical files was conducted for all children (< 15 years) who had thalassemia major and received HSCT between January, 2010 and January, 2019. The following variables were studied for all patients: age, gender, Pesaro classifications, the count of infused raw bone marrow stem cell (CD34), engraftment time, outcome and complications. Results: A total of 34 children were transplanted for thalassemia major, at an average of 4 cases per year. All underwent allogenic raw bone marrow transplantation from matched related donors. Thirteen patients (38.2%) were males and twenty one (61.2%) were females. The age ranged between 2 and 15 years, with a median age of 6.5 years. According to Pesaro classification, 31 patients were class 2 (91.2%) and 3 patients were class 3 (8.8%) while no single case met the criteria for class 1 Pesaro classification. The median CD34 count was 3.5 million/Kg of recipient weight (range, 1.5*10^6-7*10^6/Kg). The median time for neutrophil engraftment was 15.5 days. At a median follow up of 5 years (range 1- 9.5), 33 patients were alive. One patient died before 100 days post transplantation due to grade IV acute gastrointestinal Graft Versus Host Disease (GVHD). Three patients had secondary graft failure (8.8%). Six patients (17.5 %) developed mild grade 1-2 skin GVHD while another patient developed hemorrhagic cystitis due to BK virus and cytomegalovirus (CMV) which reactivated simultaneously, and was successfully managed. Conclusion: The outlook for Thalassemia major has dramatically changed after HSCT, with a considerable success in Jordan and results comparable to international data. Keywords: Hematopoietic stem cell transplantation, Thalassemia.

1. INTRODUCTION

Thalassemia is the commonest inherited hematological diseases worldwide. Around 60,000 children are born each year suffering from thalassemia major (1, 2). Thalassemia is found more commonly in Mediterranean, Middle Eastern and Asian countries (3). However, in Jordan thalassemia is common as the percentage of thalassemia carriers reaches 3.5-4% and 80-90 patients are diagnosed with Beta thalassemia major (B-thalassemia) yearly (4).

B-thalassemia patients have an abnormal accumulation of the alpha globin chain and defective erythropoiesis with hemolytic anemia which are the results of absent or reduced beta globin formation (5, 6). Supportive management is needed for each thalassemia patient including regular blood transfusion and iron chelation to maintain a safe level of iron that increases due to recurrent red blood cells transfusion or increased gastrointestinal absorption of iron (7).
Until now there is no curative treatment for B-thalassemia patients except hematopoietic stem cell transplantation (HSCT) which showed very good results in the last decade. (8-14).

As the outcome of HSCT is much better in younger candidates, HSCT is preferred to be done as soon as possible when there is an available matched related donor (15). On the other hand, Pesaro group classified thalassemia patients into 3 classes depending on three criteria’s; hepatomegaly > 2 cm below the costal margin, liver fibrosis and the compliance of the patient to deferoxamine (16-18), and the results showed better outcome for patients with class 1 and 2 in comparison with patients with advanced disease (class 3).

To introduce a comprehensive review of the results of patients underwent HSCT for B-thalassemia major, this retrospective study analyzed the data for patients who underwent HSCT for B-thalassemia from January 2010 to January 2019 in QRCH, which is the only specialized pediatric hospital in Jordan.

2. AIM

To evaluate the outcome of children who underwent allogenic hematopoietic stem cell transplantation as a curative approach for Thalassemia Major, treated at Queen Rania AL- Abdullah children Hospital (QRCH) in Amman, Jordan.

3. PATIENTS AND METHODS

This retrospective study was approved by the Ethics Committee of the Jordanian Royal Medical Services. The medical records of children (<15 years) with B-thalassemia major who underwent HSCT were reviewed at QRCH in Amman-Jordan during the period between January 2010 and January 2019.

