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Hemophagocytic syndrome: Laboratory and molecular characterization

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Abstract:

BACKGROUND: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome of fever, cytopenia, and organomegaly resulting from immune activation and cytokine storm. The syndrome can occur as a primary/familial form mostly affecting infants and young children or as an acquired form secondary to an underlying pathology (infection, malignancy, and autoimmune disease) that may have an underlying genetic predisposition, including mutations or polymorphisms.

PATIENTS AND METHODS: This case–control study was conducted in Basra, Iraq. Thirty-four pediatric and adult patients with peripheral cytopenia attributed to bone marrow (BM) hemophagocytosis enrolled with 34 healthy individuals (age and sex matched) included as a control group. Whole blood was tested for complete blood count and screened for the presence of mutations in the perforin gene by polymerase chain reaction amplification; in addition, serum samples were tested for soluble CD25, ferritin, and triglycerides (TGs).

RESULTS: The mean hemoglobin level and platelets count were significantly lower in HLH patients compared to the control group ($P < 0.001$), while there was no significant statistical difference regarding neutrophils count ($P > 0.05$). Soluble CD25 (s.IL-2R) testing revealed inconsistent results; serum ferritin and TGs were significantly higher in HLH patients compared to the control group ($P < 0.001$). About nine cases were genetically proven to have primary HLH; all were infants under the age of 6 months. Perforin gene mutations were detected in 38.8% ($n = 7$) of tested subjects. The novel frameshift mutation of the perforin gene (c.218_224del) was identified in four cases. Fifteen different perforin gene polymorphisms were detected in both case and control groups. Six out of nine infants with primary HLH did not survive, while the remaining three cases underwent BM transplantation.

CONCLUSION: Early diagnosis of HLH is often challenging; this study should increase awareness of the prevalence of familial HLH among infants; such cases require early recognition and referral to hematopoietic stem cell transplantation.

Keywords:

Basra, familial hemophagocytic lymphohistiocytosis, Iraq, PRF1

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome of fever, cytopenia, and splenomegaly resulting from immune activation and hyper-inflammation with cytokine storm.[1]

The first published report of HLH was by Scott and Robb Smith in 1939 on four adults who thought to have an atypical form of Hodgkin's disease and it was called "histiocytic medullary reticulosis." The International Histiocyte Society adopted the term "HLH" as a formal reference in 1998 and defined its diagnostic criteria, which were updated in 2004.[2] The term "hemophagocytosis" refers to histopathological findings of activated histiocytes engulfing blood cells and their precursors in the bone marrow (BM), spleen, liver, and lymph nodes.[3]

The syndrome can occur as a primary/familial form mostly affecting infants and young children or as an acquired form

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secondary to an underlying pathology (infection, malignancy, and autoimmune disease).[4]

Familial HLH (F-HLH) is caused by mutations in the perforin (*PRF1*) gene and multiple other genes (*UNC13D, STX11, STXBP2*) affecting perforin delivery and exocytosis of cytoplasmic granules, hence in the perforin-mediated killing of target cells.[1,4] Sporadic cases are encountered with other genetic immunodeficiencies, Chédiak–Higashi, Griscelli, Hermansky–Pudlak, and X-linked lymphoproliferative syndromes.[1]

Clinically, patients are present with fever, hepatosplenomegaly, lymphadenopathy, rash, and liver abnormalities with one-third of patients showing neurologic symptoms.[4]

Two main classifications are routinely used for the diagnosis of HLH: the HLH 2004 criteria were developed by Henter *et al*., and the most recently developed is the H-Score.[5,6] The HLH 2004 classification was developed for the diagnosis of pediatric forms of HLH. It includes one genetic criterion and eight clinical or biological criteria, and the diagnosis of HLH can be established either by a molecular diagnosis consistent with HLH or by five out of eight diagnostic criteria, including fever, splenomegaly, cytopenia, hyperferritinemia, hypofibrinogenemia or hypertriglyceridemia, elevated soluble CD25 (s.IL-2R), hemophagocytosis in BM, lymph nodes or other tissues, and low or absent natural killer (NK) cells activity.[2]

