Acute Graft *versus* Host Disease in Beta Thalassemia Patients Following Allogeneic Haematopoietic Stem Cell Transplantation

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ABSTRACT

Objective: To analyse the frequency, risk factors, and clinical symptoms of acute graft-versus-host disease (aGvHD) in patients with beta-thalassemia major after allogeneic haematopoietic stem cell transplantation (HSCT).

Study Design: Descriptive study.

Place and Duration of the Study: Department of Clinical Haematology, Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan, from January 2017 to December 2021.

Methodology: Data were obtained from patients diagnosed with bone and tissue malignancies (BTM) who had undergone haematopoietic stem cell transplantation (HSCT) and experienced aGVHD. Patients who experienced initial graft failure and individuals who underwent subsequent bone marrow transplantation were excluded.

Results: Total of 117 patients diagnosed with BTM underwent fully matched HSCT, including 76 (65%) males, and 41 (35%) females. The median age of the patients undergoing transplantation was 7.34±7.32 years and the donors' median age was 7.6±9.85 years. Among the donors, 53 (45.3%) were males and 64 (54.7%) were females. Gender disparity was observed in 46 (39.3%) instances as a female donor matched with a male recipient. A total of 106 individuals underwent bone marrow harvest (BMH); with 5 (4.3%) patients receiving peripheral blood stem cells (PBSC) and 6 (5.2%) patients receiving both BMH and PBSC. Acute GvHD was observed in 50 (42.7%) patients, including 30 (60%) males and 20 (40%) females. Grade I GvHD occurred in 32 (27.3%) individuals, Grade II GvHD in 16 (13.7%) patients, and Grade III GvHD in one (0.8%) patient. It had no statistically significant association with recipient/donor age, gender disparity, the source of the graft source, the dose of stem cells, or the presence of thymoglobulin (TG).

Conclusion: Acute GvHD was observed in high frequency in Beta-thalassemia patients receiving morrow harvesting proportional to their gender distribution. Associated factors were GvHD prophylaxis measure, mucositis and, CMV reactivation.

Key Words: *Beta thalassemia major patients, Acute graft versus host disease, Allogeneic haematopoietic stem cell.*

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INTRODUCTION

Beta-thalassemia major (BTM) is an inherited disorder characterised by the deficiency or absence of beta-globin chains.[1] The global yearly occurrence of BTM is estimated to be around one in ten lac individuals.[2] In Pakistan, the prevalence of thalassemia is high, with approximately 5% of the population being carriers.[3] There are about one million registered thalassemia patients in Pakistan, and over five thousand new cases are diagnosed each year.[4]

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Traditional therapies including red blood cell transfusion and iron chelation have increased thalassemia patients' quality of life and survival rates.[5] Among the limited choices, allogeneic haematopoietic stem cell transplantation (HSCT) stands as the sole viable option for these patients. However, the success and efficacy of HSCT in BTM patients can be influenced by various factors, including the age of the patient, the source of the stem cells used for transplantation, the histocompatibility between the donor and recipient, and the specific conditioning regimen employed. These factors play a crucial role in determining how well HSCT functions in addressing the needs of BTM patients.[6] Acute GvHD is a severe complication that commonly arises following transplantation and has a significant impact on the initial outcomes.[7] HLA mismatch is thought to be the most important risk factor among the many things connected to the development of aGvHD. Other factors include gender mismatch between the recipient and donor, the age of both the recipient and donor, the choice of GvHD prophylaxis, the use and dose of thymoglobulin (TG), the type of graft used, the total nucleated

cell (TNC), and CD34 dosage of the graft, as well as the recipient's cytomegalovirus (CMV) viral seropositivity.[8] The incidence of moderate-to-severe aGvHD after allo-HSCT is approximately 40%, but this can vary based on donor type and GvHD prophylaxis method.[9]

However, it is crucial to recognise that the occurrence of aGvHD and its associated risk factors can differ depending on the transplantation protocol, the characteristics of the patient population, and individual variables. Monitoring and managing these risk factors closely are crucial for reducing the occurrence and severity of aGVHD after allo-HSCT.[8] The aim of this study was to analyse the prevalence, risk factors, and clinical symptoms of aGVHD in patients with beta-thalassemia major after allogeneic HSCT.

METHODOLOGY

Patients who underwent fully matched HSCT at the Armed Forces Bone Marrow Transplant Center (AFBMTC) between January 2017 and December 2021 were inducted. The study excluded individuals who underwent a second transplant for BTM, ensuring that the analysis focused solely on the initial transplant. This study adhered to rigorous ethical guidelines, ensuring that parents or guardians of all participants provided informed consent. Additionally, the study received formal approval from the institutional review board, further ensuring the ethical conduct and oversight of the research. The collected data encompassed various factors, including the age and gender of both the recipients and donors, dosage of TG used in the conditioning regimen, source and dosage of infused stem cells, medications administered for GvHD prophylaxis, the stage and aGVHD, presence of mucositis, CMV status of the recipient, response to aGvHD treatment, and mortality outcomes. Data were retrieved from the medical records of the hospital.

