HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH REDUCED TOXICITY CONDITIONING REGIMEN IN MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOPATHY SYNDROME (MNGIE)

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Abstract word count: 199

Text word count: 2437

Short running title: Hematopoietic Stem Cell Transplantation In MNGIE

Key words: MNGIE, hematopoietic stem cell transplantation, treosulfan

Number of tables: 2

Abbreviations:

*TYMP*: Thymidine phosphorylase

MNGIE: Mitochondrial Neurogastrointestinal Encephalomyopathy

AHSCT: Allogeneic Hematopoietic Stem Cell Transplantation

VOD: Veno-occlusive disease

mtDNA: Mitochondrial DNA

GVHD: Graft versus host disease

BM: Bone marrow

PBSC: Peripheric blood stem cell

Flu/Treo: Fludarabine/Treosulfan

CSA: Cyclosporine

MTX: Methotrexate

SUMMARY

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare autosomal recessive disorder due to mutations in the *TYMP* gene. Clinical findings are characterized by neurologic manifestations and severe gastrointestinal dysfunction. The syndrome is usually fatal, the most effective treatment appears to be hematopoietic stem cell transplantation (HSCT). Aim:In this retrospective study, we evaluated HSCT that was performed using a reduced toxicicity myeloablative conditioning regimen in patients with MNGIE at our center. Results: A total of 6 allogeneic transplant procedures were performed in 4 patients. Three patients' donors had fully matched donors, and one patients’ donor was haploidentical. Treosulfan-based myeloablative conditioning regimen was applied in 5 of 6 transplants. Bone marrow was used as a stem cell source. One patient is being followed up in the 4th year of posttransplant with full chimeric and without Graft versus host disease (GVHD). One patient died of acute stage IV GIS GVHD. Two patients underwent second transplantation due to engraftment failure, one of which was the patient who had a haploidentical transplant. Conclusions: Treosulfan-based regimen is well tolerated. Engraftment failure with this conditioning regimen can be a significant problem. We share our haploidentical transplant experience, which will be the first reported case in the literature.

INTRODUCTION

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is caused by mutations in the thymidine phosphorylase gene (*TYMP*) located on chromosome 22q13.33, which results in the accumulation of the thymidine (dThd) and deoxyuridine (dUrd) substrates, nucleotide pool imbalance, and mtDNA instability with impairment of the mitochondrial genome replication and depletion, multiple deletions, and point mutations1,2. MNGIE is an ultra-rare condition, characterized by severe gastrointestinal (GI) and neurological symptoms; gastrointestinal dysmotility, cachexia, ptosis, ophthalmoparesis or both, peripheral neuropathy and leukencephalopathy3. The outcome of the syndrome is fatal, and death usually occurs before the age of 40 years, although mild/atypical phenotypes have been reported3. There are no established therapeutic options for patients with MNGIE. With conventional hemodialysis and erythrocyte-encapsulated thymidine phosphorylase infusions, plasma dThd and dUrd concentrations can be temporarily increased4,5. Currently, the only effective treatment is the allogeneic hematopoietic stem cell transplantation (AHSCT), which restores persistently TP activity and reduces circulating toxic levels of dThd and dUrd6. However, hematopoietic stem cell transplantation is still associated with high mortality in MNGIE patients7. The number of transplanted patients in the literature is limited. In this presented study we aimed to evaluate the results of our patients with MNGIE who underwent allogeneic hematopoietic stem cell transplantation.

MATERIAL AND METHODS

We retrospectively evaluated the patients who underwent stem cell transplantation with the diagnosis of MNGIE at Izmir Ege University Pediatric Bone Marrow Transplantation Center. Between January 2018 and December 2022, 6 allogeneic hematopoietic stem cell transplantation procedures were performed in four patients with MNGIE. Clinical features, mutation analysis, laboratory findings, and treatment of patients before stem cell transplantation were examined. Mutation analyzes were performed with *TYMP* gene sequencing. The pre-transplant disease findings of the patients were summarized in Table 1. Performance scores, conditioning regimens, donor characteristics, stem cell sources, engraftment characteristics, post-transplant complications, and long-term follow-up of transplant patients were evaluated. Chimerism analysis was evaluated by examining DNA sequences with tandem repeats (STR) using the molecular PCR technique. Transplant characteristics of the patients are summarized in Table 2. Additionally, all cases were reviewed in detail. Autologous peripheral blood stem cell collection was performed in all cases as a backup for engraftment failure. Here, we aimed to evaluate the outcomes of our clinical experience with MNGIE patients and our transplant results by comparison with data in the literature.