The H-score is a new diagnostic score that was developed by Fardet *et al*. for the diagnosis of acquired HLH. However, it has only been validated for the diagnosis of reactive forms of HLH in an adult cohort.[6,7]

Currently, the blurring of the boundary between primary and secondary HLH is based on evidence from studies suggesting that after certain environmental triggers, HLH-related gene polymorphisms may result in uncontrolled immune cell activation that contributes to the onset and outcome of the disease.[8]

The aim of the study is to evaluate the clinical, hematological, and biochemical profiles in patients with HLH and to study the *PRF1* gene mutations/polymorphism in relation to the clinical and laboratory findings.

Patients and Methods

This case–control study was conducted in Basra Specialized Children Hospital and Adult Hemato-Oncology Center, from November 2022 till July 2023, written consent was taken from all of the individuals who were included in the study, and it was approved by the Ethical Committee of the Scientific Council of Pathology at the Iraqi Board.

The study included 34 new cases (25 of them were children below the age of 16 years with 9 adults) with suspicion of HLH based on certain clinical and laboratory findings (fever, splenomegaly, and unexplained cytopenia), in whom BM examination revealed histomorphological finding of hemophagocytosis; cases with any chronic illness, underlying malignancy, immune suppressive conditions, or treatments that are known to be associated with HLH or BM hemophagocytosis were excluded.

Thirty-four healthy individuals (age and sex matched) were included in this study as a control group (people who attended the health-care facilities for routine clinical and laboratory checkups and those who attended the outpatient clinic for surgical consultation); any individuals with fever, recent infection or CBC abnormalities including cytopenia (except for microcytic anemia) were excluded.

Demographic and clinical data were collected from the patients' records, including age, gender, presence of fever, hepatomegaly, splenomegaly, any chronic illness, or certain drug intake.

Blood was sampled on the finding of hemophagocytosis on BM aspirate examination before starting any treatment. The complete blood count of both case and control groups was determined by the Compact 6-part differential fully automated hematology analyzer (Sysmex XN-350). Serum ferritin and triglyceride (TG) were measured by the fully automated Abbott clinical chemistry analyzer.

Soluble CD25 level for case and control groups was measured using a double-sandwich enzyme-linked immunosorbent assay (ELISA) technique with the use of YLBiont human soluble CD25 ELISA kit.

PRF1 gene sequencing

Coding exons of the *PRF1* gene (exon 2 and exon 3) were tested for the presence of mutations and polymorphisms by polymerase chain reaction (PCR) amplification of DNA before sequencing analysis of the *PRF1* gene. These tests were carried out on 30 whole blood samples (15 patients and 15 controls). Genetic test results of whole-genome sequencing (WGS) for three additional patients were obtained from patient records (total number of cases with genetic test results = 18).

DNA was extracted from peripheral blood using a gSYNC™ DNA extraction kit from Geneaid. The extracted genomic DNA was used to amplify the fragment from the PRF1 gene implementing a set of

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specific primers [Table 1]. The PCR products were sent to Macrogen Company, Korea, for sequencing. The obtained sequences were compared with the published PRF1 gene sequence.

Statistical analysis

Data were analyzed using computerized statistical software, the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY). Quantitative data were presented as mean ± standard deviation, while qualitative data were presented as frequencies and percentages. The appropriate statistical tests were performed; a Chi-square test was used for categorical variables and two-sample independent *t*-tests for the continuous variable. In all statistical analyses, the level of significance (*P* value) is set at ≤0.05, and the result is presented as tables and/or graphs.

Results

The study included a total of 34 patients (23 were male and 11 were female) in the case group and a similar number (age and sex matched) as healthy participants in the control group. The age of the studied patients ranged from 1 month to 37 years. Cases were categorized into four major age groups [Figure 1].

The clinical presentation and laboratory findings for the case group (*n* = 34 patients) are shown in Table 2.