The donors, patients and the transplantation characteristics are shown in Table 1. At the transplantation time the median age of the patients was 6.5 years (range, 2-12 years). DNA typing for human leukocyte antigen (HLA) class 1 (HLA -A,-B,-C) and class 2 (HLA -DRB1,-DQB1) was done. Gender was distributed in this study as follows: male 21 (61.8%) and female 12 (35.3%). All the donors were 10/10 HLA matched. All patients except hematopoietic stem cell transplantation as a curative approach for Thalassemia Major, treated at Queen Rania AL- Abdullah children Hospital (QRCH) in Amman, Jordan.

Supportive care and infection prophylaxis

All patients were transplanted in double door isolation rooms. Ciprofloxacin was used as an antibacterial prophylaxis, starting on day (-10) and continued until discharge from the hospital. Co-trimoxazole was used for pneumocystis jiroveci prophylaxis starting from day (+20) and continued until one year post transplant. Regarding antifungal prophylaxis, before 2016 fluconazole was used, starting from day (-10) until 2-3 months post transplant, but since 2016 micafungin was used from day (-10) until discharging the patient from hospital then fluconazole orally was continued until 2-3 months post transplant. For antiviral prophylaxis acyclovir was used from day (-10) until 5-4 months post transplant unless GVHD happened, then the antiviral was given for longer duration. For veno-occlusive disease prophylaxis Ursodeoxycholic acid was used from day (-10) until 40 days post transplant.

Intravenous immunoglobulin’s (IVIG) was given at day (-1) and every two weeks for two months then it was given according to serum immunoglobulin’s levels. As we used oral busulfan for conditioning we used to use epanutin as seizure prophylaxis starting it 24 hours prior to first dose of busulfan and the last dose of epanutin given 48 hours after the last dose of busulfan.

Blood cultures from the two lumens of the central venous catheter were done weekly. Screening for CMV in blood and urine were done twice weekly until the patient was discharged from the hospital, then it was done once weekly until day (+100) then it was done monthly until 1 year post transplant or if clinically suspected.

Antibiotics therapy was started when patients became febrile, and if fever persisted more than 96 hours or if fungal infection was clinically suspected antifungal therapy was added.

Blood was given when hemoglobin level was below 8 g/dl. Platelets were given when platelet count was below 20,000/μL or in cases of bleeding. All blood products given were irradiated.

Vaccination was given after 12 months of transplant if there were no signs of Graft Versus Host Disease (GVHD) and if the patient was on no immunosuppressant medications.

Transplantation procedure

Details about conditioning regimens and the medications used for GVHD prophylaxis were shown in Table 1. According to Pesaro classification, class 2 patients were given myeloablative conditioning regimens which consist of oral busulfan (total 16 mg/kg divided into 16 doses from days -9 to -6) without blood concentration monitoring in addition to Cyclophosphamide (total dose 160-200 mg/kg divided into 4 doses between days -5 and -2), while class 3 patients received reduced intensity conditioning (RIC) which consisted of oral busulfan (total 14 mg/kg divided into 16 doses from days -9 to -6) without blood concentration monitoring in addition to Cyclophosphamide (total dose 120 mg/kg divided into as 4 doses between days -5 and -2). Before 2016 rabbit antithymocyte globulin (ATG) was used at a total dose of 10 mg/kg divided into 4 doses from days -5 to -2, while after 2016 ATG dose was raised to 15-20 mg/kg.

GVHD prophylaxis was achieved by cyclosporine and intravenous (IV) Methotrexate. Cyclosporine IV was initiated at day -2 at a dose of 3 mg/kg/day divided into 2 doses, blood cyclosporine trough level was done twice weekly to maintain the level between 150-250 ng/ml. When the patient started to tolerate oral feeding cyclosporine was shifted to oral route with doubling the IV dose, it was continued until day +180 then tapered over 1-2 months if no signs of GVHD. Methotrexate IV was given at day (+1) with...
a dose of 15 mg/kg, then 10 mg/kg was given at days (+3), (+6) and (+11). Rescue folicic acid IV at a dose of 15 mg/kg was given 24 hours following each dose of methotrexate.

