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CASE REPORT – OPEN ACCESS

A Fatal Case of Familial Hemophagocytic Lymphohistiocytosis Associated with Fusarium Infection and Rare Mutation

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Background: Hemophagocytic lymphohistiocytosis (HLH) is classified as primary or secondary. While primary (familial) HLH is caused by genetic mutations, secondary (acquired, reactive) HLH is the type that has an underlying cause and is not associated with genetic mutations.

Case Report: We report a two-year-old female patient with a fatal course of Fusarium sepsis who was diagnosed with primary HLH. A homozygous variant of *PRF1* (c.445G > A, p.Gly149Ser) was detected. Hyphal growth was detected on Sabouraud dextrose agar and Fusarium multiplied in blood cultures. The patient's clinical course was fulminant, and she died of septic shock 4 days after admission to the hospital. Fusarium, a rare infection in HLH, was found in this case.

Conclusion: We discovered the rare *PRF1* (c.445G>A, p.Gly149Ser) mutation in HLH and the high morbidity and mortality associated with Fusarium infection.

Keywords: Children, familial hemophagocytic lymphohistiocytosis, fusarium, perforin mutation, sepsis

ABSTRACT

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INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) has two forms, familial or primary and acquired or secondary, and is a life-threatening disease in children. Primary HLH develops in early childhood and is caused by genetic mutations. Secondary HLH is a form that can occur at any age and has underlying disorders such as infections and metabolic and immunological diseases (1). In a child with fever, hepatosplenomegaly, pancytopenia, and coagulopathy, infection is usually considered. Evidence of infection does not resolve the diagnostic dilemma, as many infections can also cause disease activity in HLH. Secondary infections can cause significant morbidity and mortality in this disease (2).

Fusarium fungi species can cause localized or invasive diseases. Disseminated infections occur especially in immunocompromised patients with severe hematologic diseases (3). Few cases have investigated the specific role of infections and complications in fatal cases of primary HLH in children. Understanding these complications may allow prophylactic or preventive strategies that may improve the prognosis of this disease. We present the case of a previously healthy child who developed Fusarium sepsis and whose clinical course rapidly deteriorated due to an underlying *PRF1* gene mutation.

CASE REPORT

A two-year-old female patient was referred to our center for neck swelling, persistent fever, and pancytopenia in the blood count. She was intubated and referred to our hospital for the development of sepsis and respiratory failure. The patient had no previous health problems; the parents were first-degree cousins, and they had another child with autoimmune hemolytic anemia. Her general condition was poor, her tonsils were hypertrophic and cryptic, her abdomen was distended, her intubated lungs were ventilated bilaterally, and her liver and spleen were palpated 4 cm below the rib. Complete blood count revealed Hb:7.7 gr/dl, Hct:%21.9, white blood count: 4050/mm^3, ANC:700/mm^3, and platelets:33,000/mm^3. Other laboratory tests revealed low blood sedimentation (3 mm/h), prolongation of PT–aPTT (aPTT: 52 s, PT: 15.8 s, INR: 1.35), decrease in fibrinogen (87mg/dl), high ferritin (7890 mg/dl), and triglycerides (389 mg/dl). Mycoplasma, syphilis, and viral serology for *Toxoplasma gondii*, rubella virus, cytomegalovirus, herpes simplex virus (TORCH), Epstein–Barr virus, parvovirus, hepatitis A, B, and C virus, and human immunodeficiency virus were all negative. Brucella tube agglutination and Leishmania