Regarding laboratory features, a statistically significant difference between case and control groups was observed (*P* < 0.001); regarding each hemoglobin (Hb) level, platelets count, serum ferritin, and TG, with the exception of neutrophils count, the difference was of no statistical significance (*P* > 0.05). The s.CD25 shows a lower level among the case group in comparison to the control group [Table 3].

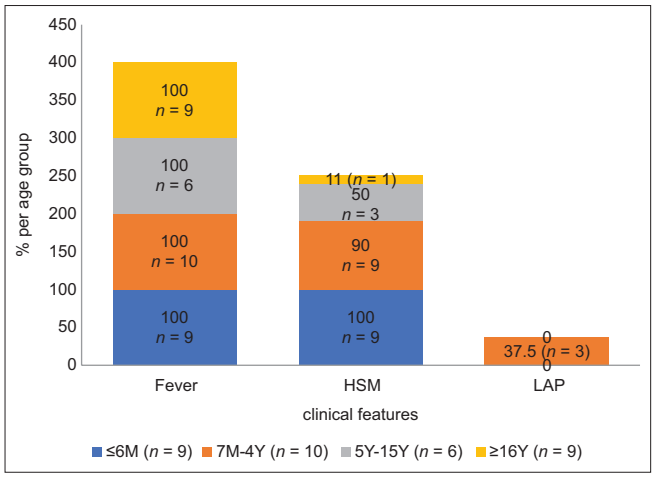

Figure 1: Main clinical features in HLH patients at diagnosis in relation to different age groups

On the application of HLH-2004 diagnostic criteria on case groups, about 24 (70%) cases have fulfilled five out of six available clinical and laboratory features to be applied (fever, splenomegaly, dicytopenia, hyperferritinemia, hypertriglyceridemia, and BM hemophagocytosis) [Table 4].

Fifteen cases with 15 age- and sex-matched control samples were tested for *PRF1* gene mutations with three additional cases whose genetic testing results were obtained from their files (the total number of cases with genetic test results is 18 cases).

Table 1: The sequence of primers used in exon 2 and exon 3 amplification of the perforin gene

Primer	Sequence (5' → 3')	Product size (bp)
PRF1-E×2-F	AAGCAGCCTCCAAGTTTGATTG	756
PRF1-E×2-R	CCCTTCCATGTGCCCTGATAATC	
PRF1-E×3-F	GAACCCCTTCAGTCCAAGCATAC	1289
PRF1-E×3-R	CCAGTCCTAGTTCTGCCCACTTAC	

PRF1=Perforin

Table 2: Clinical and laboratory features of studied cases

Clinical and laboratory features	Cases
Fever >38.5°C	100% (*n*=34)
Splenomegaly	64.7% (*n*=22)
Lymphadenopathy	37.5% (*n*=3)
Dicytopenia*	88% (*n*=30)
Pancytopenia*	44% (*n*=15)
BM hemophagocytosis	100% (*n*=34)
Hyperferritinemia >500 ng/mL	94% (*n*=32)
Hypertriglyceridemia >265 mg/dL	50% (*n*=17)

*Cytopenia below the limits set by the HLH-2004 diagnostic criteria (Hb <9 g/dL, neutrophils <1×10^9/L, platelets <100×10^9/L). HLH: Hemophagocytic lymphohistiocytosis, Hb: Hemoglobin

Table 3: The laboratory findings of the studied groups

Variables	Case (*n*=34)	Control (*n*=34)	*P*
Hb (g/dL)	8.48±1.48	11.66±1.36	0.001
Neutrophil (×10^9/L)	2.19±1.96	3.17±1.24	0.269
Platelet (×10^9/L)	78.44±20.1	344.8±118.4	0.001
Ferritin (ng/mL)	7499.48±3084.15	64.1±13.6	0.001
TG (mg/dL)	295.2±186.4	91.11±44.4	0.001
Serum CD25 (ng/mL)	1.73±1.46	2.62±2.02	0.041

Hb=Hemoglobin, TG=Triglyceride

Table 4: Number of cases who have fulfilled five out of eight hemophagocytic lymphohistiocytosis - 2004 diagnostic criteria per age group

Age	Number of cases (%)
≤6 months	8 out of 9 cases (89)
7 months–4 years	8 out of 10 cases (80)
5–15 years	4 out of 6 cases (66)
≥16 years	4 out of 9 cases (44)
Total	24 out of 34 cases (70)

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Results of *PRF1* gene analysis revealed that 6 out of 15 cases tested were genetically proven to have a primary hemophagocytosis (p-HLH). The additional three cases were also genetically proven by WGS to have p-HLH (total of cases with p-HLH = 9), while the remaining 25 cases were genetically undermined [Table 5].