For patients with acute GVHD (aGVHD) Grade II-IV, steroids were given as the first line of treatment. Engraftment was considered if absolute neutrophil count (ANC) is ≥ 500/μL and platelet count is ≥ 20,000/μL for three consecutive days without transfusion. Chimerism monitoring was done to assess engraftment only in cases of sex mismatch between donor and recipient using Fluorescent in-situ Hybridization (FISH) XY chromosome analysis, otherwise the transplant considered successful if the patient became transfusion independent.

**Definitions and endpoints**

The endpoints for the primary study were overall survival (OS), thalassemia free survival (TFS) and graft failure (GF). OS was defined as the period from transplant to death from any cause. TFS was defined as survival without graft failure. Primary GF was defined as no hematological recovery with persistent pancytopenias beyond day +28, while secondary GF was defined as decrease in the neutrophil count below 500/μL and platelet count below 20,000/μL after successful engraftment.

**Statistical analysis**

On completion of data collection phase, coded data were fed to the computer using Statistical Package for Social Sciences (SPSS version 25 and Excel 2010 (Microsoft). We chose descriptive statistical terms to characterize performance measurements, including mean, median. As statistical measurement univariate comparisons were made by using, the Chi-square test or Fisher’s exact test for dichotomous variables and Pearson’s exact Chi-square test for qualitative variables. The univariate probabilities of OS and TFS were calculated using the Kaplan–Meier estimator, and their 95% confidence intervals (CI) were constructed. Whereas comparing the probabilities of survival was calculated by using the log-rank test. Also a stratified univariate analysis of OS and TFS was performed using the Mantel–Haenszel test. Multivariate Cox regressions were performed for the variables identified as being associated with one of the endpoints.

4. **RESULTS**

A total of 34 patients with B-thalassemia were included in this study. The post-HSCT follow-ups lasted for 48 months on average due to mortality. The longest follow-up lasted for 10 years. Donor cells engraftment were achieved in all of our patients. 15.5 days was the median day for neutrophils engraftment (range 13-19 days) while 20 days was the median day for platelets engraftment (range 18-25 days). Six of our patients developed grade 2-3 skin aGVHD. One of our patients died because of grade 4 gastrointestinal aGVHD. Except the died patient, all of our patients responded very well to the first line management with steroids and increase the duration of cyclosporine therapy. Fortunately none of our patients had chronic GVHD (cGVHD).

Over a median follow up of 5 years (range 1-9.5 years), the probabilities of accumulated OS and TFS were 97.1 ± 0.03 and 88.2 ± 0.05 % respectively (Figure 1). Only one patient died at day + 65 because of grade 4 GI aGVHD, the died patient was male who was a 2 years old. Secondary graft failure happened in 3 of our patients at days (+200), (+180), (+120) respectively. All of these patients were females. Patient number one was 9 years old who had class 3 disease, patient number 2 was 11 years old who had class 2 disease, while patient number 3 was 3 years old who had class 2 disease (Table 2, Table 3). The graft loss happened gradually and was manifested as gradual drop in the hemoglobin level and mean corpuscle volume (MCV) until they became transfusion dependent; this was approved by hemoglobin electrophoresis which showed B-thalassemia major pattern.
Hematopoietic Stem Cell Transplantation in Thalassemia Patients

