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RESEARCH ARTICLE

Outcome of beta thalassemia major in pediatric patients allografted from fully matched related donors in Egypt

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ABSTRACT

Background: Thalassemia is the most common genetic disease worldwide. Allogeneic hemopoietic stem cell transplantation (HSCT) is a method available to treat transfusion-dependent thalassemia major patients. **Aim:** To report incidence of post-transplant complications and survival outcome with factors affecting it in beta thalassemia major patients. **Patients and Methods:** We conducted this retrospective study on pediatric patients (≤18 years old) who underwent their first allo-HSCT for 5 years (2015-2019). All patients received allografts from fully matched related donors. A total of 115 BTM patients' data were collected and classified into 3 classes according to Pesaro classification. **Results:** Post-transplant complications were reported in 80% of all patients where the most common one was infections (63.5%) which seen more in class III (64.7%). Also, acute graft versus host disease (AGVHD), chronic GVHD, cardiovascular toxicity, endocrinopathies and graft failure were more common in class III patients (35.3%, 11.8%, 11.8%, 17.6% and 11.8%, respectively). While pulmonary complications, neurotoxicity, and hemorrhagic cystitis were reported more in class II patients (40%, 21.8% and 21.8% respectively). Three-year overall survival (OS) and thalassemia free survival (TFS) of all patients were 80% and 77.4%, with the worst outcome reported in class III patients (58.8% for both OS and TFS). Patients' age group 5-10 years and received MTX included GVHD prophylaxis were associated with better survival outcomes than others. **Conclusion:** Addition of fludarabine to the conditioning regimen in class III BTM patients didn't improve the occurrence of GVHD, graft failure or survival outcomes in comparison to class I and II patients.

Keywords: Allogeneic hemopoietic stem cell transplantation, Free survival, HSCT, overall survival, Pesaro classification, Thalassemia

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INTRODUCTION

Beta thalassemia major (BTM) is hemolytic anemia caused by genetic disorder producing absent or reduced beta globin chain synthesis. Patients are treated with blood transfusions and iron chelation therapy to prevent iron overload in multiple organs (Olivieri & Brittenham, 1997). This management avoids consequences of fulminant hemolytic anemia and complications resulting from expansion of the bone marrow space (Davis et al., 2000). However, it's difficult to maintain this management in a chronic manner with advancing age (Olivieri and Brittenham, 1997). This is due to occurrence of several late effects; as endocrine dysfunctions, cardiomyopathy, progressive liver fibrosis, and consequences of posttransfusion viral infections that affect the quality of life and increase the mortality risk with age (Zurlo et al., 1989 and Olivieri et al., 1994). Allogeneic HSCT is the only cure for thalassemia, a part of gene therapy, which corrects the genetic defect in the hematopoietic system by using allogeneic stem cells from immunologically acceptable (Roberts, 1997 and Li et al., 2002). Risk for marrow transplantation in thalassemia patients can be categorized into three classes; class I, II and III (Lucarelli et al., 1993 & Lucarelli et al., 2002) with class III has the greatest risk (Lucarelli et al., 1996). The survival outcome of treated BTM patients with allo-HSCT has been improved especially after recent progress of transplantation technologies as the overall

survival (OS) and thalassemia free survival (TFS) rates reach around 90% and 80%, respectively (Choudhary et al., 2019 and Huang et al., 2018). The transplant outcomes are affected by several factors; include age at transplantation, donor type, degree of HLA matching, pre-transplant risk class, source of stem cell, and type of conditioning regimen (Issaragrisil and Kunacheewa, 2016).

Thalassemia is the most common cause of chronic hemolytic anemia in Egypt, accounts nearly 85.1% of hemolytic anemias and causes worse outcome (Mokhtar et al., 2013 and El-Danasoury et al., 2012). A transplant program was promoted in Egypt at the beginning of twenties; to decrease the huge psychological, social and financial burden on both the patient and his family, also to offer a radical cure or at least a stable chimeric state that makes avoidance of regular blood transfusion and chelation therapy (Mahmoud et al., 2014). In this study, we reported the frequency of post-transplant complications, survival outcome and factors affecting it in BTM pediatric patients.

PATIENTS & METHODS

Data collection

This retrospective study was carried on 115 BTM pediatric patients (≤ 18 years old) who underwent their first allogeneic HSCT at bone marrow transplantation unit of Nasser Institute Hospital for research and treatment, Cairo, Egypt; in the period from 2015 till 2019. All donors were siblings or other family members and at least 6/ 6 HLA matched (HLA-A, B and DR). Data collected from patients' files were included: baseline patient-related variables and transplantation-related data. We classified all studied patients according to Pesaro classification in which patients with enlarged liver ≥ 2 cm below costal margin, on irregular chelation therapy and with portal fibrosis diagnosed on liver biopsy are classified as class III; patients with one or two of these characters are classified as class II and patients who hadn't any one of these characters are classified as class I (Lucarelli et al., 1993). We assessed their survival rates and the complications reported in each class of patients after allo-HSCT. Approval from ethical committee at South Egypt Cancer Institute, Assiut, Egypt and the internal review board of Nasser Institute Hospital for research and treatment, Cairo, Egypt was received.