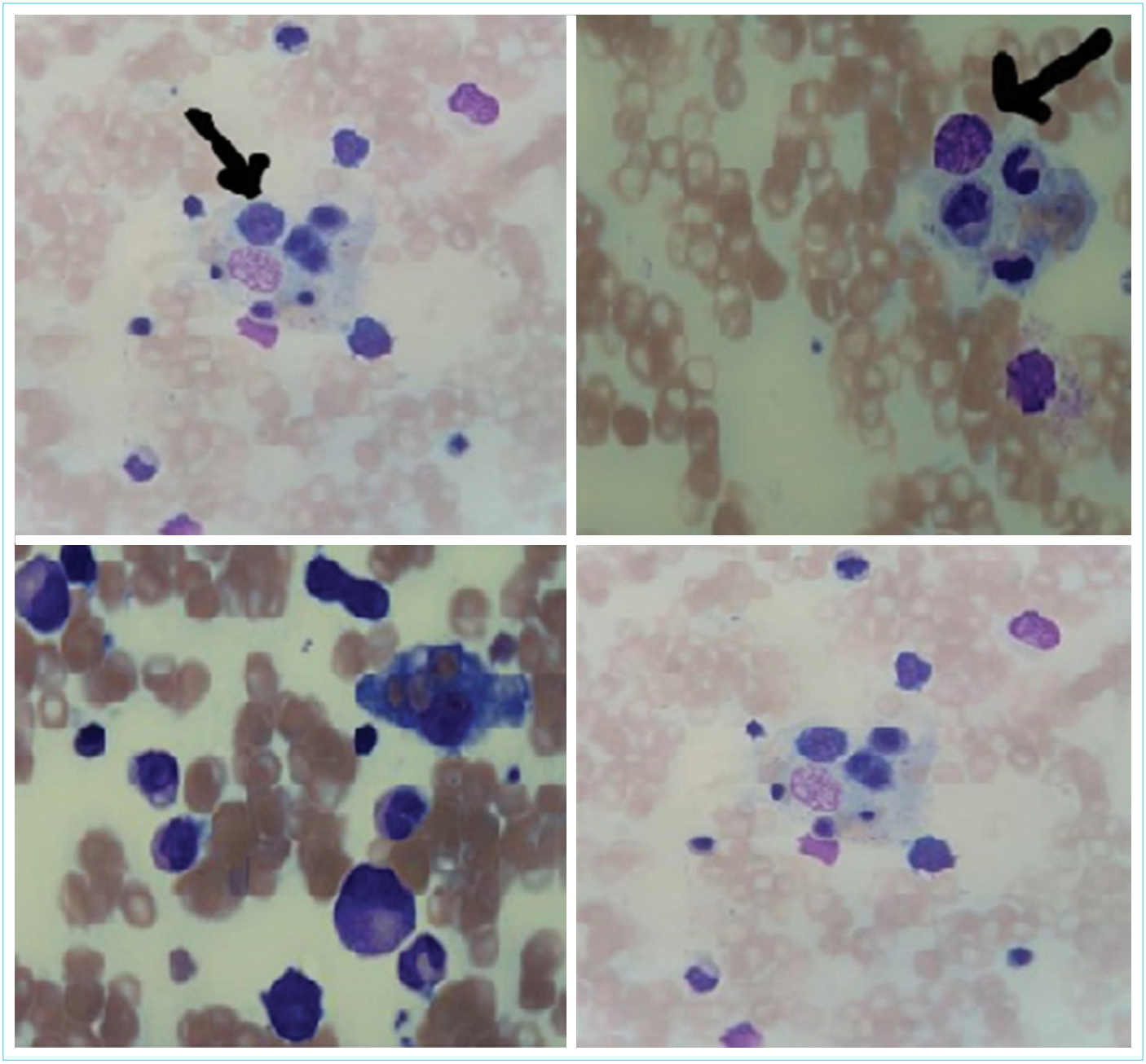

Figure 1. Histiocytes showing hemophagocytosis in bone marrow aspiration

tests were negative. Lymphocyte subgroups and immunoglobulins were within the normal range. Histiocytes with hemophagocytosis were observed in bone marrow aspirate (Fig. 1). The patient with suspected HLH was administered intravenous immunoglobulin at a dose of 1 g/kg/day for 2 days and a total of 2 g/kg.

The patient's blood culture was sent to the microbiology laboratory and incubated in BACT/ALERT 3D (Biomerieux, France) Microbial Identification System. Positive blood culture samples are inoculated on 5% sheep blood agar (BD, USA) and eosin methylene blue lactose sucrose agar (BD, USA). Conventional methods were used for routine identification (Fig. 2). After incubation at 37°C for 48 h, hyphal growth was observed (Fig. 2), and microscopic examination revealed fungal hyphae and macroconidia (Fig. 3). The samples were cultured on Sabouraud dextrose agar (BD, USA) without the addition of gentamicin and cycloheximide and incubated at 35°C. In vitro antifungal susceptibility testing for Fusarium was performed using the 9.3.1 broth microdilution method of the European Committee for Antimicrobial Susceptibility Testing. The minimum inhibitory concentrations were 8 µg/ml for amphotericin B, 128 µg/ml for fluconazole, 8 µg/ml for itraconazole and 8 µg/ml for voriconazole.