| Variables                      | OS, % | P  | TFS, % | P  |
|--------------------------------|-------|----|--------|----|
| Gender, mean ± SE              | 0.35  | 0.04|        |    |
| Male                           | 147.9±56.1 | 75.0±9.6 |  |    |
| Female                         | 131.4±45.1 | 48.9±7.6 |  |    |
| Age at transplantation, mean ± SE | 0.00  | 0.56|        |    |
| <= 7 y                         | 120.13±47.2 | 61.4±7.7 |  |    |
| > 7 y                          | 174.5±30.9  | 55.3±11.5 |  |    |
| Donor type, mean ± SE          | 0.12  | 0.63|        |    |
| Brother                        | 129±45.8  | 52.8±8.3 |  |    |
| Sister                         | 139±49.5  | 64±10.7 |  |    |
| Father                         | 216±0.0   | 84±37.3 |  |    |
| Mother                         | 216±0.0   | 84±37.3 |  |    |
| Pesaro Class                   | 0.51  | 0.26|        |    |
| Two                            | 135.9±51.4 | 61.1±6.5 |  |    |
| Three                          | 156.0±12.0 | 36.2±21.1 |  |    |
| Conditioning regimen group, mean ± SE | 0.27  | 0.45|        |    |
| BU/CY16/200                    | 134.8±50.2 | 57.4±6.6 |  |    |
| BU/CY14/160                    | 168.0±31.7 | 72.2±21.2 |  |    |
| CD34*10^6                      | 146.0±47.7 | 66.0±36.05 | 0.05|    |
| <=5                            | 114.6±59.5 | 39.3±32.17 |  |    |

Table 2 Univariate analysis of OS and TFS. Univariate analysis of OS and TFS. Univariate analysis showed that age at transplantation was potential key factor for OS: the impacts of gender, donor type, Pesaro class, CD34*10^6, and conditioning regimen on OS were not significant (p>0.05). Univariate analysis also showed that gender was potentially related to TFS; the effects of age, donor type, pesaro class, CD34 count and conditioning regimen on TFS were not significant. These results are displayed in Table 2.

| Variable           | HR   | 95%CI  | P Value |
|--------------------|------|--------|---------|
| Gender             | 0.55 | 0.051 – 6.01 | 0.063 |
| Pesaro Class       | 0.09 | 0.009 – 0.89 | 0.04  |
| Age at transplantation | 1    | 0.095 – 11.1 | 0.98  |
| <= 7 y             |      |         |        |
| > 7 y              |      |         |        |

Table 3. Multivariate Cox Regression analysis of TFS Cox Regression. A multivariate COX regression analysis indicated that pesaro class was leading factor to difference in TFS [Hazard Ratio (HR = 0.09, 95% CI 0.009 – 0.89, P = 0.04)]. While gender and age at transplantation not potentially affect of TFS. These results noticed in Table 4.

These 3 patients are still alive and they are transfusion dependant as their parents refuse second transplant. At the time of this study, of the remaining 30 patients, with a median follow up duration of 10 years are alive and thalassemia free. Ten patients (29%) developed bacteremia during hospitalization in the bone marrow transplant unit, 8 of them (80%) had gram positive bacteria while 2 of them (20%) had gram negative bacteria. None of our patients had a positive culture for fungal infection. Three patients (9%) developed CMV infections, which was symptomatic in one of them as he developed severe hemorrhagic cystitis at day (>30) which was treated by gancyclovir, over hydration, bladder irrigation and forced diuresis. This patient was found to have BK virus infection in addition to the CMV at the time of hemorrhagic cystitis. Fortunately no one of our patients developed veno-occlusive disease (VOD) despite the use of oral busulpan without blood level monitoring.

5. DISCUSSION

In our study, we analyzed retrospectively all patients with B-thalassemia who underwent HSCT in QRCH in Jordan between January 2010 and January 2019. Our study is the first one in Jordan about HSCT in thalassemia patients. The median follow up for the patients were 5 years (range, 1-9.5 years). Despite the excellent outcome of HSCT in thalassemia patients, the number of the transplants is far under satisfactory in developing countries including our centre where thalassemia prevalence is high (19, 20). This is because of the few number of doctors who are specialized in pediatric HSCT and the small capacity of our bone marrow transplant unit. Transplant was performed as soon as possible after we found a matched related donor, so most of our HSCT were performed early in childhood; the median age at the time of transplant was 6.5 years (range, 2-12 years).