On comparing cases of p-HLH cases with the genetically undetermined ones, there was a statistically significant association regarding the presence of splenomegaly in cases of p-HLH and those who are genetically undetermined ($P < 0.05$); for laboratory features, there was no significant difference regarding the Hb, neutrophil count, platelets counts, and serum ferritin ($P > 0.05$). For serum TGs, there is a significant statistical association observed, between primary cases and those who are genetically undetermined ($P < 0.05$) [Table 6].

Mutation analysis of the *PRF1* gene revealed that 6 out of 15 cases tested, in addition to the three cases who have had WGS, were proven to have p-HLH (total number 9 out of 18 cases), PFR1 gene mutations were not detected in control samples.

Out of the nine cases with primary HLH, seven cases had mutated *PRF1* gene (FHLH-2), one case had two different pathogenic mutations of the STXBP gene (FHLH-3), and the last case had a mutation of RAB27A gene associated with Griscelli type 2 which is liked to familial form of HLH. The mean age of diagnosis was 2.7 months [Table 7]. *PRF1* gene analysis revealed different mutations, the most frequent one is the novel (7 nucleotide deletion: c.218_224delAGGGTGC) which was observed in four cases, three of which were homozygous for the deletion (Case No. 2, 4, 12), and the fourth case was heterozygous (Case No. 15); this deletion has neither been reported previously nor listed in the genomic databases. Five different missense mutations of the *PRF1* gene were detected in a single case (Case No. 5), two of these mutations were homozygous and three were heterozygous (c.274C>T, c.202C>G, c.16C>T, c.299C>T, c.536A>C), with the last two mutations being novel and have not been reported in the published literature.

Fifteen different intragenic polymorphisms were detected in the *PRF1* gene [Table 8], all of which have been previously described. The Ala274Ala was the most frequent one, identified in five healthy control samples and two cases followed by the His300His variant identified in four control and one case samples. The remaining polymorphisms were observed only once mostly in control samples.

Table 5: The demographic characteristics of the 15 cases tested for perforin gene mutations

Age	Number of cases tested per age group	Gender	Number of cases with p-HLH
≤6 months	6*	4 males and 2 females	6
7 months–4 years	3	2 males and 1 female	0
5–15 years	3	1 male and 2 females	0
≥16 years	3	2 males and 1 female	0

*Genetic results (WGS tests) for three additional cases of p-HLH (2 males + 1 female) obtained from their files, were also infants below 6 months of age (total number of cases of p-HLH is 9). HLH=Hemophagocytic lymphohistiocytosis, WGS=Whole-genome sequencing

Table 6: The clinical and laboratory features of the two case group categories

Variables	Primary (*n*=9)	Genetically undetermined (*n*=25)	*P*
Fever, *n* (%)	9 (100)	25 (100)	0.96
Lymphadenopathy, *n* (%)	0	3 (12)	0.27
Splenomegaly, *n* (%)	9 (100)	13 (52)	0.01
Hb (g/dL)	8.1±1.3	8.6±1.55	0.41
Neutrophil (×10^9/L)	1.7±0.91	2.3±1.1	0.74
Platelet (×10^9/L)	40.6±4.01	92.0±24.22	0.21
Ferritin (ng/mL)	7004.5±2371.3	7677.6±2216.6	0.86
TG (mg/dL)	409.5±216	254±159.7	0.029