Stem cells collection procedure

PBSC Donors were injected subcutaneously with granulocyte-colony stimulating factor (G-CSF, 10 µg/kg daily for 5 days) and mobilized PBSC was collected at day of last injection. One to 2 apheresis procedures were planned by means of COBE Spectra continuous cell separator (Gambro, Lakewood, CO, USA) using Spin–Nebraska protocol. For *BM donors*, aspirations were performed under general anesthesia from posterior ileum region. Enumeration of total WCC, MNC and CD34 +ve cells was done by flow cytometry (Coulter EPICS, Coulter electronics, Hialeah, FL, USA) using anti CD34 monoclonal antibody HPCA2 (BD, San Jose, CA, USA). The aim was to collect at least 5 x 10^8 mononuclear cells (MNC) and/or 3 x 10^6 viable CD34+ cells/kg recipient's body weight. The products of PBSC apheresis or BM harvest were infused to patients on the same day of collection (day 0 of conditioning regimen).

Transplant procedure

All BTM patients received the following conditioning regimen; Bu/Cy regimen consisted of busulfan 5 mg/kg/day orally for 4 days to patients ≤ 8 years or 4 mg/kg/day orally for 4 days to patients > 8 years and cyclophosphamide at a dose of 30 mg/kg/day for 4 days (Mahmoud et al., 2014). Fludarabine with a dose of 30 mg/m2/d from day -17 through day -13; was added to 15 patients of class III.

GVHD prophylaxis: all patients received cyclosporine A (CSA) at a dose of 3 mg/ kg/day IV given from day -1 until oral intake was initiated then shifted to oral dose 5 mg/kg/day divided on two daily doses and maintained till day 180 post-transplant then gradually tapered off (Mahmoud et al., 2014). Methotrexate (MTX) was given to almost all class I patients (mainly in patients received bone marrow stem cells) at a dose of 15 mg/m2 IV on day +1, 10 mg/m2 on days +3, +6, and +11. Anti-thymocyte globulin (ATG) was added to class II and III patients (mainly in patients received peripheral blood stem cells) at dose of 30 mg/kg/day (from

day-3 to day-1). Methyl prednisone at dose 2 mg/kg (MP) starting from day -7 till day +4 then tapered gradually over two weeks; was added to some patients (mainly in class II and III).

Anti-microbial prophylaxis was started from the beginning of conditioning regimen till the end of immunosuppression; levofloxacin is the used antibacterial prophylaxis, fluconazole is the used anti-fungal prophylaxis, acyclovir as anti-herpes prophylaxis. Also, trimethoprim /sulfamethoxazole as anti-pneumocystis jiroveci prophylaxis was started two days before start of conditioning regimen till day -2 and then reinitiated after engraftment to the end of immunosuppression. Once Febrile neutropenia occurred, empirical therapy with piperacillin/ tazobactam or cefepime and amikacin was added until results of culture sensitivity. If the fever persists with no results yet, shift of the antibiotics to imipenem (or meropenem) and broad-spectrum antifungals as amphotericin-B or voriconazole were added. Irradiated and filtered Packed red blood cells and platelet transfusions were given to maintain Hb level ≥ 8 g/dl and platelet count ≥ $20x10^9$ /L respectively.

Post-transplant engraftment of neutrophil and platelet were defined by absolute neutrophilic count (ANC) ≥ $0.5x10^9$ /L and platelet count ≥ $20x10^9$ /L in three successive days (without transfusion). To assess engraftment, degree of chimerism in patients was monitored at D+28 and D+56 post-transplant by Fluorescent In-situ Hybridization (FISH) XY chromosome analysis in case of sex mismatch and by PCR for variable number tandem repeats (VNTR) analysis in case of sex similarity between donor and recipient (Mahmoud et al., 2014).

Outcome definitions

End point of the study on 30th June, 2020. OS was defined as the time from HSCT to death or the last follow-up. TFS was defined as the time from HSCT to recurrent transfusion-dependent thalassemia (graft failure), death or the last follow-up (Bernardo et al., 2012). Many patient and transplant related factors were analyzed to detect risk factors of OS and TFS.

