Pediatric Hemophagocytic lymphohistiocytosis: A rarely diagnosed entity in a developing country

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Abstract

Introduction: Hemophagocytic lymphohistiocytosis (HLH) is an exaggerated inflammatory reaction secondary to a host's inadequate immune response causing a self-perpetuating loop of altered regulation. Signs and symptoms of HLH are compatible with other common diseases and are nonspecific. Underdiagnosis makes it difficult to estimate the real incidence of HLH, especially in developing countries. Materials and Methods: Retrospective, descriptive study of hospitalized pediatric patients admitted to a high-complexity institution in Cali, Colombia between 2012 and 2019 with HLH diagnosis. Medical history review to complete a virtual database. A secondary descriptive analysis was carried out. The study was approved by the Institutional Ethics Committee. Results: Twenty-one patients were included. 52.4% of the population was male with a median age of 9.3 years [IQR (3.0-13.7years)]. More than half of patients (66.6%) had viral isolation at diagnosis, the most frequent being Epstein-Barr Virus (EBV) (52.3%) and dengue (14.3%). Three patients had gene mutations (LYST, XIAP, and UNC13D). Ninety-five percent of the patients were treated with the HLH 2004 protocol, half of them received incomplete protocol with IgIV at high doses and/or systemic steroids, while the other half received the complete protocol including etoposide and cyclosporine. More than three-fourths (76.2%) required admission to an ICU with a median stay of 14 days [IQR (11-37 days)] and a median hospital stay of 30 days [IQR (18-93 days)]. 14.3% (n = 3) of patients died. Conclusions: HLH is an underdiagnosed pathology that requires greater sensitization in developing countries in order to make early diagnoses and obtain better outcomes.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an exaggerated and ineffective inflammatory reaction secondary to a host's inadequate immune system response causing a self-perpetuating loop of altered immune system regulation.[1,2] In HLH there is overactivation of T cells, natural killer (NK) cells and macrophages causing an uninhibited release of cytokines.[2,3] The term hemophagocytosis describes the pathognomonic findings where highly activated macrophages and lymphocytes, surround erythrocytes, leukocytes, platelets and different tissues, producing excessive cytokines and an uncontrolled inflammatory reaction.[1]The term HLH encompasses a wide range of disorders including primary HLH [which includes familial HLH (FHLH), familial erythrophagocytic lymphohistiocytosis] and secondary HLH [infection-associated hemophagocytic syndrome and autoimmune-associated macrophage activation syndrome (MAS)].[1,3]

Signs and symptoms of HLH are compatible with other common diseases such as infections, tumors and rheumatological diseases and are often nonspecific.[4,5] HLH should be included in the differential diagnosis of other clinical conditions such as: 1) fever of unknown origin, 2) hepatitis with coagulopathy (30% of patients with HLH present an increase of transaminases above 100 U/L), 3) sepsis with multiple organic failure, 4) lymphocytic encephalitis.[6]

The first diagnostic guidelines for HLH were published by the Histiocyte Society in 1991 and included clinical,

laboratory and histopathological criteria.[6,7] In 2004 the guidelines were modified due to the fact that some patients did not fully complete all criteria and different criteria may develop throughout the course of the disease and as genetic testing has become more readily available.[6]Just as well, other criteria have been tested including the H-score.[8,9]

Currently, underdiagnosis makes it difficult to estimate the real incidence of HLH. Some reports estimate 1.2 cases per million people per year.[1,10] In Sweden the incidence rate is 1 in 50,000 live births for primary HLH.[11] These entities occur in children under 1 year of age in 70%-80% of cases and the incidence rate is 1 per 1 million newborns per-year.[5,12–14]Some reports described a male predominance but there is not a clear association.[14,15] There are no reports of epidemiology in developing countries in Latin America.

We aimed to determine the frequency of hemophagocytic syndrome and describe the demographic, clinical and outcome characteristics of pediatric patients who were hospitalized a high complexity institution in Cali, Colombia between 2012 and 2019.

Materials and Methods

This is a retrospective, observational study evaluating patients younger than 18 years of age hospitalized with HLH diagnosis between 2012 and 2019 in Fundación Valle de Lili (FVL), a high-complexity institution in Cali, Colombia. FVL is the pediatric reference center for complex pathologies in southwestern Colombia.

Data were obtained from the institutional statistics of hospitalized patients under 18 years of age with ICD-10 (D7.6) diagnoses, in addition to the pediatric hematooncology and immunology services records. Subsequently, a medical history review was carried out and a database in BD Clinic, an institutionally-created, virtual platform for research data bases, was completed by a trained pediatrics resident. The database included 120 variables including sociodemographic, clinical, laboratory, treatment/management and outcome variables. During the study period a total of 182,372 patients consulted the pediatric emergency department at our institution, allowing us to calculate total incidence of pediatric HLH to be 0.01% (21/182,372).