All patients underwent a myeloablative conditioning regimen (MAC), which consisted of fludarabine (Flu), busulfan (BU), and cyclophosphamide (Cy). Thymoglobulin (TG) was optionally included in the regimen. Mucositis, characterised by oral cavity inflammation resulting in oral pain and difficulties in food consumption, was assessed based on the presence of erythema or ulcers. World Health Organization (WHO) criteria was used for grading of oral mucositis.[10]

CMV polymerase chain reaction (PCR) tests were conducted on day 14 post-transplantation, and subsequent tests were performed every two weeks. Recipients with a CMV viral load exceeding 2000 copies/ml were classified as experiencing CMV reactivation. Using the Glucksberg-Seattle criteria, aGvHD that affects skin, gut, and liver was assessed.

Topical steroids were the exclusive mode of therapy for Grade 1 of aGvHD. Systemic therapy was necessary for higher degree of aGvHD. The primary treatment approach involved administering a high dose of methylprednisolone/prednisolone at a dosage of 2 mg/kg/day for a duration of 7-14 days. After that dosage of steroids was gradually decreased. aGvHD was categorised as steroid-refractory if there was insufficient response within 7-14 days or if it got worse within 5 days of starting treatment.

SPSS version 24 was used to analyse the data gathered for this study. The age of the donor and recipient, TNC (total nucleated cell) count, and CD34 dose were considered as quantitative variables. The mean values and standard deviation of these variables were calculated to understand their central tendency and variability. On the other hand, qualitative variables, such as, patient and donor gender, graft type, GvHD prophylaxis, presence of aGVHD, aGVHD grades, response to steroid treatment, mucositis, and CMV status of patients, were analysed to determine their frequencies and percentages, providing insights into the distribution and prevalence of these variables in the study population.

To examine the relationship between aGVHD and other risk variables, a post-stratification chi-square test was conducted. The statistical analysis conducted in this study revealed a strong correlation exists between the variables, which was determined by a p-value of ≤ 0.05 or lower. This finding underscores the importance of the relationship between the variables and highlights their potential impact within the context of the study.

RESULTS

Among the participants, 77 (65.8%) were males, while 40 (34.2%) were females. The patients undergoing the transplantation procedure had an average age of 7.34±7.32 years. In comparison, the donors contributing haematopoietic stem cells had an average age of 7.6±9.85 years. Table I provides detailed information about the conditioning protocol, stem cell dose, and other relevant transplant details.

Table I: Transplant details.

Source of stem cell	
Bone marrow harvest	106 (90.6%)
Peripheral blood stem cells only	5 (4.3%)
Mixed (BMH + PBSC)	6 (5.1%
Gender mismatch	
No gender mismatch	48 (41%)
Gender mismatch	69 (59.%)
Female donor to male patient	46 (39.3%)
GVHD prophylaxis	
CSA+MTX	98 (83.7%)
CSA+MTX+MMF	19 (16.3%)
Conditioning protocol	
Flu120, BU14, Cy160	18 (15.3%)
Flu120, BU16, Cy160	72 (61.5%)
Flu150, BUIV16, Cy160	14 (12.0%)
Flu150, BuIV16, Cy160,TT10	02 (1.7%)
Flu150, Bu14, Cy200	11(9.4%)
TG / ATG in conditioning regimen	
Yes	99 (84.6%)
No	18 (15.4%)
Mean TNC/MNC dose x 10^8/kg	5.40±2.14
Mean CD34 dose x 10^6/kg	7.78±4.9

Acute GvHD was identified in 50 cases, representing 42.7% of the total cases. Among those affected, 32 (27.3%) patients had Grade I aGVHD, 16 (13.6%) patients had Grade II, and only one (0.8%) had aGVHDs of Grade III. Eighty percent of patients (n=40) involved skin involvement, including 12 instances in stage I, 17 in Stage II, 10 in Stage III, and just one in stage IV of skin GVH. Gut GvHD was noted in 9 (18%) patients, with 4 patients in Stage I, 2 in Stage II, 2 in Stage III, and 1 in Stage IV. Eight patients (16%) experienced both gut and skin GvHD (Grade II), while one (2%) patient was found to have stage I hepatic GvHD.

Among the individuals who received this combination therapy, there were 16 (32%) cases of aGVHD, whereas in the group that received only CSA and MTX, there were 34 (68%) cases. These findings underscore the potential benefits of incorporating MMF into the prophylactic regimen, suggesting its role in reducing the incidence of aGVHD in transplant recipients. This difference in incidence rates was determined significant, with a $p<0.001$. No statistically significant associations were found after looking at a number of factors related to aGVHD, including recipient or donor age, gender mismatch, graft source, stem cell dosage, and TG. These factors did not demonstrate a significant influence on the incidence of aGVHD within the study population.