Detection of complications post-transplant

Post-transplant complications were reported; include blood stream bacterial infections (BSI), viral infections as cytomegalovirus, proven fungal infections (Donnelly et al., 2020), pulmonary complications, AGVHD (Schoemans et al., 2018), sinusoidal obstructive syndrome (SOS), capillary leak syndrome (CLS), transplant associated micro-angiopathy (TAM), engraftment syndrome (ES), hemorrhagic cystitis (HC), neurological comorbidities, CGVHD, cardio-vascular complications, endocrinopathies and secondary malignancy. Primary graft failure was considered when the graft of donor-origin after infusion failed to establish efficient hematopoiesis. While, secondary graft failure was considered when absolute neutrophil count (ANC) < 5×10^9 /L after initial engraftment (Mahmoud et al., 2014).

Statistical analysis

Continuous variables are reported as medians with interquartile ranges; while categorical variables are reported as counts and percentages. The baseline characteristics of subjects with complications were compared using the chi square test or Fisher's exact test for categorical variables and independent t-testing for parametric variables. Survival rates from time of transplant (OS and TFS) were estimated using the Kaplan-Meier method and comparisons between groups were made using the log-rank test. Significant variables in univariate analysis Variables were tested in multivariate analysis to find the independent prognostic factors affecting survival after transplant using cox regression analysis. *P*-value was always two-tailed and significant at 0.05 level. All statistical analyses were performed using SPSS software version 26.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients' characteristics and transplant related data

We studied 115 pediatric patients with BTM; most of them were in class II (47.8%), while class I & III included 37.4% and 14.8% of the patients respectively. Median age of the total group was 5.5 years, with a significant difference in the age distribution of included patients reported between the three classes (P<0.001). Males were more predominant in all classes with no statistical significance. The median interval time

from diagnosis to transplant was 53 months, that was significantly higher in class III (P<0.001). Regarding time of engraftment of polymorphonuclear (PMN) cells and platelets; it was significantly different between three classes (P=0.039 and P=0.008, respectively), with the shortest median engraftment time of both PMN cells and platelets was reported in class III patients. Patients' characteristics and transplant related data (Table 1).

Outcome

Twenty-three (20%) of the included patients died post-transplant. Class II patients represented most of deaths (47.8%). AGVHD reported as the most common cause of death among patients (30.2% of died patients), especially those in class III (17.4%). Sepsis (26.1%), chest infection (17.4%) and SOS (13%) represented other causes of death. Table 2 showed causes of death of included patients. Three-year OS of all patients was 80%; that was significantly different among the three patients' classes (P=0.036) as shown in Figure 1, with class III had the lowest 3-years OS 58.8% (95% CI, 33%-82%), while class I had the highest OS 88.4% (95% CI, 74%-90%). Regarding 3-year TFS of all patients; it was 77.4%, class I & II patients were 83.7% & 78.2% respectively, while class III patients had the lowest (58.8%) with no statistical significance (*P*=0.113) as shown in Figure 2.

Patient's age, conditioning regimen type, GVHD prophylaxis and Pesaro classes were statistically significant prognostic factors affecting the 3-year OS of the study group in the univariate analysis (*P*< 0.001, 0.031, 0.008 and 0.036 respectively). In multivariate analysis; only patient's age and type of GVHD prophylaxis were found to be significant independent risk factors, where patients age group (5-10 years) had more OS than patients aged > 10 years) (P= 0.001) and MTX based regimen had better outcome compared to regimen based on ATG & steroid (P= 0.004). Regarding 3-year TFS; patient's age and type of GVHD prophylaxis were significantly affect it (P= 0.001 & 0.041 respectively). With multivariate analysis patient's age and GVHD prophylaxis still had a significant effect on TFS; where patients at age group (5-10 years) had better TFS than those aged > 10 years (P= 0.009). Also, patients received MTX within their GVHD prophylaxis regimen had TFS better than patients received ATG and steroid (P= 0.029). Prognostic risk factors affecting 3-year OS and TFS (Table 3).

Post-transplant complications

Post-transplant complications reported in 80% of all patients with no significant difference reported in incidence and distribution between the three patients' classes. Nearly 64% of all patients reported to have infectious complications, BSI (55.6%) was the most common. BSI and proven fungal infections reported more commonly in class II patients (60% and 3.6%, respectively), while viral infections reported more in class III (17.6%). Pulmonary complications and neurotoxicity were more common in class II patients (40% and 21.8% respectively). As regard AGVHD; 22.6% of all patients developed AGVHD mainly in class III (36.3%). Also, SOS, CLS and TAM were more common in class III patients (11.8%, 5.9% and 11.8%, respectively). ENS occurred more in class I patients (1.7%), while HC more in class II (21.8%). Other complications were higher in class III patients in the form of; CGVHD, cardiotoxicity, endocrinopathy and secondary malignancy represented in 11.8%, 11.8%, 17.6% and 5.9% respectively. Also, graft failure occurred more in class III patients (11.8%). Incidence of post-transplant complication in studied patients (Table 4).