The peripheral blood mononuclear cell (PBMC) cytotoxicity test was defective compared with the healthy control (Fig. 4). Whole exome sequencing was performed at Dr. von Hauner Sequencing Facility. The patient's underlying genetic defect was investigated. The patient was found to have a homozygous variant of *PRF1* (c.445G > A, p.Gly149Ser). A de novo event was confirmed in the Sanger sequencing analysis of the family.

Figure 2. **Hyphal growth on 5% sheep blood agar**

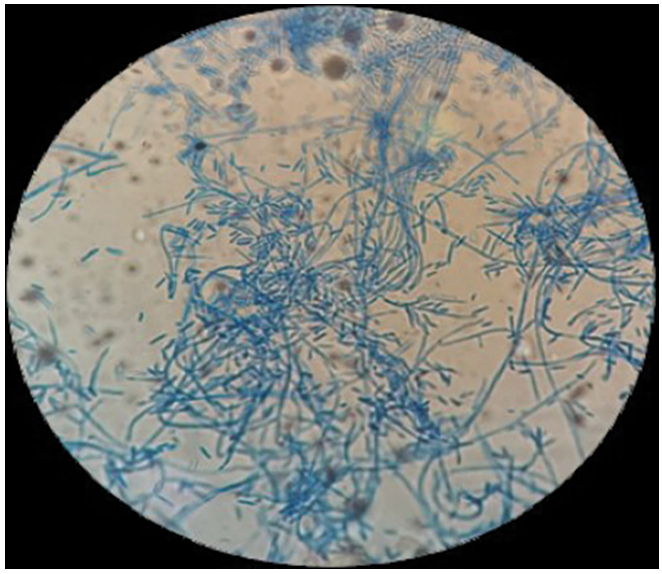

Figure 3. **Microscopic examination of fungal hyphae and macroconidia**

As a result of whole exome sequencing, the patient was found to have the previously reported pathogenic homozygous *PRF1* variant. The parents and healthy siblings were found to be heterozygous carriers. The patient was diagnosed with primary hemophagocytic syndrome with fusariosis. Due to severe sepsis, treatment with vancomycin, meropenem, voriconazole, and amphotericin B was started. In our case, multiple organ failure developed rapidly due to the Fusarium infection. The high-risk child