Clinical and laboratory variables were included on diagnosis, 24-48 hours afterwards, at 4 weeks and at 8 weeks. Not all patients included have data for 4 weeks and 8 weeks, because of adequate clinical response they were discharged from hospital. Most patients did not continue follow-up after discharge as follow-up consults are not authorized by health care providers in our health system hindering adequate long-term follow-up and management.

Diagnosis of HLH was based on the HLH-2004 Criteria:[6]

With 5 out of 8 criteria the diagnosis is made:

1. Fever (Temperature $> 38.5\ ^\circ$ C, $>$ 7 days)
2. Splenomegaly ($>$ 3 cm below the costal margin)
3. Cytopenias that affect at least 2 of 3 lineages in the peripheral blood and are not caused by hypocellular bone or marrow (neutrophils $<$ 1 109/L, hemoglobin $<$ 9 g/dL, platelets count $<$ 100 X10^9/L)
4. Serum Triglycerides $>$ 2.0 mmol/L and/or serum fibrinogen $<$ 150 mg/dL
5. Phagocytosis in bone marrow (BM), spleen, or lymph nodes
6. Soluble CD $>$ 2,400 U/m (*not available in our institution)
7. Low NK cell number or reduced activity
8. No evidence of malignancy

OR A molecular or genetic test confirming the presence of primary HLH

With the data, a descriptive and secondary analysis of the data was carried out using STATA 12.1 ©. Frequency, central tendency and dispersion measures were used according to the classification of each of the variables and their non-normal distribution.

The study was approved by the Institutional Ethics Committee of FVL.

Results

A total of 21 pediatric patients between 2012 and 2019 were diagnosed with some form of HLH (either primary or secondary) in our institution. A total of 182,372 patients consulted through the emergency department during this time period, this makes the total incidence of pediatric HLH to be 0.01% during the study period.

Males were 52.4% of the population with a median age of 9.3 years [IQR(3.0-13.7 years)]. The majority of patients were from Cali (the city where FVL is located) (38.1%); however, we also had patients from other parts of the Valle del Cauca state (23.8%), Cauca department (19%), Nariño state (9.5%), Choco state (4.8%) and Caquetá state (4.8%). All patients were febrile on admission with a median of 17 days (IQR 6-30 days) while 1/3 of patients had splenomegaly (Table 1).

Additionally, on diagnosis the most common blood cell alterations were anemia and thrombocytopenia (Table 2).

Two patients (9.5%) had bacterial growth in cultures, one patient with abdominal *Mycobacterium tuberculosis* and another patient with*Pseudomona aeruginosa* and *Klebsiella pneumoniae* in blood. Fourteen patients (66.6%) had viral isolation, with EBV (11 patients, 52.3%) and dengue (3 patients) being most frequent viral etiologies. Three patients (14.3%) had reported genetic mutations, mutations in the LYST, XIAP, and UNC13D genes were found. The patients with the LYST and UNC13D mutations had acute EBV infection and the patient with the XIAP mutation had acute cytomegalovirus (CMV) infection, triggering the HLH. The patients with XIAP and UNC13 mutations were taken to BMT, while the patient with LYST mutation had a heterozyte form and responded adequately to IgIV management after an EBV infection. (Figure 1)

Most patients received management with HLH 2004 protocol (95.2%), 47.6% received incomplete protocol (only human intravenous immunoglobulin and/or dexamethasone at high doses) while the other half of the patients received the complete protocol (etoposide, cyclosporine and dexamethasone at high doses). One patient received methotrexate at another institution before referral to our institution and was taken to bone marrow transplant (BMT).

Three patients were taken to BMT (14.2%), of which one was diagnosed with FHLH with the c.9049g> a homozygote mutation in the UNC13D gene, another with c.1045G>T p.Glu349* variant a on the XIAP gene and the other was diagnosed with severe combined immunodeficiency.

More than 76% of patients (76.2%) required admission to an intensive care unit with a median stay of 14 days [IQR (11-37 days)] and a hospital stay of 30 days [IQR (18-93 days)]. Three patients (14.3%) died before genetic testing could confirm final diagnosis, two of them had elevated EBV viral loads with active infection, one died before completing 4 weeks of HLH- 2004 protocol. The third patient did not have any infectious etiology, a rheumatological disease was suspected, however, he had a fulminant course of the disease and died two weeks after diagnosis was made.