DISCUSSION

In Egypt, B-thalassemia is considered the commonest hereditary hemolytic anemia, with a carrier state varying between 6% and 10% (El-Beshlawy et al., 1995). Allo-HSCT, apart from gene therapy, is the only curative therapy for thalassemia (Issaragrisil and Kunacheewa, 2016). Pesaro classification is an established system of classifying BTM patients prognostically into 3 classes, has been described and tested in patients younger than 16 years of age (Lucarelli et al., 1993). With analysis of 115 pediatric patients, most of them reported as class II (47.8%). Around 60% of the patients received PBSC as the source of stem cells as there are higher T-cell content of PBSC grafts, which in turn leads to less rejection rate.

Table1. Patients' characteristics and transplant related data.

	Total N. 115 (100%)	Class 1 43 (37.4%)	Class II 55 (47.8%)	Class III 17 (14.8%)	P. Value
Age:					
Median (range) years	5.5 (1.8-18)	3.9 (1.8-7)	6.4 (2.5-12.5)	13.4 (7.6-18)	**< 0.001****
< 5		38 (88.4%	14 (25.5%)	0	
5-10		5 (11.6%)	38 (69%)	3 (17.6%)	
> 10		0	3 (5.5%)	14 (82.4%)	
Sex:					
Male	73 (63.5%)	26 (60.5%)	34 (61.8%)	13 (76.5%)	0.479
Female	42 (36.5%)	17 (39.5%)	21 (38.2%)	4 (23.5%)	
Time interval from diagnosis-transplant:					
Median (range) months	53 (5-210)	35 (5-78)	65 (6-144)	154 (53-210)	**<0.001****
Ferritin level:					
< 1500	56 (48.7%)	27 (62.8%)	23 (41.8%)	6 (35.3%)	0.058
> 1500	59 (51.3%)	16 (37.2%)	32 (58.2%)	11 (64.7%)	
CMV IgG r/d:					
P/P	95 (82.6%)	33 (76.7%)	48 (87.3%)	14 (82.4%)	
P/N	7 (6.1%)	3 (7%)	3 (5.5%)	1 (5.9%)	0.603
N/P	11 (9.6%)	6 (14%)	4 (7.3%)	1 (5.9%)	
N/N	2 (1.7%)	1 (2.3%)	0	1 (5.9%)	
Conditioning regimen:					
BU/CY	100 (87%)	43 (100%)	55 (100%)	2 (11.8%)	**< 0.001****
FLU/BU/CY	15 (13%)	0	0	15 (88.2%)	
GVHD prophylaxis:					
CSA, MTX	45 (39.1%)	42 (97.7%)	2 (3.6%)	1 (5.9%)	
CSA, steroid	3 (2.6%)	0	3 (5.5%)	0	**< 0.001****
CSA, ATG	22 (19.2%)	0	21 (38.2%)	1 (5.9%)	
CSA, ATG, steroid	45 (39.1%)	1 (2.3%)	29 (52.7%)	15 (88.2%)	
Stem cell source:					
BMSC	47 (40.9%)	42 (97.7%)	5 (9.1%)	0	**< 0.001****
PBSC	68 (59.1%)	1 (2.3%)	50 (90.9%)	17 (100%)	
CD34 count:					
Median (range)	7.1 (1.7-50)	5.8 (1.7-24)	7.7 (1.7-50)	7.4 (2.8-48)	0.065
1.3-4		10 (23.3%)	4 (7.3%)	3 (17.6%)	
4.1-7		16 (37.2%)	19 (34.5%)	4 (23.5%)	
> 7		17 (39.5%)	32 (58.2%)	10 (58.8%)	
GCSF received:	75 (65.2%)	42 (97.7%)	28 (50.9%)	5 (29.4%)	**< 0.001****
PMN engraftment:					
Median (range) days	14 (10-29)	14 (11-23)	15 (11-29)	13 (10-23)	**0.039***
PLT engraftment:		20 (12-63)	19 (9-43)	13.5 (10-28)	**0.008****
Median (range) days	19 (9-63)				

* Statistically significant difference (p<0.05)**, **** Highly statistically significant difference (p<0.01). CMV: cytomegalovirus, BU: busulfan, CY: cyclophosphamide, FLU: fludarabine, GVHD: graft versus host disease, CSA: cyclosporin A, MTX: methotrexate, ATG: anti-thyroglobulin G, BMSC: bone marrow stem cells, PBSC: peripheral blood stem cells, CD34: cluster of determinants 34, PMN: polymorphonuclear cells, PLT: platelet.