Most of our patients received bone marrow from MSD (94%) while the remaining received bone marrow from MRD (3% from father and 3% from mother). The stem cell source used was unmanipulated raw bone marrow for all of our patients. We depend on raw bone marrow as the incidence of cGVHD is much less with raw bone marrow in comparison to peripheral blood stem cell (PBSC) (21, 22), this lower incidence of cGVHD has an effect on long term quality of life and decrease the incidence of secondary malignancy post HSCT (23). On the other hand, raw bone marrow has superior long term clinical outcome and lower cost compared to PBSC (24, 25).

Our patients received myeloablative conditioning regimens including oral busulfan, Cyclophosphamide and ATG. Oral busulfan was used instead of IV form as it is less costy and the potential advantage of IV busulfan compared to oral busulfan is not well established in standard risk thalassemia HSCT (26). However, in spite of using oral busulfan for all of our patients without blood level monitoring we didn’t have any case of veno-occlusive disease (VOD), depending on this outcome a prospective multicentre study is needed to be done to explain the low incidence of VOD in Jordanian population.

In our study the OS was 97% and TFS was 88.2% after a median of follow up of 5 years (range, 1-9.5 years). These results showed better outcome in our center compared to multiple centers results which showed OS and TFS of about 90% and 80% respectively for MRD HSCT for B-thalassemia patients (16, 27).

This may be explained by the younger age of the patients at the time of transplant compared to other centers as the median age of transplant at our centre was 6.5 years. This conclusion was published in multiple studies as they conclude the adverse effect of the older age on TFS and OS in B-thalassemia patients who underwent HSCT (28-32). On the other hand, our results maybe explained also by the fact...
that most of our patients were Pesaro class 2 (91.2%) while Pesaro class 3 patients were just 3 (8.8%). This conclusion was published by multiple studies as they noticed that the OS and TFS is much better in class 1 and 2 patients in comparison to class 3 patients (2, 33). The transplanted related mortality happened in just one patient (3%) which was due to grade IV GI aGVHD, which presented as severe diarrhea and vomiting which was associated with ascites and electrolyte disturbances. This aGVHD was confirmed by the gastrointestinal biopsies taken during upper and lower endoscopy. This aGVHD was refractory to high dose steroids, infliximab, cyclosporin and the addition of mycophenolate.

Secondary graft failure was the main problem we had in our center which happened in three of our patients; upon Pesaro classification one of these patients was class 3 and the other two patients were class 2. Two of these three patients were receiving blood in peripheral hospitals and they were given blood at a hemoglobin level of less than 8 mg/dl, these levels of hemoglobin have inadequate suppression of erythroid marrow expansion, so we expect that their expanded erythropoietic marrow played a major role in the rejection of the new bone marrow they received. This explanation was observed in the French experience (34).

Grade I-III aGVHD incidence was 17.6% and grade IV aGVHD was 3%, these results are similar to that reported by others (35). None of our patients developed chronic GVHD which is an impressive outcome compared to the international studies worldwide including the 30 years study of patients with β-thalassemia undergoing H SCT conducted by Caocci G and colleagues (36). This may be explained by the fact that all of our patients received raw bone marrow and most of our patients received stem cells from MSD. This was reported by the international survey done by Chunfu Li and his colleagues (6).

The most common complications happened to our patients were bacterial infections, mucositis, hypertension and CMV virus reactivation. The most serious complication was a severe hemorrhagic cystitis which happened to a male patient at day (+50), he developed gross hematuria with clots, this hemorrhagic cystitis was due to CMV infection as the titer was high in blood and urine, the patient also had BK virus in the urine at the same time. We managed this patient by platelets transfusion to keep the platelets count more than 100,000/μL, over hydration, bladder irrigation, diuretics and Gancyclovir IV. The patient improved with this aggressive management, the symptoms resolved completely after 8 days of this management, but the patient was kept in hospital for the IV Gancyclovir which continued for 3 weeks until 2 negative readings of CMV was observed and then he was discharged from the hospital as the patient recovered completely.