Case 1

The female patient, who had recurrent diarrhea and pseudo-obstruction signs, was diagnosed with MNGIE at the age of 15. *TYMP-1* mutation analysis of the patient showed homozygous p.P131L (c.392C>T) mutation. She had a severe cachectic appearance (body weight SDS= -4.65 ), bilateral ptosis, hypertrichosis, clubbing and drop foot. Deep tendon reflexes could not be obtained in the lower extremities. The patient was diagnosed with Type 1 Diabetes mellitus two years before the diagnosis of MNGIE and was using an insulin pump. Grade III hepatic steatosis, sensorineural hearing loss, sensorineural myopathy and leukoencephalopathy demonstrated by cranial MRI scan were other major clinical findings (Table 1). AHSCT was performed when the patient was 18.5 years old. The donor was a fully matched male cousin who was also a heterozygous carrier of MNGIE. The source of stem cells was G-CSF stimulated bone marrow (BM) + peripheral blood stem cell (PBSC). The myeloablative conditioning regimen was reduced intensity/toxicity using Fludarabine/Treosulfan (Flu/Treo), and anti-thymocyte globulin (ATG). Graft versus host prophylaxis was performed with cyclosporine (CSA)+ short course methotrexate (MTX) (Table 2). Defibrotide was used for veno-occlusive disease (VOD) prophylaxis. The patient started on a high protein and fat and low carbohydrate (ketogenic diet) before the transplant and continued the same diet throughout the transplant (details of the diet in MNGIE is to be reported in a separate paper). Neutrophil (Absolute neutrophil count; ANC) engraftment occurred on day 12 after HSCT, and platelet engraftment on day 15 after HSCT. Gram-negative bacterial sepsis and adenovirus gastroenteritis occurred in the post-transplant follow-up, which was successfully treated. Acute GVHD did not develop. She was discharged from hospital 57 days after transplant. Chimerism in the first month was 100% , and follow-ups showed full chimerism. After HSCT, the patient's need for insulin therapy was decreased. She started menstruating 19 months after HSCT. The patient’s most recent follow-up was 50 months after transplantation. Plasma dThd and dUrd levels were measured before transplant, but were not followed up after transplantation. There was a slight weight gain and gastrointestinal symptoms persist intermittently; however, the patient is now completely fed orally and is not hospital dependent. There is improvement in her polyneuropathy. Chronic GVHD did not develope, and she is continues with a 100% donor chimerism.

Case 2

Patient 2 was followed up with recurrent attacks of diarrhea, inability to gain weight and hepatomegaly since infancy. With the detection of homozygous p.P131L (c.392C >T) mutation in the *TYMP-1*gene, he was diagnosed with MNGIE at 18-years of age. Examination before HSCT revealed cachexia, hepatomegaly, hepatosteatosis, bilateral ptosis, sensorineural hearing loss and polyneuropathy. Brain MRI showed diffuse periventricular and bilateral parietooccipital white matter changes (Table 1). The patient underwent AHSCT at the age of 20-years. The donor was 10/10-matched sister, who was a heterozygous carrier of MNGIE. The conditioning regimen included Flu/Treo and ATG, and for GVHD prophylaxis, CSA+MTX was administered. Defibrotide was used for VOD prophylaxis. The source of stem cells was G-CSF stimulated BM+PBSC. Neutrophil engraftment occurred on day 13 after HSCT, and platelet engraftment on day 13 after HSCT. Donor chimerism was 100% one month after transplantation. Fifty days after stem cell transplantation, acute GVHD Stage IV GIS GVHD developed without liver and skin involvement. The daily stool volume was >200 cc/kg/day, and severe bloody diarrhea and abdominal pain occurred. The endoscopy-colonoscopy of the patient was compatible with acute GVHD. A high-dose steroid + tacrolimus therapy was rapidly initiated, but without response. On day 74 after HSCT, the patient died due to steroid-resistant GIS GVHD and massive gastrointestinal bleeding (Table 2).