Three-year OS was 80% and TFS was 77.4%, with significant difference in OS between the three disease classes. This finding is lower than that documented on both EBMT registry at 2016 (OS and EFS from MSD was 91% and 83% respectively) and the Chines study (OS and TFS were 92% & 90.8% respectively) (Baronciani et al., 2016 and Huang et al., 2021). This could be attributed to more time delay in referral of patients to BMT centers in developing countries with increasing age at time of transplant that has independent worse impact on outcome in our study. Also, less compliance of iron chelation therapy, which lead to aggravation of thalassemia toxicities and more complications after HSCT. Class III BTM patients had the worst OS and TFS in our study; this was in contrast to the Italian study, 2012 which didn't show any effect of class of risk, or the donor type on the outcome.

Table 2. Causes of death among the study group (115 patients).

	Total N. 23 (20%)	Class I 5 (21.7%)	Class II 11 (47.8%)	Class II 7 (30.4%)	P. Value
AGVHD	7 (30.4%)	2 (8.7%)	1 (4.3%)	4 (17.4%)	0.368
Sepsis	6 (26.1%)	0	5 (21.7%)	1 (4.3%)	0.392
Chest infection	4 (17.4%)	3 (13%)	1(4.3%)	0	0.119
SOS	3 (13%)	0	2 (8.7%)	1 (4.3%)	0.666
Graft failure	2 (8.7%)	0	1(4.3%)	1(4.3%)	0.669
Seizures	1 (4.3%)	0	1 (4.3%)	0	-

AGVHD: acute graft versus host disease, SOS: sinusoidal obstruction syndrome.

Table 3. Prognostic factors affecting 3-year overall survival and thalassemia free survival.

	Thalassemia free survival				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate	
	TFS±SE	P-value	HR	P-value	OS±SE	P-value	HR	P-value
Patient gender:								
Male	82.2+/-7%	0.136			83.6+/-4%	0.235		
female	69+/- 4%				73.8+/- 7%			
Age:								
< 5 years	78.8+/- 6%		0.695	0.520	82.7+/- 5%		0.338	0.122
5-10 years	87+/- 5%	***0.001***	0.231	***0.009***	89.1+/- 5%	***0.000***	0.094	***0.001***
>10 years	47.1+/-12%		Ref		47.1+/-13%		Ref	
Pesaro class:								
Class I	83.7+/- 7%				88.4+/-5 %			
Class II	78.2+/- 6%	0.1133			80+/- 5%	***0.036***		
Class III	58.8+/-13%				58.8+/-13%			
Ferritin category:								
< 1500	80.4+/- 5%	0.434			83.2+/- 5%	0.526		
> 1500	74.6+/- 6%				78.5+/- 6%			
CMV IgG r/d:								
P/P	76.8+/- 4%				80+/- 4%			
P/N	71.4+/-19%	0.529			68.6+/18%	0.528		
N/P	90.9+/-9%				90.9+/-9%			
N/N	50+/- 35%				50+/- 35%			
Conditioning regimen:								
BU/CY	80+/- 4%	0.061			83.5+/- 4%	***0.031***	3.104	0.101
FLU/BU/CY	60+/- 14%				61.5+/-13%		Ref	
GVHD prophylaxis:								
CSA, ATG	86.4+/- 7%		0.483	0.287	86.4+/- 7%		0.480	0.281
CSA, MTX	86.7+/- 5%	***0.041***	0.273	***0.029***	91.1+/- 5%	***0.008***	0.154	***0.004***
CSA, steroid	66.7+/-35%		1.120	0.918	100%		0.000	0.981
CSA, steroid, ATG	64.4+/- 7%		Ref		64.4+/- 7%		Ref	
Graft source:								
BMSC	80.9+/- 7%	0.506			85.1+/- 6%	0.292		
PBSC	75+/- 5%				76.5+/- 5%			
CD34 count:								
1.3-4	70.6+/-11%				70.6+/-11%			
4.1-7	79.5+/- 7%	0.711			82.1+/- 7%	0.580		
> 7	78+/- 6%				81.4+/- 5%			

CMV: cytomegalovirus, BU: busulfan, CY: cyclophosphamide, FLU: fludarabine, GVHD: graft versus host disease, CSA: cyclosporin A, MTX: methotrexate, ATG: anti-thyroglobulin G, BMSC: bone marrow stem cells, PBSC: peripheral blood stem cells, CD34: cluster of determinants 34, OS: overall survival, TFS: thalassemia free survival.

Table 4. Incidence of post-transplant complication in studied patients.