6. CONCLUSION

HSCT provides the best option for the treatment of B-thalassemia considering the excellent results and high success rate. The results we achieved shows that the outcome of HSCT in QRC in Jordan are comparable to the results reported in the international literatures. Best outcome is achieved if HSCT is performed early in life as iron overload and related organs toxicity are low.

• Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms.
• Author's contribution: M. Jazairi, O. Aljarrah gave a substantial contribution to the conception and design of the work. M. Qatawneh, M. Al tarawneh gave a substantial contribution of data. M. Mustafa, M. Qatawneh, R. Oudat gave a substantial contribution to the acquisition, analysis, or interpretation of data for the work. M. Mustafa, M. Qatawneh, R. Al hazaimeh, R. Al majali had a part in article preparing for drafting or revising it critically for important intellectual content. M. Mustafa, M. Qatawneh, R. Al hazaimeh, R. Al majali gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
• Conflicts of interest: There are no conflicts of interest.
• Financial support and sponsorship: Nil.

REFERENCES

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008 Jun; 86(6): 480–487. doi: 10.2471/blt.08.0356675.
2. Mohamed SY. Thalassemia Major: Transplantation or Transfusion and Chelation. Hematol Oncol Stem Cell Ther. 2017; 10(4): 290–298. doi:10.1016/j.hemonc.2017.05.022.
3. Lucarelli G, Isgrò A, Sodani P, Gaziev J. Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. Cold Spring Harb Perspect Med. 2012 May; 2(5): a011825. doi: 10.1101/cshperspect.a011825.
4. Ministry of Health, Jordan, www.moh.gov.jo/ Non Communicable Disease Directorate.
5. Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med. 2005 Sep 15; 353(11): 1155–1146. doi: 10.1056/NEJMra050436.
6. Li C, Mathews V, Kim S, George B, Hebert K, Jiang H, Li C, et al. Related and unrelated donor transplantation for β-thalassemia major: results of an international survey. Blood Adv. 2019 Sep 10; 3(17): 2562-2570. doi: 10.1182/bloodadvances.2019000291.
7. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica. 2004 Oct; 89(10): 1187-1195.
8. Thomas ED, Buckner CD, Sanders JE, Papayannopoulou T, Borgna-Pignatti C, De Stefano P, Sullivan KM, Clift RA, Storb R. Marrow transplantation for thalassaemia. Lancet. 1982 Jul 31; 2(8292): 227-229. doi: 10.1016/s0140-6736(82)90319-1.
9. Lucarelli G, Gaziev J. Advances in the allogeneic transplantation for thalassemia. Blood Rev. 2008 Mar; 22(2): 53-63. doi: 10.1016/j.brr.2007.10.001.
10. Di Bartolomeo P, Santarone S, Di Bartolomeo E, Olioso P, Bavaro P, Papalinetti G, et al. Long-term results of survival in patients with thalassaemia major treated with bone marrow transplantation. Am J Hematol. 2008 Jul; 83(7): 528-530. doi: 10.1002/ajh.21175.
11. Gaziev J, Lucarelli G. Allogeneic cellular gene therapy for hemoglobinopathies. Hematol Oncol Clin North Am. 2010 Dec; 24(6): 1145–1163. doi: 10.1016/j.hoc.2010.08.004.
12. Gaziev J, Isgrò A, Sodani P, Marziali M, Paciaroni K, Gallucci C, et al. Optimal Outcomes in Young Class 3 Patients With Thalassemia Undergoing HLA-Identical Sibling Bone Marrow Transplantation. Transplantation. 2016 Apr; 100(4): 925-932. doi: 10.1097/TP.0000000000000928.
13. Goussetis E, Peristeri I, Kitra V, Vessalas G, Paisiou A, Theodo-
Hematopoietic Stem Cell Transplantation in Thalassemia Patients

14. Angelucci E, Baroni D. Allogeneic stem cell transplantation for thalassemia major. Haematologica. 2008 Dec; 93(12): 1780-1784. doi: 10.3324/haematol.2008.001909.