Hb=Hemoglobin, TG=Triglyceride

Discussion

HLH is a hyperinflammatory heterogeneous disorder of immune dysregulation affecting the function of cytotoxic T-lymphocytes and macrophages, resulting in tissue injury and organ failure.[9,10] Although HLH is considered to be a disease of infancy,[11] it can present beyond the age of 1 year. A study by Shabrish *et al*. reported that 34% of cases of F-HLH were diagnosed beyond 1 year of age.[10] In the present study, nine cases with HLH-related gene mutations were identified, all of which were under the age of 6 months at the time of diagnosis. The male gender was the predominant one, with a male:female ratio of 2:1, which is exactly similar to a study of HLH among pediatric cases by Saud *et al*. conducted at Basra Children's Hospital.[12] A higher male:female ratio has also been reported by other studies.[10,13]

HLH is characterized by many clinical signs, including fever, organomegaly, cytopenia, and coagulopathy.[1] In this series, fever was the most common presentation present in all cases [Figure 1] which is consistent with the literature data,[12,14] followed by splenomegaly in 64.7% of cases.

Hematological abnormalities in the form of cytopenia below the limits proposed by the HLH-2004 diagnostic criteria are present in all of the cases, and cytopenia affecting two cell lines was found in 88% of cases; this is in agreement with the literature data.[10,15] Pancytopenia

Table 7: The genetic mutations identified in patients

Mutation	HLH related gene	AA change	Type of mutation	Frequency	Case number
c.218_224delAGGGTGC†	PRF1	p.(Thr72Pro)	Frameshift	4/18	2, 4, 12, 15
c.274C>T*	PRF1	p.(Leu92Phe)	Missense	1/18	5
c.202C>G*	PRF1	p.(Arg68Gly)	Missense	1/18	5
c.16C>T*	PRF1	p.(Leu6Phe)	Missense	1/18	5
c.299C>T**	PRF1	p.(Ser100Phe)	Missense	1/18	5
c.536A>C**	PRF1	p.(Tyr179Ser)	Missense	1/18	5
c.844_846delAAG**	PRF1	p.(Lys285del)	In-frame del	1/18	10
c.533T>C**	RAB27A	p.(Leu178Pro)	Missense	1/18	16^
c.880del**	PRF1	p.(Gln294Lys)	Frameshift	1/18	17^
c.828-2A>C*	STXBP2	p.?	Splicing	1/18	18^
c.84G>A*	STXBP2	p.(Trp28X)	Nonsense	1/18	18^

*Heterozygous, **Homozygous, †Three cases were homozygous for the mutation and one was heterozygous, ^Cases numbers 16, 17, and 18 results were obtained from their files. PRF1=Perforin, HLH=Hemophagocytic lymphohistiocytosis

Table 8: Polymorphisms of the perforin gene

Exon	Nucleotide change	AA effect	Frequency	Sample
2	c.462 A>G	Ala154Ala	1/30	Case
3	c.900 G>A	His300His	3/30	2 controls + 1 case
	c.822 G>A	Ala274Ala	6/30	4 controls + 2 cases
	c.821 G>A	Ala274Val	1/30	Control
	c.1078 G>A	Leu360Leu	1/30	Control
	c.1305 C>T	Thr435Thr	1/30	Case
	c.1311 C>A	Ala437Ala	1/30	Case
	c.1090 G>A	Leu364Leu	1/30	Control
	c.609 C>A	His203Gln	1/30	Control
	c.615 G>A	Asn205Asn	1/30	Control
	c.591 G>A	Leu197Leu	1/30	Control
	c.1376 G>A	Pro459Leu	1/30	Control
	c.723 G>A	Thr241Thr	1/30	Control
	c.706 G>A	Leu236Phe	1/30	Control
	c.618 G>A	Ala206Ala	1/30	Control

