Hemophagocytic Lymphohistiocytosis in Children with Griscelli Syndrome Type 2: Genetic, Laboratory findings and Treatment

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Abstract
Griscelli syndrome is a rare autosomal recessive inherited syndrome that causes immunodeficiency. Hemophagocytic lymphohistiocytosis (HLH), which is characterized by high mortality, may develop due to Griscelli syndrome type 2 (GS2). We aimed to share our experience in diagnosis and treatment methods of patients who developed HLH secondary to GS2. GS2 patients

Introduction
Griscelli syndrome (GS) is a rare autosomal recessive disorder that causes immunodeficiency and is phenotypically characterized by silver-gray hair and partial albinism. Hemophagocytic lymphohistiocytosis (HLH) is a syndrome with a high mortality rate triggered by severe systemic hyperinflammation (1). This inflammation occurs as a result of unregulated immunologic activation of natural killer (NK) cells and cytotoxic T cells and causes a variety of symptoms in the body (2). Fever, organomegaly, liver damage, consumptive coagulopathy, hypertriglyceridemia, cytopenias, neurological dysfunction, dermatological abnormalities, and increases in acute phase reactants (particularly serum ferritin) may be observed in clinical and laboratory findings (3). In addition, inherited diseases and immunodeficiency syndromes such as Chediak-Higashi syndrome, Griscelli syndrome-2 (GS2), X-linked lymphoproliferative syndrome (XLP), Wiskott-Aldrich syndrome may be associated with HLH (4). Here, we aimed to share our experience in diagnosis and treatment methods of patients who developed HLH secondary to GS2.

Methods
We retrospectively analyzed pediatric patients with Griscelli, who were diagnosed and treated with HLH in the 2017 to 2022 period at Cukurova University, Division of Pediatric Allergy and Immunology. The diagnosis of GS2 was made by microscopic examination of the hair shaft, clinical features and molecular genetic testing of RAB27A gene via next generation sequencing. Variant assessments were performed accordingly with American College of Medical Genetics and Genomics (ACMG) Criteria (5).

In 5 years, 15 patients presented with GS2, and 11 of them developed HLH. Eleven patients fulfilled at least 5 of 8 diagnostic criteria of HLH 2004 (6). Symptoms and laboratory parameters (White Blood Cell (WBC), neutrophil, lymphocyte, hemoglobin, platelet counts, lactate dehydrogenase (LDH), fibrinogen, ferritin, triglyceride, albumin, alanine transaminase (ALT), aspartate aminotransferase (AST) ) at the time of diagnosis of HLH and laboratory parameters during treatment were evaluated. HLH 2004 protocol was
used in the treatment. Hematopoietic stem cell transplantation (HSCT) was performed in patients who had a suitable donor. ESID guidelines for hematopoietic stem cell transplantation was used for conditioning regime (7).

Statistical Analysis

Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and minimum-maximum where appropriate. The normality of distribution for continuous variables was confirmed with the Shapiro-Wilk test. For comparison of two related (paired) continuous variables, the paired-samples t-test or Wilcoxon Signed Rank test was used depending on whether the statistical hypotheses were fulfilled or not. All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package. The statistical level of significance for all tests was considered to be 0.05.

Results

Clinical Features, Laboratory Parameters and Genetics

Eleven patients were enrolled in the study. Six were male and five were female. The mean age at diagnosis was 29.3 (min. 3 – max. 137) months. As the first presentation finding, HLH was detected in 8 (72.7%) patients. 1 (9%) patient presented with CNS involvement and 2 (18.1%) patient with recurrent febrile diseases. The mean HLH development time was 35.5 ± 6.2 months in patients without HLH at first presentation. At admission, 90% (n = 10) of patients had cytopenia in at least two hematological series. Hypofibrinogenemia was detected in 54.5% (n = 6). Ferritin elevation was detected in all and 36.3% (n = 4) had high triglyceride levels. Hemophagocytosis in the bone marrow was documented in all. Hemaphagocyte was detected in the CSF samples of 1 patient (9%). The parents of patient 1, 3, 4, 7 were cousins. There is no consanguinity between the other patients’ parents. Genetic mutation was RAB27A in 10 patients. All the RAB27A mutations were homozygous. Nonsense mutations were detected in 63.6% (n = 7), missense mutations in 8.3% (n = 1) and large deletion in 8.3% (n = 1) (Details of clinical features, laboratory parameters and genetic testing results were given in Table 1. Pathogenicity classifications were given in Table 2.). All patients’ hair shaft was inspected microscopically, and irregular clumps of melanin pigment were detected (Figure 1).