15. Lucarelli G, Andreani M, Angelucci E. The cure of thalassemia by bone marrow transplantation. Blood reviews. 2002; 16: 81-5. 10.1054/br.2002.0192.

16. Galambrun C, Pondarré C, Bertrand Y, Loundou A, Bordigoni P, Frange P, et al. French Rare Disease Center for Thalassemia; French Society of Bone Marrow Transplantation. French multicenter 22-year experience in stem cell transplantation for beta-thalassemia major: lessons and future directions. Biol Blood Marrow Transplant. 2013 Jan; 19(1): 62-68. doi: 10.1016/j.bbmt.2012.08.005.

17. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baroni D, Giardini C, et al. Bone marrow transplantation in patients with thalassemia. N Engl J Med. 1990 Feb 15; 322(7): 417-421. doi: 10.1056/NEJM199002153220701.

18. Giardini C, Angelucci E, Lucarelli G, Galimberti M, Polchi P, Bar- onciani D, Bechelli G. Bone marrow transplantation for thalassemia. Experience in Pesaro, Italy. Am J Pediatr Hematol Oncol. 1994 Feb; 16(1): 6-10.

19. Baroni D, Angelucci E, Potschger U, Gaziev J, Yesilipek A, Zecca M, et al. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry. 2000-2010. Bone Marrow Transplant. 2016 Apr; 51(4): 536-541. doi: 10.1038/bmt.2015.295.

20. Mohamed SY, Fadhil I, Hamldaji RM, Hamidieh AA, Fahmy O, Laedeb S, et al. Hematopoietic stem cell transplantation in the Eastern Mediterranean Region (EMRO) 2008-2009: report on behalf of the Eastern Mediterranean Bone Marrow Transplantation (EMBMT) Group. Hematol Oncol Stem Cell Ther. 2011; 4(2): 81-95. doi: 10.1016/j.hematol Oncol Stem Cell Ther. 2011; 4(2): 81-95. doi: 10.1016/j.hematol.2011.81.

21. Eapen M, Horowitz M, Klein J, Champlin R, Loberiza F, Ringdén P, et al. Comparison of Intravenous with Oral Busulfan in Allogeneic Hematopoietic Stem Cell Transplantation With Myeloablative Conditioning Regimens for Pediatric Acute Leukemia. Biol - thorapy Journal. 2017; 2. 10.1016/j.phoj.2017.12.002.

22. Kumar R, Kimura F, Ahn KW, Hu ZH, Kuwatsuka Y, Klein JP, et al. Comparing Outcomes with Bone Marrow or Peripheral Blood Stem Cells as Graft Source for Matched Sibling Transplants in Severe Aplastic Anemia across Different Economic Regions. Biol Blood Marrow Transplant. 2016 May; 22(5): 932-940. doi: 10.1016/j.bbmt.2016.01.012.

23. La Nasa G, Caocci G, Efficace F, Dessi C, Vacca A, Piras E, et al. Long-term health-related quality of life evaluated more than 20 years after hematopoietic stem cell transplantation for thalassemia. Blood. 2013 Sep 26; 122(15): 2262-2270. doi: 10.1182/blood-2013-05-502658.

24. Preusssler JM, Denzen EM, Majhail NS. Costs and cost-effectiveness of hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2012 Nov;18(11):1620-8. doi: 10.1016/j.bbmt.2012.04.001.

25. Ramprakash S, Agarwal R, Dhanya R, Marwah P, Low-cost matched sibling bone marrow transplant for standard-risk thalassemia in a limited-resource setting. Pediatric Hematology Oncology Journal. 2017; 2. 10.1016/j.pho.2017.12.002.