Additionally, survival of pediatric patients with HLH diagnosis was 65.5% after a hundred days sin diagnosis. (Figure 2)

Discussion

HLH is an underdiagnosed pathology especially in developing countries where lack of resources and awareness of this disease prevails. The total incidence in our study was 0.01%, this is far below what is reported in literature in developed countries.[5,12–14]Awareness of this pathology has increased, especially in our institution where diagnosis has increased over the last couple of years because of an active search for the disease in critically ill patients.

HLH has a broad spectrum of etiologies, including infections which have a wide range of clinical presentations with high morbidity and mortality rates secondary to the host's immune predisposition.[1,3,16,17] Acute infection with EBV was the most frequent infection in our population, which is in accordance with what's reported in literature.[16,18] Dengue was the second most common virus associated with HLH we consider that it may be secondary to an active search of these infections due to our local epidemiology, and this is in accordance to literature in developing countries.[19]

Most of our patients had hypertriglyceridemia, hypofibrinogenemia and hyperferritinemia. Upon admission, 28% of our population had ferritin >10,000 ng/ml which has reported a sensitivity and specificity above 80%.[20,21]

Hemophagocytes were found in bone marrow (BM) in 57% of patients which is in accordance with literature reports depending on the time of the BM aspirate, this finding is reported between 40% and 80%.[2,22] In our population hemophagocytes were not sought in tissues other than BM since this practice is not protocolized in our institution.

The rapid onset of immunosuppressive therapy to control host immune hyperreactivity is necessary to obtain better results.[1–3,6,23]. The first-line treatment is dexamethasone based on its cytotoxic effect on lymphocytes, inhibition of cytokine production and dendritic cell. [1–3,6] This should be combined with cyclosporine A, which interferes with the activation of lymphocyte and macrophage function. [1–3,6] The etoposide is used as it destroys the antigen presenting cells.[1–3,6] The HLH-94 trial, published in 2011, was the largest prospective diagnostic/therapeutic study of HLH.[24] They evaluated the results of an 8-week course of treatment with dexamethasone, cyclosporine and etoposide and reported a reduction in the mortality rate from 95% to 30%.[4]While this study was being conducted, the HLH-2004 protocol was initiated with revised diagnostic criteria to achieve better remission by adding cyclosporine to the treatment and intensifying the induction regimen. [4] In our patients, 42.5% received a complete protocol with cyclosporin A and etoposide, of which 2 died before reaching 8 weeks of treatment and without genetic studies to clarify the origin of HLH, while three patients were taken to BMT. Patients with genetic causes, persistent/resistant HLH treatment or reactivation of the disease should have a BMT as soon as possible to improve their survival and morbidity results.[6]

Three of our patients were taken to BMT, two patients with genetic mutations associated to FHL. The first one was a male with a homozygous UNC13D gene mutation in c.9049G>A with persistent EBV infection, he presented occlusive vein disease of the liver immediately after BMT but recovered and was discharged after a prolonged hospitalization. Three years after BMT he had regained adequate immune cell and immunoglobulin levels, so antibiotic prophylaxis and Ig IV was suspended.

The second patient was a 7-month female patient with a SCID diagnosis that was initially treated in another city and was referred for BMT. She was taken to allogenic BMT (father was the donor). She was diagnosed with gastrointestinal graft-vs-host disease by clinical criteria, and immunosuppressive therapy was increased. Seven months after BMT she had 50% total chimerism and 97% of T-cells chimerism.

The third patient was a fourteen-month old male that had a c.1045G>T p.Glu349* variant in the gene XIAP2 and had an acute CMV infection which triggered the appearance of HLH. He was taken to haploidentical BMT (brother was the donor) less than a year ago. He was diagnosed with cutaneous graft-vs-host disease after skin biopsy three months after BMT and is currently being managed with topical corticoids and follow-up by dermatology.

Patients with HLH resistant to etoposide-based therapy have a poor prognosis because there are currently few treatment alternatives with little evidence available. Alemtuzumab, a monoclonal antibody directed to the CD52 antigen in lymphocytes, monocytes, macrophages, and dendritic cells, and rituximab has recently been proposed as an alternative treatment.[25,26]

The Hybrid Immunotherapy for Hemophagocytic Lymphohistiocytosis (HIT-LHL) study is an open label phase II trial that was carried out by Cincinnati group in patients with HLH < 18 years of age.[27] They evaluated the safety and effectiveness of a treatment regimen combining ATG with dexamethasone, intrathecal methotrexate, etoposide and hydrocortisone.[27] The primary outcome was complete response rate at 8 weeks (clinicaltrials.gov NCT01104025).[27] Euro-HIT-LIT is a phase II/III trial and a Cincinnati study extension performed in Europe (ClinicalRegister.eu 2011-002052-14).[28] There still no result of both trials, but preliminary data is promising on the effectiveness of treating HLH with hybrid immunotherapy.[27,28]

Limitations

Some of the limitations with our study includes its retrospective nature over 7 years, which makes it difficult to gather some of the data that wasn't recorded is medical records and could include information or selection bias in our data.