Furthermore, mucositis was observed in 50 patients, accounting for 42.7% of the total cases. Among those patients, 43 (86%) developed aGVHD. The study findings demonstrated a significant correlation between mucositis and GvHD ($p<0.001$).

The duration of steroid treatment for aGVHD varied from 10 to 50 days. The average response time to treatment was 8±12 days. Among the patients, 46 (92%) showed a positive response to steroid treatment, while four (8%) cases experienced steroid-refractory aGvHD that necessitated second-line therapy involving Infliximab and Ruxolitinib.

Out of the total of 117 patients, 21 (18%) unfortunately passed away, resulting in an overall survival rate of 82%. Among the total cases (n=19), the majority (90.4%) experienced a range of complications that ultimately resulted in their death. Only 9.6% of cases of aGVHD were shown to be the cause of mortality directly attributable to the patient.

CMV reactivation was noted in 29 (24.7%) of the cases. It shows a strong association between the occurrence of aGVHD and CMV reactivation ($p<=0.001$). This finding indicates that the administration of corticosteroids for aGVHD management may contribute to the reactivation of CMV in transplant recipients.

DISCUSSION

Acute aGvHD was shown to have a frequency of 42.7% among the patients receiving bone marrow transplants in this study. This finding is consistent with the documented results in international literature, which also reported a similar incidence rate of 40%.[11] The age of either the recipient or the donor did not appear to have a notable impact on the likelihood of developing aGVHD in this study population. These findings align with the results reported by Jagasia *et al*. In addition, these results showed no statistically significant association between the genders of the donor and recipient in terms of a higher risk of aGVHD.[12] Likewise, the source of the transplanted stem cells did not demonstrate a significant correlation with the likelihood of developing aGVHD. These results are in line with Flowers *et al*.[13] This study's findings also agreed with those of another study, which found no connection between the CD34 dosage and the probability of getting aGVHD.

According to this study's results, the TG group's cumulative incidence rate of grade 2-4 aGVHD was significantly lower (13.7%) than the control group's (27.0%).[14] Furthermore, this study demonstrated a noteworthy finding which was not reported in similar studies. Patients who were administered Mycophenolate-Mofetil (MMF) along with Cyclosporine A (CSA) and Methotrexate (MTX) as a prophylactic measure against GvHD exhibited a lower frequency of developing acute GvHD compared to those who received only CSA and MTX. This suggests that the addition of MMF to the prophylactic regimen may have a beneficial effect in reducing the occurrence of aGVHD.

Furthermore, this study finding revealed a significant association between the presence of mucositis and an increased risk of developing aGVHD. Patients who experienced mucositis were more likely to develop aGVHD compared to those who did not encounter mucositis during their treatment.

Similarly, patients who developed aGVHD had a higher risk of subsequent reactivation of CMV. This can be attributed to the fact that systemic corticosteroids, which are commonly used to treat aGVHD, are known to be significant risk factors for CMV reactivation. Furthermore, it is worth noting that the occurrence of CMV reactivation was found to be associated with the subsequent development of aGVHD. This suggests a potential link between CMV reactivation and the pathogenesis of aGVHD, highlighting the importance of monitoring and managing CMV reactivation in transplant patients to mitigate the risk of aGVHD. In simpler terms, the presence of CMV reactivation increased the risk of developing grade 2-4 aGVHD, which aligns with findings reported in an international study.

Mucositis was linked to a higher frequency of aGVHD, while aGVHD itself increases the likelihood of CMV reactivation. These findings shed light on the intricate relationship between mucositis, aGVHD, and CMV reactivation in transplantation.[15]

The main limitation of this study is that it was conducted in just one centre. In addition, the study only included a small number of patients and was carried out on a modest scale.

CONCLUSION

Individuals who got MMF together with CSA and MTX as prophylaxis for GvHD had a lower probability of developing aGVHD. Patients who experienced mucositis also had a risk of aGVHD. Usage of MMF is suggested as a method to lower the risk of GvHD. Additionally, the prevention and early management of

mucositis have shown potential in lowering the risk of aGVHD. It is worth noting that CMV reactivation is associated with the treatment of aGVHD using steroids.

ETHICAL APPROVAL:
The study was conducted after obtaining official approval from the institutional review board (IRB#007/AFBMTC/Approval/2018).

PATIENTS' CONSENT:
Informed consent was obtained from the participants' parents or guardians to publish the data concerning their cases.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
NR: The acquisition, drafting of the manuscript, and 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.
AA: Conception and design of the work.
TAK: Revising it critically for important intellectual content.
TG: Analysis and interpretation of data for the work.
MBA: Revising it critically for important intellectual content.
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