Statistically significant decreases were found in ferritin (p < 0.01), CRP (p = 0.028) levels and a significant increase in fibrinogen (p = 0.016) level was detected in the mean of the second week of treatment (Table 3).

Treatment

In the treatment, the HLH-2004 protocol was used. During the HLH active phase, all patients received intravenous immunoglobulin (IVIG) and corticosteroid treatment. Four patients received just corticosteroid and IVIG therapy. Three individuals with CNS involvement received intrathecal methotrexate therapy. Aside from these patients, two patients underwent plasmapheresis therapy (Table 4).

Allogeneic HSCT was performed in 2 patients who have suitable family donors, 2 patients underwent haploidentical HSCT; and 1 patient underwent unrelated HSCT. Remission was observed in 4 of the 5. Unrelated HSCT was performed for the second time in one patient due to the lack of engraftment, and remission was observed after the second transplantation. Treosulfan (14 mg/m²/day for 3 days) + fludarabine (30 mg/m²/day for 5 days) + thiotepa (10 mg/kg/day for 1 day) + antithymocyte globulin (10 mg/kg/day for 4 days) were administered to two patients. One patient received fludarabin (45 mg/m²/day for four days) + busulfan (3.5 mg/kg/day for four days) + ATG (5 mg/kg/day for three days). One patient received fludarabine (30 mg/m²/day for 5 days) and treosulfan (12 mg/m²/day for 3 days). In the first conditioning regimen of the patient whose engraftment could not be achieved in the first HSCT, fludarabine (30 mg/m²/day for 5 days) + treosulfan (14 mg/m²/day for 3 days) + alemtuzumab (0.2 mg/kg/day for 5 days); in the second HSCT, fludarabine (40 mg/m²/day for 4 days) + busulfan (4.9 mg/kg/day for 4 days) and antithymocyte globulin (10 mg/kg/day for 3 days) was used. Cyclosporin and short-term methotrexate were administered for GVHD prophylaxis in four patients, while cyclophosphamid and cyclosporin were given to one patient who had haploidentical transplantation. After HSCT, the patients were followed for 23.3 ± 5.2 months. There
were no signs of acute GVHD or chronic GVHD. EBV and CMV PCR positivity was not detected in patients who performed transplantation (Table 5).

Survive

The first clinical presentation of a patient was CNS involvement, and the patient died 11 months after diagnosis. CNS involvement with strabismus and seizure symptoms developed in two patients during the follow-up period. Among these patients, the patient who could not undergo HSCT due to the lack of a suitable donor died within an average of 12 months, while regression in neurological symptoms was observed in the patient who underwent HSCT.

Three patients died a mean of 13 months after the diagnosis of HLH, and the cause of death was septic shock. 5 patients who were underwent HSCT survived.

Discussion

Griscelli syndrome is divided into subgroups: GS1 with more neurological findings, GS2 with predominant immunological findings, and GS3 with only hypopigmentation (8). RAB27A deficiency in GS2 can impair NK and T lymphocyte cytotoxicity, resulting in the release of lytic granules and the development of HLH (9). In our study, the majority of patients’ first clinical manifestation was HLH.

In Griscelli syndrome, there may be a link between the beginning of the disease, its clinical course, and heredity. In a study by Mesache et al., T cells from two patients with a missense mutation in MYO5a showed normal anti-CD3-induced cytotoxic activity when compared with control T cells. It has been stated that the genetic mutation in the RAB27A gene that causes the premature codon causes very low cytotoxic activity, and the $454G'C$ mutation (10), which has the same mutation as the patient 7 in our study, causes decreased cytotoxicity. For this reason, it has been stated that genetic mutations will cause different phenotype and genotype characteristics in patients with GS2. In addition, in the above-mentioned study, it was stated that the most reported RAB27A mutations associated with GS2 were nonsense or frameshift mutations. The loss of $CAAGC$ between nucleotides 514_518 caused a frameshift mutation in 54.5% of patients ($n=6$), which was the most prevalent mutation in our analysis.