26. Kato M, Takahashi Y, Tomizawa D, Okamoto Y, Inagaki J, Koh K, et al. Comparison of Intravenous with Oral Busulfan in Allogeneic Hematopoietic Stem Cell Transplantation with Myeloablative Conditioning Regimens for Pediatric Acute Leukemia. Biology of blood and marrow transplantation. 2013; 19: 10.1016/j.bbmt.2013.09.012.

27. Lawson SE, Roberta IA, Amirola P, Dokal I, Szydllo R, Darbysheir PJ. Bone marrow transplantation for beta-thalassaemia major: the UK experience in two paediatric centres. Br J Haematol. 2005 Jan; 120(2): 289-95. doi: 10.1046/j.1356-2141.2003.04065.x.

28. Mathews V, George B, Vishwambaya A, Abraham A, Ahmed R, Ganapule A, et al. Improved clinical outcomes of high risk β thalassemia major patients undergoing a HLA matched related allogeneic stem cell transplant with a treosulfan based conditioning regimen and peripheral blood stem cell grafts. PLoS One. 2015 Apr 26; 8(4): e61657. doi: 10.1371/journal.pone.0061657.

29. Sabloff M, Chandy M, Wang Z, Logan BR, Ghavamzadeh A, et al. HLA-matched sibling bone marrow transplantation for β-thalassemia major. Blood. 2011 Feb 3; 117(5): 1745-1750. doi: 10.1182/blood-2010-09-306829.

30. Mathews V, George B, Deotare U, Lakshmi KM, Vishwambaya A, Daniel D, et al. A new stratification strategy that identifies a subset of class III patients with an adverse prognosis among children with beta thalassemia major undergoing a matched related allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2007 Aug; 13(8): 889-894. doi: 10.1016/j.bbmt.2007.05.004.

31. Horan J, Wang T, Haengenson M, Spellman SR, Deyn J, Eapen M, et al. Evaluation of HLA matching in unrelated hematopoietic stem cell transplantation for nonmalignant disorders. Blood. 2012 Oct 4; 120(14): 2918-2924. doi: 10.1182/blood-2012-03-41758.

32. Eapen M, Wang T, Veys PA, Boelens JJ, St Martin A, Spellman S, et al. Allele-level HLA matching for umbilical cord blood transplantation for non-malignant diseases in children: a retrospective analysis. Lancet Haematol. 2017 Jul; 4(7): e325-e333. doi: 10.1016/S2352-3026(17)30104-7.

33. Choudhary D, Doval D, Sharma SK, Khandelwal V, Setia R, Han-doo A. Allogeneic Hematopoietic Cell Transplantation in Thalas-semia Major: A Single-center Retrospective Analysis From India. J PediatrHematol Oncol. 2019; 41(5): e296-e301. doi:10.1097/MPH.0000000000001475.

34. Cazzola M, Borgna-Pignatti C, Locatelli F, Ponchio L, Beguin Y, De Stefano P. A moderate transfusion regimen may reduce iron loading in beta-thalassemia major without producing excessive expansion of erythropoiesis. Transfusion. 1997 Feb; 37(2): 135-140. doi: 10.1046/j.1537-2995.1997.37297203514.x.

35. Gaziev D, Polchi P, Galimberti M, Angelucci E, Giardini C, Bar- onciani D, et al. Graft-versus-host disease after bone marrow transplantation for thalassemia: an analysis of incidence and risk factors. Transplantation. 1997 Mar 27; 63(6): 854-860. doi: 10.1097/00007899-199703270-00011.

36. Caocci G, Orofino MG, Vacca A, Pirroddi A, Piras E, Addari MC, et al. Long-term survival of beta thalassemia major patients treated with hematopoietic stem cell transplantation compared with survival with conventional treatment. Am J Hematol. 2017 Dec; 92(12): 1505-1510. doi: 10.1002/ajh.24898.