	Total N. 115 (100%)	Class 1 43 (37.4%)	Class II 55 (47.8%)	Class III 17 (14.8%)	P. Value
All complications:	92 (80%)	31 (72.1%)	47 (85.5%)	14 (82.4%)	0.251
All infections:	73 (63.5%)	27 (62.3%)	35 (63.6%)	11 (64.7%)	0.990
BSI	64 (55.6%)	22 (51.2%)	33 (60%)	9 (52.9%)	
Proven fungal infection	3 (2.6%)	1 (2.3%)	2 (3.6%)	0	
Viral infections	12 (10.4%)	6 (14%)	3 (5.5%)	3 (17.6%)	
Pulmonary complications:	44 (38.3%)	16 (37.2%)	22 (40%)	6 (35.3%)	0.926
AGVHD:	26 (22.6%)	7 (16.3%)	13 (23.6%)	6 (35.3%)	0.449
Grade I-II	17 (14.8%)	4 (9.3%)	10 (18.2%)	3 (17.6%)	
Grade III-IV	9 (7.8%)	3 (7%)	3 (5.4%)	3 (17.6%)	
CGVHD:	8 (6.9%)	1 (2.3%)	5 (9.1%)	2 (11.8%)	0.293
Limited	5 (4.3%)	0	4 (7.3%)	1 (5.9%)	
Extensive	3 (2.6%)	1 (2.3%)	1 (1.8%)	1 (5.9%)	
Neurotoxicity:	21 (18.2%)	7 (16.3%)	12 (21.8%)	2 (11.8%)	0.447
Seizures	17 (14.8%)	7 (16.3%)	8 (14.5%)	2 (11.8%)	
Headache	2 (1.7%)	0	2 (3.6%)	0	
Neuropathy	2 (1.7%)	0	2 (3.6%)	0	
SOS:	8 (6.9%)	2 (4.6%)	4 (7.3%)	2 (11.8%)	0.616
CLS:	4 (3.5%)	1 (2.3%)	2 (3.6%)	1 (5.9%)	0.792
ENS:	3 (2.6%)	2 (4.7%)	1 (1.8%)	0	0.523
TAM:	12 (10.4%)	4 (9.3%)	6 (10.9%)	2 (11.8%)	0.949
HC:	17 (14.8%)	3 (7%)	12 (21.8%)	2 (11.8%)	0.113
Cardio-vascular toxicity:	9 (7.8%)	2 (4.6%)	5 (9.1%)	2 (11.8%)	0.334
Cardiac dysfunction	3 (2.6%)	0	2 (3.6%)	1 (5.9%)	
Hypertension	2 (1.7%)	0	2 (3.6%)	0	
Thrombo-embolic	4 (3.5%)	2 (4.6%)	1 (1.8%)	1 (5.9%)	
Endocrinopathy:	7 (6.1%)	2 (4.7%)	2 (3.6%)	3 (17.6%)	0.095
Secondary malignancy:	1 (0.8%)	0	0	1 (5.9%)	0.055
Engraftment failure:	7 (6.1%)	2 (4.6%)	3 (5.4%)	2 (11.8%)	
Primary	1 (0.8%)	0	1 (1.8%)	0	0.459
Secondary	6 (5.2%)	2 (4.6%)	2 (3.6%)	2 (11.8%)	

Chi-square test. BSI: blood stream infection, AGVHD: acute graft versus host disease, CGVHD: chronic graft versus host disease SOS: sinusoidal obstruction syndrome, CLS: capillary leak syndrome, ENS: engraftment syndrome, TAM: transplant associated micro-angiopathy, HC: hemorrhagic cystitis.

This could be attributed to the use of reduced intensity conditioning regimen containing treosulfan in their study (Baronciani et al., 2016). On the other hand, Sodani et al found that; adding fludarabine to the conditioning regimen of class III patients leads to improvement of the probability of thalassemia-free survival from 58% to 85% together with a reduction from 30% to 8% of the probability of the return of the thalassemic hematopoietic clone after transplantation (Sodani et al., 2004). Conversely, we reported that adding fludarabine didn't improve survival of class III patients.

Age of the patient and type of GVHD prophylaxis were found as a significant independent risk factors affecting both OS & TFS at our study. EBMT registry also reported that; age of the patients was significantly affecting survival outcome of BTM patients, with the optimal transplant outcomes was associated with a threshold age of around 14 (Baronciani et al., 2016), here we found the age group 5-10 years was associated with the best outcomes among all patients. Although ATG was added to decrease alloreactivity and graft failure, its use was associated with lower survival outcomes.

