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RESEARCH **CASE REPORT**

Novel ZNFX1 Variant Mutation In Hemophagocytic Lymphohistiocytosis Patient-A Case Report

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KEYWORDS - ZNFX1; hemophagocytic lymphohistiocytosis; hepatosplenomegaly; immunodeficiency.

BACKGROUND

The interferon-stimulated response is an important part of innate immunity's role against viral infections, this response is triggered upon identifying viral structural nucleic acids [1]. NFX1-type zinc finger-containing 1 (ZNFX1) is an Interferon (IFN)-stimulated double-stranded RNA (dsRNA) sensor that limits viral replication in infected host cells. Consequently, a deficiency in ZNFX1 increases the liability to severe viral infections, a high mortality rate, and multisystem inflammatory syndrome in children (MIS-C) and adults, which have been clinically compatible with Hemophagocytic lymphohistiocytosis (HLH) [1],[2].

HLH is described as a syndrome of abnormal immune activation, that is contributed to genetic and/or environmental causes. It is subject to complex diagnostic criteria making it challenging to diagnose even if it's a life-threatening condition. We report this case of a 2.5-month-old infant who exhibited symptoms consistent with HLH, along with an identified autosomal recessive ZNFX1 deficiency upon genetic testing. This report provides information regarding the immune-hematological abnormalities that arise from ZNFX1 deficiency in HLH. It also includes a discussion on the coexisting molecular and genetic abnormalities, as well as an exploration of the clinical manifestations observed with HLH disorder. Furthermore, we emphasize considering genetic screening for ZNFX1 mutations in patients with early onset severe viral infections and HLH symptoms [3],[4].

CASE PRESENTATION

A 2.5-month-old Jordanian female patient born to second-degree consanguineous parents, with no prior neonatal intensive care unit (NICU) admission, presented to the emergency room with respiratory distress associated with vomiting and diarrhea of 2 days duration. The vomit was non-projectile, non-bilious, and non-bloody and occurred after each breastfeeding. The diarrhea was large in amount, foul-smelling, with no blood or mucous, and occurred approximately 3-4 times per day. A history of fever (38 °C, axillary) was reported by the mother. The history revealed a positive ill contact, along

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List of Abbreviations:

HLH: Hemophagocytic lymphohistiocytosis
ZNFX1: NFX1-type zinc finger-containing 1
IFN: Interferon
NICU: Neonatal intensive care unit
LDH: Lactate dehydrogenase

with a family history of three unfortunate deaths among her siblings which were due to a relapse of HLH in one sister, cardiac disease in another sister, and liver cirrhosis in a brother at the ages of 4.5 years old, 1-year-old, and 5 months old, respectively. Her 4.5-year-old sister was previously clinically diagnosed with HLH based on the modified HLH-2009 criteria and was on infliximab and cyclosporine until her condition suddenly deteriorated and passed away afterwards.

Upon admission, the patient was afebrile (36.5°C), with a pulse of 150 beats per minute, mean arterial pressure (MAP) of 75 mmHg, respiratory rate of 45-50 breaths per minute, oxygen saturation on room air was 75%-85% that improved to 99% on a 3L nasal cannula. Physical examination revealed an ill-looking, irritable patient with nasal discharge, grunting, subcostal, and suprasternal retractions. Upon palpation, the abdomen was soft and lax with no tenderness. Auscultation of the chest revealed decreased bilateral air entry with no added sounds. Examination for hepatosplenomegaly was inconclusive, with no rash, bleeding, or lymphadenopathy. The neurological exam was normal and the rest of the examination was unremarkable. The initial clinical diagnosis was sepsis with gastroenteritis and bronchiolitis with radiological features showing pulmonary congestion and fissures edema that improved after administering furosemide.

The patient was admitted to the pediatric ICU, and a full septic workup was done, where she was given IV fluids and empirical antibiotics (IV Ceftriaxone 50 mg/kg/day every 8 hours for 1 day and IV Vancomycin loading dose of 20 mg/kg then a maintenance dose of 15 mg/kg every 8 hours for 5 days) but her condition rapidly deteriorated. Blood cultures, COVID-19 by RT-PCR, Respiratory syncytial virus, Clostridium difficile toxin, Rotavirus, Cryptosporidium, and stool culture tests were all negative.

Hematological investigations revealed a lactate dehydrogenase (LDH) to be 1311 U/L (on 29/08). Three days later, LDH was 1335 U/L and ferritin was 613 ng/ml. This required the patient to be transferred to the hospital's pediatric hematology/oncology department for HLH full evaluation. Detailed laboratory investigations are shown in Table 1. A pre-therapy HLA typing was performed to search for a hematopoietic cell transplant (HCT) donor, and the matching results were (3/6), (3/6), (2/6) for her mother, father, and all her siblings respectively. Lumbar puncture and echocardiogram did not show any abnormal findings. Abdominal ultrasonography showed homogenous liver and spleen with no focal lesions, a 6.2cm long axis for the liver, and 5.8cm for the spleen. Both kidneys appeared normal in size, shape, and echotexture with no hydronephrosis seen. A partially contracted gallbladder was visualized, with no intra- or extra-hepatic biliary dilatation.