Case 3

Patient 3, was diagnosed with MNGIE when he was 12-years of age. The patient had severe vomiting attacks, vomiting around 1500 ml per day. He had classical MNGIE symptoms including cachexia, sensorineural hearing loss, polyneuropathy, bilateral foot drop, and loss of deep tendon reflexes in the lower extremities (Table 1). *TYMP-1* mutation analysis showed a homozygous p.P131L (c.392C>T) mutation. No matched tissue type donor was found for the patient. At the age of 14 years, the patient underwent ASCT from his haploidentical uncle who was not a carrier of the disease of stem cells The conditioning regimen included ATG 30 mg/kg (-10,-9,-8) /Flu 150mg/kg (-7,-6,-5,-4,-3)/Treo 36 gr/m2 (-7,-6, -5). Cyclophosphamide 50 mg/kg/day (+3,+4), Mesna 200% dose, Tacrolimus and prednisolone were used for GVHD prophylaxis. G-CSF stimulated BM+PBCS were used as the source of stem cells . No complications developed during and after the conditioning regimen. Neutrophil engraftment occurred on day 14 after HSCT, and platelet engraftment on day +13. Acute GVHD did not develop. Donor chimerism was 85%, one month after transplantation. Donor chimerism gradually decreased, declining to 0% three months after transplantation. Secondary engraftment failure developed and an autologous stem cell backup was performed due to pancytopenia. A second stem cell transplant was performed with the same haploidentical donor 6 months after the first HSCT. The second conditioning regimen was a non-myeloablative haploidentical (Baltimore) protocol using only PBSC as the stem cell source. The patient's donor chimerism was 0% in the 1st month after transplantation, and the patient was considered a primary engraftment failure. The patient's MNGIE symptoms persisted, and died from disease progression 16 months post-transplant (Table 2).

Case 4

The patient had recurrent diarrhea-vomiting episodes, weight loss and muscle weakness since the age of 7 and was diagnosed with MNGIE at 17-year-old. *TYMP-1* mutation analysis showed a homozygous c.215G>A (p.G72E) mutation. The clinical complaints of the patient were mainly secretory diarrhea, severe abdominal pain, signs of pseudo-obstruction and muscle weakness. Physical examination revealed bilateral ptosis, bilateral drop foot, contracture deformity in the ankles, clubbing and hepatomegaly (Table 1). AHSCT was performed at the age of 18.5 years. The donor was a 10/10 matched-unrelated donor. The conditioning regimen was reduced toxicity (Flu/Treo/ATG) and GVHD prophylaxis was with CSA+MTX. Defibrotide was administered for VOD prophylaxis. The source of stem cells was bone marrow. Due to the major ABO blood group mismatch, erythrocyte depletion in the bone marrow product was accomplished using an automated method. White blood cell engraftment occurred at day +15, platelet engraftment at day 27 following transplant. During follow-up, no severe infection or acute GVHD were observed. Chimerism was found at 72% in the first month after the HSCT, but decreased to 10% in the 3rd month after transplantation. Secondary engraftment failure developed. Six months after the first transplant, a second stem cell transplantation was made from the same donor. The conditioning regimen was Busulfan-based and the stem cell source was again bone marrow. However, after the second transplantation procedure, primary engraftment failure developed. The patient's cytopenia continued, so on the 45th post-transplant day, an autologous stem cell infusion was made. The patient is currently being followed up for the symptoms of MNGIE disease (Table 2).

DISCUSSION

Allogeneic hematopoietic stem cell transplantation (AHSCT) is a well-defined treatment option for MNGIE8-10. HSCT is effective in permanently restoring the biochemical imbalance11. It requires chemotherapy and immunosuppressive therapy and is associated with a high risk for complications and mortality related to therapy, including graft vs host disease6. In this retrospective study, we report clinical experience on the transplantation at our center.

A total of 6 transplantation procedures for four patients were performed. In the study published by Kalkan Uçar *et al*. in 2022, patients diagnosed with MNGIE at Ege University Faculty of Medicine, Department of Pediatrics, Department of Pediatric Metabolism and Nutrition were evaluated12. In this patient group, we performed AHSCT on four patients. In this study, three patients who underwent stem cell transplantation had a homozygous p.P131L (c.392 C > T) *TYMP* mutation, and one patient had a homozygous p.G72E (c.215G > A) mutation. We concluded that the “Mediterranean”/homozygous p.P131L (c.392 C > T) mutation is associated with a rapidly progressive clinical course and poor prognosis12. The mean age at diagnosis of our patients was 16.4 years and all our patients had persistent gastrointestinal manifestations and neurological deficit findings at the time of diagnosis. Rapid progression was the major clinical observation outcome in our patients’ group. It was well described in the literature that transplantation before the development of permanent gastrointestinal symptoms can increase the success of transplantation6,13.