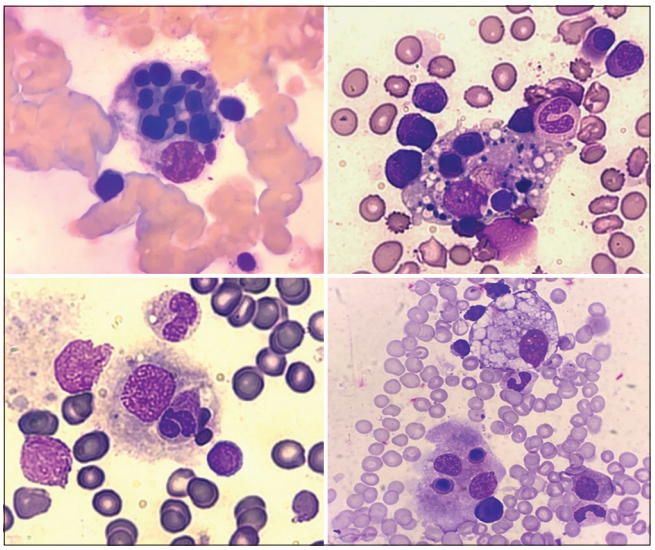

Figure 2: Hemophagocytosis showing histiocytes with ingested hematopoietic cells, including erythroblasts, a neutrophil, platelets, diff-quik, oil emersion

was found in 44%. Cytopenia occurs secondary to the release of pro-inflammatory cytokines leading to impairment of hematopoiesis and apoptosis. Moreover, cytopenia results from consumptive hemophagocytosis in the BM, spleen, and other tissues.[16]

Hemophagocytosis in BM aspirate samples was found in all cases [Figure 2]; however, to this time, there is no consensus on the differentiation of pathological and physiological hemophagocytosis with no threshold proposed for the degree of hemophagocytosis creating an uncertainty among pathologists when evaluating hemophagocytosis.[17,18] In a recent study, it was found that HLH was strongly associated with hemophagocytosis of granulocytes, nucleated erythrocytes, and at least one hemophagocyte engulfing multiple nucleated cells.[18] An unexpected paradox in this study was the degree of hematophagocytosis found in cases of p-HLH which was reported to be mild/occasional by the hematopathologists, while other adult cases, which were likely to acquire a secondary form of the disease, were reported to have marked histiocytic hyperplasia with florid hemophagocytosis [Figure 2]. Gupta *et al*. combined the results of a series of studies and found that BM hemophagocytosis is likely to occur in secondary rather than p-HLH ($P = 0.046$).[18]

In the present study, 94% of cases had hyperferritinemia of more than 500 ng/ml, which is consistent with the literature data.[10,19] Ferritin is also an acute phase reactant that may increase in response to any inflammatory state. Studies have demonstrated that a ferritin level >10,000 ng/ml in pediatric cases was 90% sensitive and 96% specific for HLH.[20] A TGs cutoff >265 mg/dL is used in HLH-2004;[5] 50% of cases in our study had an elevated TG level of more than the limit proposed by the International Histiocyte Society, and these were similar to results reported by other studies.[21,22]

Although the elevated level of soluble interleukin-2 receptor (sCD25) has been recognized as a diagnostic criterion since the HLH-2004 protocol, this study did not yield consistent results regarding sIL-2R, where both case

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and control groups had a mean value of sIL-2R within the reference range for healthy controls. Methods for measurement of sIL-2R other than the ELISA may be more reliable in producing consistent results (such as immunochemiluminometric assay) since ELISA-based assays are liable for interferences by certain factors, including technical issues and reagents stability during shipment and storage.