We also consider the loss of follow-up after discharge to be a major limitation, however, this is common in our health system which is plagued by bureaucratic processes making it sometimes difficult for patients to access specialty care.

It is worth mentioning that genetic testing is done in specific cases, usually in patients under five years of age where primary HLH would be most likely. In our institution and country, genetic testing is expensive, making it poorly accessible and many times a tool that is applied late in the course of disease.

Conclusions

Education about HLH and its early inclusion in differential diagnoses could reduce its insufficient and late diagnosis and improve patient outcomes. Diagnosis can be made with clinical and paraclinical criteria with high sensitivity and specificity, and delays in genetic testing should not delay treatment. New therapies are emerging to improve the prognosis and improve the quality of life of patients with HLH.

Conflicts of Interest Statement

Authors declare no conflict of interest.

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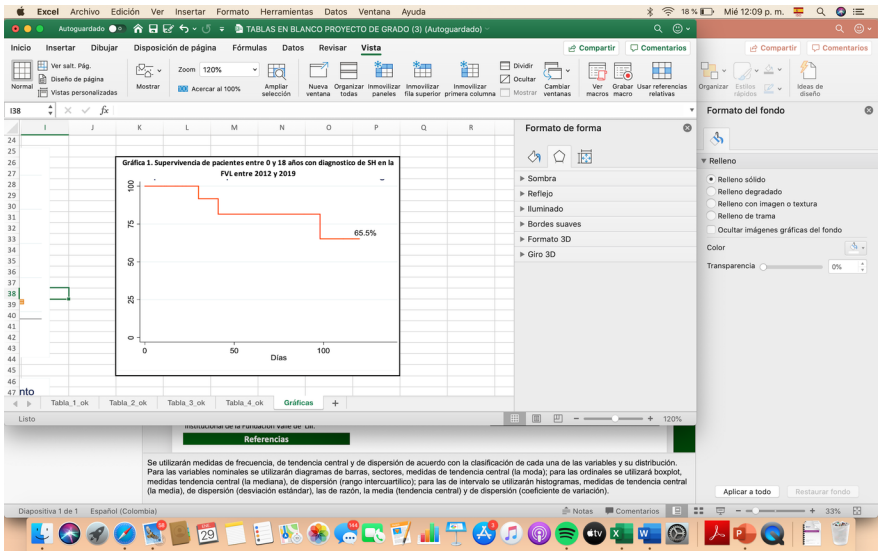

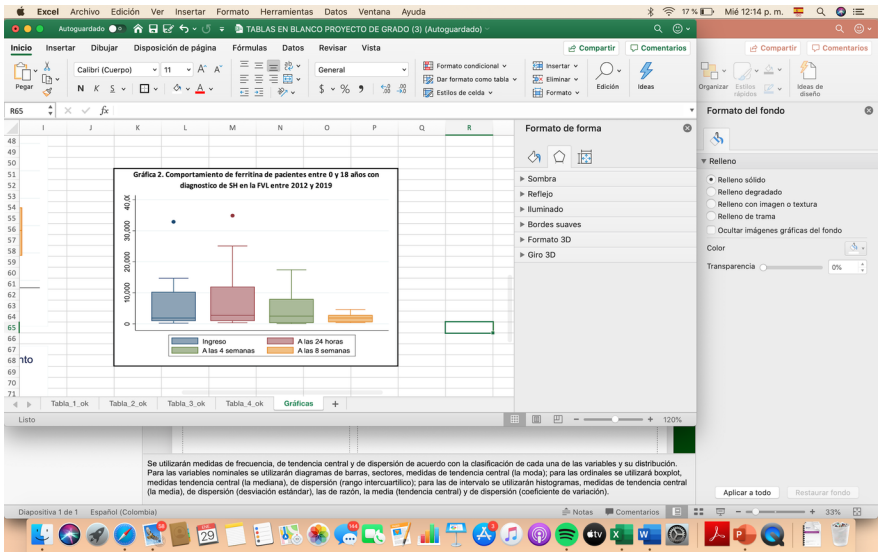

Legends:

Table 1. Diagnostic criteria at admission in patients between 0 and 18 years with a diagnosis of HLH in the FVL between 2012 and 2019

Table 2. Laboratory values of patients between 0 and 18 years of age diagnosed with HLH in FVL between 2012 and 2019

Figure 1. Different etiologies and triggers of patients between 0 and 18 years of age diagnosed with HLH in the FVL between 2012 and 2019

Figure 2. Survival of patients between 0 and 18 years of age diagnosed with HLH e in the FVL between 2012 and 2019

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