HLH was suspected given the patient's family history, critically ill condition, cytopenia, and high serum ferritin. Whole-genome sequencing was done revealing a homozygous frameshift deletion variant of ZNFX1 (NM_021035.3: c.5362_5369del, NP_066363.1:p.Val1788CysfsTer6) causing the ZNFX1 related immuno-hematological abnormalities seen in our patient.

After her initial admission, the patient underwent multiple admissions for recurrent respiratory and gastrointestinal infections. During her most recent admission, she experienced severe metabolic acidosis and respiratory distress, which led to a cardiopulmonary arrest and death. Figure 1 illustrates a timeline from the patient's birth until her passing away.

DISCUSSION

In this report, we present a unique case of a 2.5-month HLH patient that presented with a unique genetic variant of a mutated ZNFX1 gene. HLH is characterized by multisystem inflammation resulting from prolonged activation of phagocytic cells and lymphocytes, which can manifest idiopathically or secondary due to predisposing comorbidities.

Secondary or acquired HLH usually manifests in a strong immunological activation background resulting from severe infection or malignancy, whereas primary HLH occurs in a genetic background in which a mutation is present at birth and manifestation appear in the first years of life [5].

Our case report further highlights the importance of genetic testing in the diagnosis of primary HLH and related disorders. As more genes are identified and associated with immune dysregulation, genetic testing may become an increasingly important tool in diagnosing and managing these disorders. One study done by Cetica et al. revealed that a genetic diagnosis was possible in more than 90% of patients with Familial HLH[6].

Our case report also advances knowledge of the genetic underpinnings of HLH and associated immune-hematological abnormalities. The development of HLH is influenced by a variety of genetic and environmental factors, including mutations in genes related to immune control and

hematopoiesis. Our report suggests that ZNFX1 may play a crucial role in regulating the interferon-stimulated response, and it calls for more investigation into the potential therapeutic implications of immune-hematological abnormalities linked to ZNFX1.

In our case, whole-exome sequencing revealed a frameshift deletion mutation in the ZNFX1 gene, which is a member of the helicase superfamily 1 stimulated by IFN. ZNFX1 has a prominent antiviral function as it recognizes viral RNA and interacts with mitochondrial antiviral signaling protein to induce a type 1 IFN response [7].

The literature on ZNFX1 involvement in immunodeficient states has been growing. It has been first addressed in 13 patients with severe viral infections [1]. Despite prior evidence which demonstrated that ZNFX1 deficiency in mice or human cell lines does not predispose to DNA-virus infection, this study had six patients infected with DNA-viruses. [1]. The authors owed this to the insufficient resolution of the IFN response to infection and the heavy viral load [1],[7]. Another study reported four patients with intermittent monocytosis and mycobacterial infections who were found to be homozygous for loss of function ZNFX1 variants. Hematopoietic and nonhematopoietic cells from these patients' cell lines responded normally to IFN-gamma indicating that ZNFX1 is associated with stress granules, and is necessary for monocyte hemostasis and mycobacteria immunity [8].

Recently, in a study from the middle east exploring ZNFX1-related familial immunodeficiency, nine patients were identified from seven families. [9] Complete protein loss was predicted to occur in two ZNFX1 variants (p.(Arg472Glyfs*16) and p.(Tyr362*)) through nonsense-mediated decay. Patients with these variants have also manifested more severe clinical phenotypes including liver, spleen, and kidney involvement, developmental delay, as well as death, compared to patients who were able to produce truncated proteins missing only the C-terminal region. [9] Interestingly, only the family from Jordan had the same variant as the one detected in our case (NM_021035.3: c.5362_5369del, NP_066363.1:p.Val1788CysfsTer6).

Other genetic conditions predisposing to HLH include familial causes through the involvement of PRF1, UNC13D, STX11, and STXBP2 genes. [5] Pigmentary disorders were also found to be associated with HLH through defective lymphocyte granule-mediated cytotoxicity with RAB27A, LYST, and AP3B1 gene involvement. [6] With the recently emerging evidence, the ZNFX1 should be considered as well.