The first allogeneic transplant in MNGIE was performed by Hirano *et al*. on two patients and in this study there was evidence of improvement in biochemical abnormalities9. The first multicenter study, evaluating 24 MNGIE patients undergoing AHSCT reported a survival rate of 37.5% after a median follow-up of almost 4 years7. The number of patients transplanted in MNGIE is still limited13,14,15. Nevertheless, HSCT is effective in the long term in improving quality of life, although limited by the high post-transplant mortality rate (63%) in severely symptomatic adult patients7.

Engraftment failure and GVHD have been determined as the most critical problems in MNGIE transplants7. Therefore, it has been reported that HSCT should be considered in pediatric patients and young adults with mild or no GI symptoms6. The mean age of our group at the time of transplantation was 17.9-years-old, and all of them had severe permanent GI symptoms before transplantation. However, our patient, who underwent HSCT at 18 years and six months (patient 1), did not develop any transplant-related complications, and a significant improvement was achieved in her quality of life.

It is recommended to use a fully compatible donor for stem cell transplantation in MNGIE10. The donors of our three patients, two of them from family members, were fully matched donors. Haploidentical stem cell transplantation was performed in a patient whose GI and neurological symptoms were severe, despite his young age, because a fully matched donor could not be found. Unmanipulated bone marrow and peripheral stem cells were used as a source. Chemotherapy-related toxicity did not develop in patient who received a treo-based conditioning regimen with posttransplant cyclophosphamide. However, our patient, the first reported case of haploidentical stem cell transplantation, developed engraftment failure twice. The patient died of disease progression after autologous stem cell backup.

In the consensus report published in 2011, it was recommended to use bone marrow as a source of stem cells10. We employed bone marrow in 5 of 6 transplants as a stem cell source and in three transplants, supplemented these with peripheral blood stem cells.

The consensus report also recommended a Fludarabine/Busulfan conditioning regimen 10. In most literature cases, this conditioning regimen experience has been published, and also it has been shown to be associated with an increased toxicity in this patient group. Due to our patients developing severe GI and neurological deficits due to disease prior to transplantation, we aimed to reduce the toxicity of the conditioning regimen. By employing a treo-based regimen in five transplantation procedures. A second transplant for a patient with secondary engraftment failure was performed with a busulfan-based conditioning. One of patients’ currently being followed up thoroughly and has shown no severe organ toxicity and GVHD in the 50th month after transplantation (patient 1). One patient died with acute Grade IV GIS GVHD on post-transplantation early period (patient 2). Engraftment failure developed in 2 patients, one of whom had a haploidentical transplant (patients 3 and 4). Experience with a treo-based preparation regimen at MNGIE, was first reported in the study of Zaidman et al13. Similar to our study, they reported engraftment failure in two of four patients. They reported that, a reduced intensity/toxicity conditioning is not sufficient for this group of patients13. In our study, although the number of patients was limited, the treo-based regimen toxicity was also low in this patient group. However, this conditioning regimen may lead to engraftment failure. Therefore, we support the busulfan-based conditioning regimen , particularly in patients without severe organ toxicity. The addition of thiotepa to the treosulfan-based regimen is also debatable in the patient group with severe organ involvement.

Allogeneic stem cell transplantation is still the only curative treatment option for MNGIE syndrome. In conclusion, based on our clinical experience we retrospectively evaluated the transplantations performed at our centre using a Treosulfan-based reduced toxicity conditioning regimen. We also report on our experience with haploidentical transplants, the first reported case in the literature. Although the number of cases are limited, it is the treosulfan-based regimen is well tolerated. However, engraftment failure with this conditioning regimen is a significant problem. As previously reported in consensuses, we believe that early transplantation with the most compatible donor, without the development of permanent severe GIS symptoms, is vital for increasing the success of transplantation in MNGIE.

FUNDING INFORMATION

No external funding was received for this study.

CONFLICT OF INTEREST

Gülcihan Ozek , Serap Aksoylar Sema Kalkan Uçar , Ebru Canda , Mediha Akcan, Ozgür Cartı, Zuhal Onder Siviş, Yeşim Oymak, Havva Yazıcı , Bridget Bax ,Fatma Derya Bulut , Merve Yoldaş Çelik , Fehime Erdem, Mahmut Çoker, Savaş Kansoy declare that they have no conflict of interest.

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