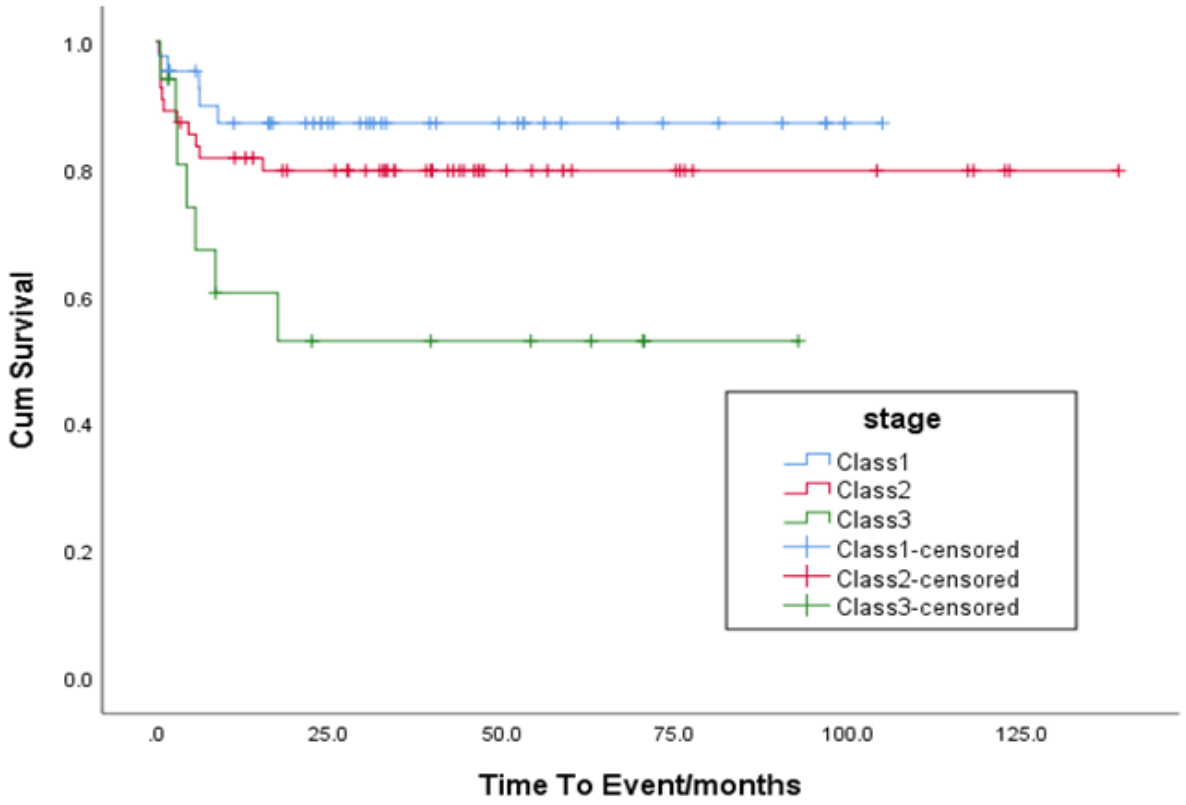

Figure 1. Three-year overall survival for the three classes of beta thalassemia major of the studied patients.