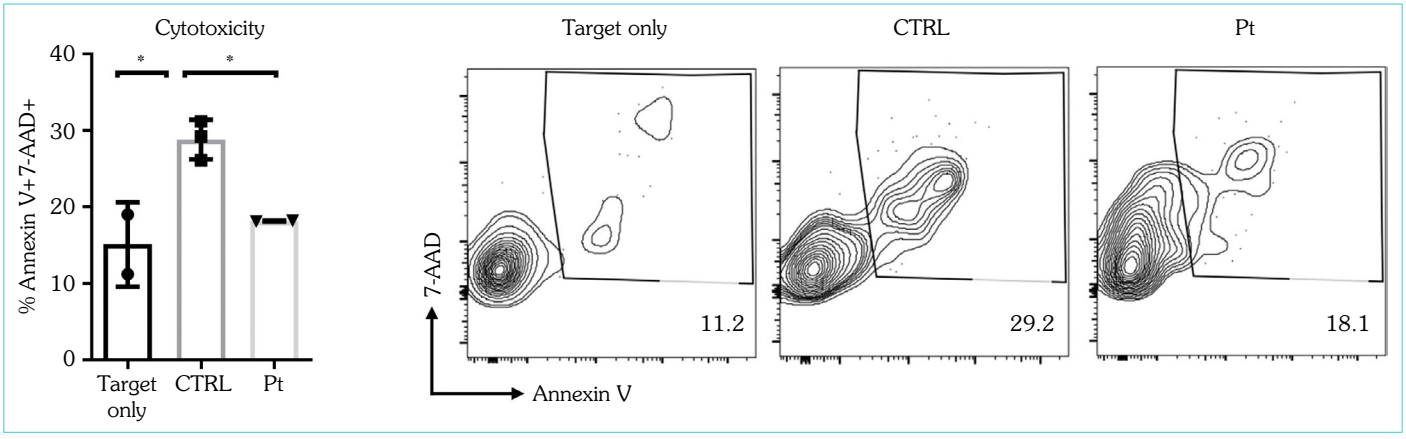

Figure 4. **Peripheral blood mononuclear cells (PBMC) isolated from the patients showed reduced cytotoxicity to the target cells. (Control (CTRL), patient (Pt)) *p<0.05. To prepare the target cells K562 cells were cultured 1 week before the experiment to confluency in Iscove's Modified Dulbecco's Medium supplemented with 10% FBS (complete medium). PBMC was purified from 5 cc of blood via Ficoll Paque. K562 cells were labeled with tag-it-violet (Biolegend#425101) according to the manufacturer's guidelines. The staining of target cells (K562) was performed in 1 ml PBS (supplemented with 5% FBS) for 10 min in dark at room temperature at a concentration of 5 μM tag-it-violet. The cells were washed three times with a complete medium. PBMCs and target cells were mixed in a 96-well round bottom plate in a 50:1 ratio, spun for 5 min at 200g at room temperature, and cultured for 4 h at 37°C, in a 5% CO_2 incubator. Twenty thousand target cells were seeded. The cocultures were then stained for FITC-ANNEXIN V and 7-AAD for 15 min according to the manufacturer's instructions (Biolegend#640922) and run on a FACSAria III. Tag-it-violet negative cells were excluded and apoptosis on tag-it-violet+ cells was quantified based on ANNEXINV/7-AAD positivity. The PBMC cytotoxicity test was defective compared with that of the healthy control**

PBS: Phosphate buffered saline, FBS: Fetal bovine serum, FITC-ANNEXIN V: Flourescein isothiocyanate-Annexin V, 7-AAD: 7-AminoActinomycin-D

diagnosed with septic shock or sepsis-related organ dysfunction was started on fluid and inotropic therapy. On the fourth day of treatment, a cardiopulmonary arrest occurred and the patient died despite resuscitation attempts.

DISCUSSION

Familial HLH is an autosomal recessive inheritance that can be triggered by various stimuli that disrupt immune response. It has the same phenotype as secondary HLH, with more than 70% of patients developing the disease by about 1 year of age (4). Our case is 2 years old, and a mutation was found in the gene encoding perforin. Immune activation by infection is a common trigger in genetically predisposed patients and in sporadic cases where the genetic cause cannot be identified (5). Park et al. (6) discovered familial HLH associated with rotavirus infection after hematopoietic stem cell transplantation in an eight-month-old child. The patient with the *UNC13D* mutation died of multiple organ failure and septic shock, just as in our case. In the study by Ramzan et al., (7) septic shock and death were observed in patients with primary HLH, especially in children after stem cell transplantation. Severe invasive fungal infections are an important cause of mortality in familial HLH. Fusarium species have virulence factors by producing mycotoxins. They can cause cell destruction by suppressing humoral and cellular immunity (8). In our case, the causative infectious agent was Fusarium, a rare fungal pathogen not previously reported in primary HLH. Additionally, we investigated and ruled out frequent viral, parasitic, and bacterial causes. Our case, which initially appeared to be secondary HLH due to infection, turned out to be primary HLH based on genetic blood testing. The coexistence of primary HLH and Fusarium infection is a rare case. It is not clear whether Fusarium infection is fatal due to HLH or whether Fusarium infection affects the occurrence of primary HLH.

Genetic information can help determine the likelihood of disease recurrence, the need for hematopoietic cell transplantation, and the risk of HLH in family members. Most of the genes involved encode components of perforin-dependent cytotoxicity. These genes act in an autosomal recessive manner, and many cases are related by consanguinity (9). *PRF1*/*Perforin-FHL2* is caused by the *PRF1* mutations that encode perforin. Perforin is released as cytolytic granules and creates pores in the membrane of target cells. Mutations in other genes affecting perforin gene expression have also been reported (10). Our patient had first-degree consanguinity and all exon sequencing was performed. In countries where consanguineous marriages are common, primary HLH should be considered. The results of the genetic determination of the patient and his family members showed *PRF1* mutation, one of the mutations in *PRF1, UNC13D, STX11*, and *MUNC* 13-4 genes that can lead to primary HLH. In children with primary HLH with *PRF1* gene mutation, lethal infectious agents such as Fusarium can appear suddenly and be fatal.

Familial HLH triggered by any microorganism can be life-threatening in children. Early diagnosis and prompt treatment are important to reduce morbidity and mortality. It should be noted that Fusarium infection, which is uncommon in HLH, may have a fatal course. We also contributed to the literature by identifying the *PRF1* (c.445G>A, p.Gly149Ser) mutation, which is a rare HLH mutation.

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Informed Consent: Written, informed consent was obtained from the patient's family for the publication of this case report and the accompanying images.

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Conflict of Interest: The authors have no conflict of interest to declare.

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