Treatment options for ZNFX1 deficient patients are still limited. In a cohort study by Vavassori et al., treatment with immunosuppressants led to transient benefit, while hematopoietic stem cells transplantation led to the complete arrest of HLH flares along with regression of magnetic resonance imaging (MRI) white matter changes. [1]

CONCLUSION

In conclusion, we report a homozygous ZNFX1 variant as the underlying cause of HLH in this patient. HLH proposes a diagnostic challenge as its signs and symptoms are concurrent with other differential diagnoses, so the need for high clinical suspicion cannot be understated. Further studies on the novel ZNFX1 variant (NM_021035.3: c.5362_5369del, NP_066363.1:p.Val1788CysfsTer6) can help in understanding the potential role of ZNFX1 in its related immuno-hematological abnormalities and HLH etiology, this insight will warrant enhanced targeted therapeutics in this subset of HLH patients.

INFORMED CONSENT

Written informed consent was obtained from the parents to publish this report in accordance with the journal's patient consent policy.

AUTHOR CONTRIBUTIONS

MA Manuscript writing and revision, figure, table creation

AA Manuscript writing and revision, figure, table creation

HAS Manuscript writing and revision

FAZ Manuscript writing and data collection

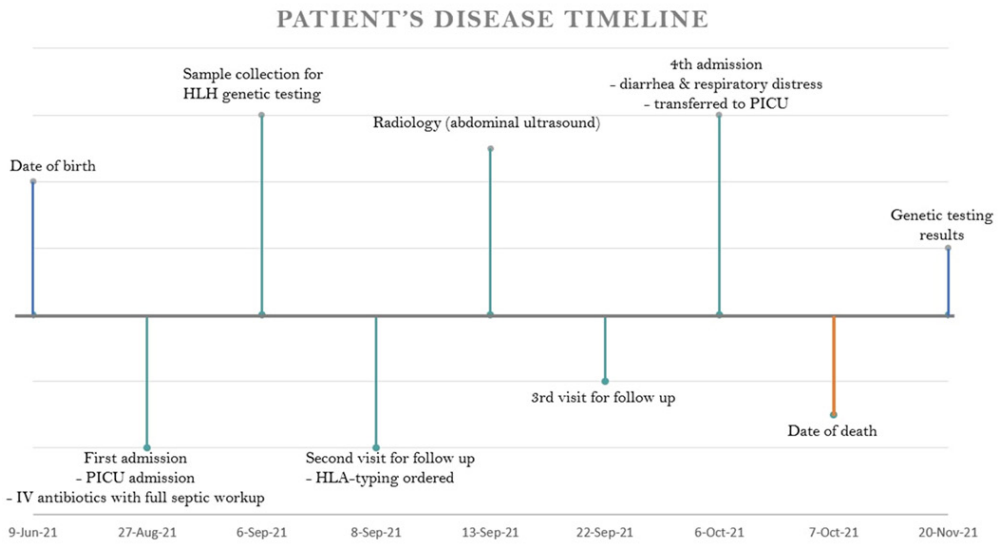
HA Patient care and data collection

SAS* Overall review, direct patient care

Table 1. Laboratory investigations throughout the patient's follow-up. Abbreviations - Hb: Hemoglobin, HCT: Hematocrit, WBCs: White Blood Cells, PMN: Polymorphonuclear leukocytes, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl Transferase, LDH: Lactate dehydrogenase, PT-INR: Prothrombin Time - International Normalized Ratio, TG: Triglycerides.

	27/08/2021	08/09/2021	22/09/2021	06/10/2021	07/10/2021
Hb	9.2 g/dl	11.2 g/dl	11.3 g/dl	11.6 g/dl	10.1g/dl
HCT	28.8%	35.1%	34.3%	35.3%	32.6%
Reticulocytes	5.48%				
WBCs	37.3 10*3/mm*3	28.05 10*3/mm*3	17.3 10*3/mm*3	22.63 10*3/mm*3	52.5 10*3/mm*3
Platelets	261 10*3/mm*3	265 10*3/mm*3	105 10*3/mm*3	262 10*3/mm*3	351 10*3/mm*3
PMN	29%	13.6%	20.7%	32.9%	35%
Lymphocytes	64%	79%	71.6%	58.6%	59%
Monocytes	4%	6.4%	6.1%	6.8%	4%
Serum ferritin			502.55 ng/ml		
Fibrinogen	187 mg/dl		259 mg/dl		
AST	49 U/L				37.6 UL
ALT	15 U/L				11.6 U/L
ALP	281 U/L				274 U/L
Albumin	34 g/L				29.8 g/L
GGT	32 U/L				17 U/L
LDH	1168 U/L	984 U/L			933 U/L
Total Bilirubin	7 umol/L				3.4 umol/L
Direct bilirubin	2 umol/L				0.1 umol/L
PT-INR	1.14				
TG					1.27 mmol/L
Blood NH3					89.5 umol/L
Creatinine	21 umol/L			21 umol/L	31 umol/L
Na	141 mmol/L			139 mmol/L	134 mmol/L
K	4.9 mmol/L			5.41 mmol/L	6 mmol/L

Figure 1. Timeline of sequential progression from the patient's birth until death.



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