In this study, direct sequencing of PCR products of coding exon 2 and 3 of the *PRF1* gene revealed mutations in 7 out of 18 cases tested (38.8%) and is predicted to affect the coding sequence and function of the protein [Table 7]. *PRF1* mutation is associated with F-HLH2 with a reported frequency of 20%–40% of F-HLH.[23] A novel frameshift mutation of the *PRF1* gene was identified in four cases (c.218_224del), two of which were the result of consanguineous marriage and one case had a deceased sibling with the same presentation. Three of those cases harbored the c.218_224del in the homozygous form, while the fourth case was heterozygous for the deletion; no other mutations were detected in the perforin gene for the latter case except for three polymorphisms in the exon 3 of the gene [Table 8], two of which were previously reported to be likely benign (1305C>T, 900G>A) and the third one (c.1311C>A) was a novel polymorphism that has not been described previously. Of note, this case was a 5-month-old neonate, who presented with pancytopenia and hepatosplenomegaly and had met the 2004 HLH diagnostic criteria, despite having the c. 218_224del in the heterozygous rather than the homozygous form. One explanation is that heterozygosity for the latter mutation may result in a decrease in perforin expression together with a second hit or environmental factors that may contribute to the development of the disease; moreover, the polymorphisms detected in the gene may act as genetic modifiers creating certain predisposition for HLH. An in-frame deletion was identified in one case (c.844_846del), the mutation resulted in the removal of 1 amino acid but otherwise preserves the integrity of the reading frame. It has been reported before in the literature in children with F-HLHL.[23,24] A third frameshift mutation was found in a case and has been described before to be associated with F-HLH2.[25] Five different missense mutations of exon 2 of the *PRF1* gene were found in the same case, two of which are the novel c.299C>T and c.536A>C; these missense mutations detected may affect the synthesis, stability, or function of perforin. One case was found to be a compound heterozygous for STXBP gene mutation, one of which (p. Trp28*) created a premature stop codon and would be predicted to give rise to a truncated, nonfunctional protein. Finally, a homozygous missense mutation of RAB27A was found in one patient who presented with gray hair and eyelashes; this led to the diagnosis of Griscelli syndrome which is a hypopigmentation immunodeficiency syndrome that has been associated with pHLH.

PRF1 gene polymorphisms have been widely studied to investigate its possible role in the pathogenesis of pHLH or sHLH; studies revealed that A91V is the most common *PRF1* gene polymorphism with an allele frequency of 3%–17%.[26,27] In this study, A91V polymorphism was not detected in either cases or control groups; however, Ala274Ala was found to be the most frequent polymorphism of the *PRF1* gene, occurring in about five controls and two cases (23.5% of tested samples), which is slightly higher than the frequency (15%) reported by Molleran *et al*. They also found out that the most common polymorphism was His300His with a frequency of 67%;[26] in this study, His300His polymorphism was observed in one case and four control samples with a frequency of 16.6% only [Table 3]. The presence of this polymorphism in this study does not completely exclude their pathogenic role as a recent study in China suggested that sHLH may be caused by a two-hit model, where the first hit can be heterozygous variants or polymorphisms in HLH-related genes, and that the second hit from an exogenous trigger required to induce HLH (e.g. a viral infection).[28]

F-HLHL is a life-threatening disease with a poor prognosis, especially for those diagnosed at a very young age; median survival was reported to be around 1–2 months.[29,30] Regarding the outcome of cases with p-HLH in this study, about 66% are deceased ($n = 6$), and three cases underwent BM transplantation.

The limitation of this study is the small sample size due to the rarity of the disease; furthermore, we did not undertake other important tests for the diagnosis of HLH, including tests for NK cell activity and fibrinogen level. Finally, genetic test results were available for a limited number of cases; therefore, it was not possible to classify the remaining cases whether primary or secondary.

Conclusions

HLH is a multisystemic hyperinflammatory disorder caused by certain genetic diseases or secondary to an underlying trigger. Common findings of HLH are fever, cytopenia, hyperferritinemia, and organomegaly. We have presented a functional analysis of mutations and polymorphisms of the *PRF1* gene. All patients below 6 months of age were found to have p-HLH ($n = 9$). The novel *PRF1* c.218_224del was detected in four cases which may be confined to this region of Iraq. This observation may require further extensive studies on larger sample size. *PRF1* gene polymorphism was found

more frequently among healthy population, and it most likely appears to have no impact on disease development. Findings in this should increase the awareness of the clinical and laboratory presentation of the disease and the prevalence of the primary form of HLH. Screening for p-HLH by flow cytometry-based assay with genetic testing is advisable for all children with HLH, especially those under the age of 1 year for early diagnosis and urgent management of this life-threatening disease.

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Conflicts of interest
There are no conflicts of interest.

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