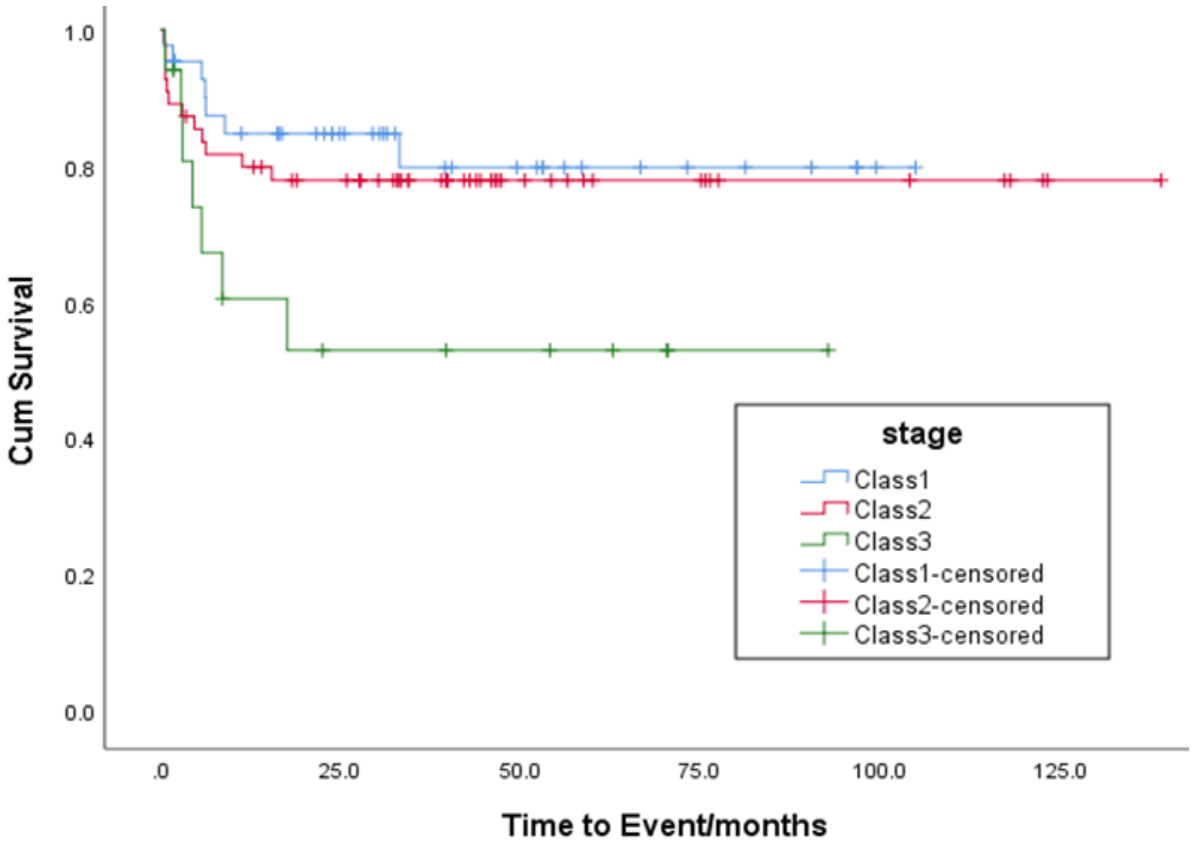

Figure 2. Three-year thalassemia free survival for the three classes of beta thalassemia patients of the studied patients.

MTX prophylaxis showed better outcomes in group I patients, this can be explained by better biology and less toxicity of disease before HSCT in this group.

Infection rate in our study was 63.5%; mainly bacterial infections (55.6%), with no significant difference between the three disease classes. Goussetis et al., reported incidence of infections was (67.5%) with higher viral infection (53.3%) and less bacterial infections (14.2%) (Goussetis et al., 2015). Using ATG based conditioning regimen associated with higher rates of infections (especially viral reactivation), which was reported by Nishihori et al, (Nishihori et al., 2016). This's explains why infection rate was higher in Goussetis et al., study, where ATG used in all of their patients and only in 58% our patients.

Fludarabine was added in conditioning regimen of class III patients, because its known marrow suppressive and high immunosuppressive activities (Sodani et al., 2004). In this study, with addition of fludarabine and ATG to the conditioning regimen of class III patients was not associated with lower incidence of GVHD and graft rejection in comparison to class I & II patients. Overall incidence of AGVHD and CGVHD was 22.6% & 6.9% in the current study; which was higher than reported in an earlier study carried in the same center from 1997 to 2012, where AGVHD (only grade II-IV, where grade I not reported) reported in 15% and CGVHD in 12% of the patients (Mahmoud et al., 2014). All patients of the former study received BU/CY/ATG without fludarabine.

Late cardiac toxicity and endocrinopathy were also detected more in class III patients, mostly due to extensive iron overload in these organs with inappropriate chelation therapy before transplant. In French national experience published in 2018, they found late endocrinological complications occurred in around 40% of the included patients (compared to 6.1% reported in our patients); this could be due to longer follow up time in the French study (12 years), compared to following up patients in this study (3 years), usually endocrinal complications take long time to appear. In contrast, the French study detected only 2% of their patients experienced late cardiac toxicity, which was lower than incidence in our study (7.8%); this could be due to late presentation to BMT unit after previous repeated blood transfusion with higher ferritin level before transplant found in our study and its cardiomyopathic effect (Rahal et al., 2018).

Engraftment failure was reported in 6.1% of our patients, this finding was comparable to the incidence of graft failure in Bernardo et al., 2012 study (8%), despite they use reduced intensity conditioning regimen (Bernardo et al., 2012). As a retrospective study; based mainly on registry, results might be prone to information or selection bias. Large scale studies are needed for further evaluation of allo-HSCT from fully matched versus mis-matched donors; where the latter is more usual and applicable, especially after adoption of haplo-identical technique.

CONCLUSION

Survival rates of BTM patients after transplantation are still lower than literature, class I & II BTM patients has better outcome than class III. Referral of BTM patient to allo-HSCT before age of 10 years lead to better outcome. Adding fludarabine to the conditioning regimen of class III patients had no impact in decreasing the occurrence of GVHD nor graft failure, and didn't improve survival in comparison to class I and II patients.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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