In another study in which 9 patients were examined, CNS involvement developed in 4 patients and it was stated that 2 of these patients had a genetic mutation c.514_518del (11). This mutation was the same as in patients with CNS involvement in our study. Moreover, c.514_518del variation was submitted in 5 patients whom 3 of them reported dead. Likewise, our patients who carry the same genetic change also died. In addition to the fact that it is the most common genetic alteration in our patients, we emphasize that c.514_518del may be associated with CNS involvement and poor prognosis.

When the studies on GS2 are examined, it has drawn our attention that genetic mutations are concentrated in some regions. The c.514_518del mutation in Turkey and Iran (11), (current study) the c.550Tmutation in a study by Singh et al. in India, and the c.244C > T mutation in a study involving 6 families in Qatar by Al-Sulaiman et al. (12), (13). However, predisposing any founder affect based on the findings and literature knowledge is less likely due to the lack of population specific databases. In order to speculate a possible founder affect, more population-wide studies that were focused on haplotype analysis is suggested.

A ferritin cut-off value of 500 ng/ml was included in the 2004 HLH guidelines, and values of 10,000 ng/ml were stated to provide higher sensitivity and specificity for the diagnosis of pediatric HLH (14). It has been proposed that lowered ferritin levels following HLH therapy are a promising prognostic sign in critically ill patients (15). CRP is not required for a conclusive diagnosis of HLH. It is, nevertheless, a prominent acute-phase protein whose concentration can rise under inflammatory situations (16). Furthermore, low fibrinogen levels constitute an additional laboratory marker in the diagnosis of HLH (17). When we evaluated our patients’ laboratory parameters before to HLH treatment, we found that they all had high ferritin levels and almost half had low fibrinogen. A statistically significant decrease in ferritin and CRP levels, as well as a substantial rise in fibrinogen values, two weeks following HLH treatment show that these indicators can be used to assess early response to treatment.
Conditioning regimens for allogeneic HSCT are broadly categorized into myeloablative conditioning (MAC), non-myeloablative conditioning, and reduced-intensity conditioning (RIC) regimens (18). MAC regimens result in long-term high-level donor chimerism but are more toxic and have long-term problems (19), (20). Although long-term complications and acute toxicity after transplantation are less prevalent with the RIC regimen, graft rejection is more likely (21). Reduced toxicity regimens are preferred for lower rates of acute and long-term complications (22). In a study by Kuskonmaz et al., ten patients with GS2 who underwent HSCT with myeloablative regimen and the survival rate was found as 80% (8/10) (23). In another study, M. Al-Mofareh et al. performed HSCT with myeloablative regimen to 35 patients and found a survival rate of 37.1% (13/35) after 87.7 months of follow-up (24). Schmid et al. evaluated ten patients with GS2 who underwent HSCT at their center. The patients were followed for a mean of 5.2 years, and seven survived, three died, and four of the survivors achieved complete remission (25). In another study, HSCT was performed on 5 patients with GS2 using an HLA-matched related donor RIC regimen, and the patients were followed for an average of 19 months, with an 80% (4/5) survival rate (26). In our study, a reduced toxicity regimen was received to all patients who underwent HSCT, GVHD was not observed, and all patients continued their lives in remission. The optimal conditioning regimen for HSCT in GS2 has yet to be defined. However, in the studies mentioned above and in our study, it is revealed that regardless of the conditioning regimen, HSCT is the main treatment for survival and disease remission in GS2 patients who develop HLH. Furthermore, the fact that patients in our study had 100% survival and no GVHD development may be due to the fact that the patients in our research had a shorter mean follow-up duration than the patients in the studies mentioned above.

Consequently, approximately 150 cases have been reported in the literature so far as to the Genetic and Rare Diseases Information Center (27). Fifteen cases with this rare disease are our patients. The genetic variants found in our patients showed that deletion of CAAGC at nucleotides 514_518 in GS2 individuals is associated with CNS involvement and a poor prognosis. HLH may be the first sign of presentation in patients with GS2, caution should be exercised in this regard. Ferritin, fibrinogen, and CRP can be used as a marker in the evaluation of response to treatment. Although further research is needed, regardless of the conditioning regimen utilized, early HSCT remains the primary therapy option for preventing mortality in HLH caused by GS2.

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