The Role of Pre-B-cell Colony-Enhancing Factor in Egyptian Children with Hemophagocytic Lymphohistiocytosis

Thesis

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بسم الله الرحمن الرحيم

﴿وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ

فَضْلُ اللهِ عَلَيْكَ عَظِيمًا﴾

صدق الله العظيم

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List of Abbreviations

Abb.	Full term
aa	Amino acids
ALI	Acute lung injury
ALL	Acute lymphocytic leukemia
BM	Bone marrow
BMT	Bone marrow transplantation
Cdna	Complementary DNA
CHS	Chediak-Higashi syndrome
CKD	Chronic kidney disease
CMV	Cytomegalovirus
CREB	cAMP response element binding protein
CSF	Cerebrospinal fluid
CTLs	Cytotoxic T lymphocytes
EBV	Epstein-Barr virus
ELISA	Enzyme-Linked Immunosorbent Assay
FHL	Familial HLH
GNHRH	Gonadotropin releasing hormone receptor
GS2	Griscelli syndrome type 2
HIF-1α	Hypoxia-inducible factor 1α
HLH	Hemophagocytic lymphohistiocytosis
HPS2	Hermansky-Pudlak syndrome type 2
HREs	HIF-responsive elements
HRP	Horse Radish Peroxidase
HUVECs	Human umbilical endothelial cells
IFN	Interferon
IL	Interleukin
ITK	Inducible T cell kinase
IUIS	Union of Immunological Societies
Kb	Kilobases

List of Abbreviations Cont...

Abb.	Full term
MAPK	Mitogen activated protein kinase
MAS	Macrophage activation syndrome
mPGES-1	Microsomal PGE synthase 1
NAADP	Nicotinic acid adenine dinucleotide
NAD	Nicotinamide adenine dinucleotide
NAMN	Nicotinic acid mononucleotide
ND1	NADH dehydrogenase subunit 1
NHD	Non Hodgkin lymphoma
NK	Natural killer
NMN	Nicotinamide mononucleotide
PBEF	Pre-B-cell colony-enhancing factor
PBMCs	Peripheral blood mononuclear cells
PI3K	Phosphatidylinositol 3-kinase
PIDs	Primary immunodeficiency
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
s HLH	Secondary HLH
SMCs	Smooth muscle cells
T2DM	Type 2 diabetes
TNF	Tumor necrosis factor
UTR	Untranslated region
VEGF	Vascular endothelial growth factor
VEGFR2	VEGF receptor 2
VILI	Ventilator-induced lung injury
VSMC	Vascular smooth muscle cell
XLP	X-linked lymphoproliferative syndrome

Abstract

Background: Hemophagocytic lymphohistiocytosis (HLH) is a clinical syndrome caused by a highly active but ineffective immune response, including impaired or absent function of natural killer cells and cytotoxic T cells, and the release of pro-inflammatory cytokines. Pre-B-cell colony-enhancing factor (PBEF) is an inflammatory cytokine involved in several inflammatory diseases and it has been identified to react with several cytokines involved in HLH.

Objective: we aimed to evaluate the role of PBEF as a diagnostic and prognostic marker in patients with HLH.

Subjects and Methods: The study was conducted at the pediatric hematology oncology unit, Ain Shams University. Fifteen patients were recruited and underwent through clinical assessment lying concentration on disease manifestation, classification, treatment and prognosis. Plasma concentration of PBEF was determined using an enzyme-linked immunosorbent assay.

Results: PBEF level was measured in the patients group, it was highly significantly increase for patients group than control group. Four patients were classified as primary HLH, seven patients were classified as secondary HLH and four patients had unknown classification due to waiting for genotyping. Seven patients of the study group were died and eight patients still alive. PBEF level showed a significant positive correlation with serum ferritin and triglycerides level and negative correlation with fibrinogen level.

Conclusion: An elevated PBEF level was observed in pediatric HLH, indicating that it may be involved in its inflammatory process. PBEF was correlated with the widely available biochemical markers for diagnosis of HLH.

Keywords: Childhood, Cytokine, Hemophagocytic lymphohistiocytosis, Pre-B-cell colony-enhancing factor.

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a clinical syndrome caused by a highly active but ineffective immune response, including impaired or absent function of natural killer cells and cytotoxic T cells, and the release of pro-inflammatory cytokines ***(Freeman and Ramanan, 2011).***

Patients with HLH present with a wide spectrum of clinical mamifestations and their conditions may rapidly deteriorate, resulting in considerable morbidity and mortality. The primary form, familial HLH, typically seen during infancy and early childhood, is inherited as a recessive trait ***(Henter et al., 2007).*** Adult-onset HLH is often secondary to an underlying disease, such as infection, malignancy, or autoimmune disease ***(Janka, 2007).***

Treatment of patients with HLH aimed to suppress the severe hyperinflammation, and to kill pathogen-infected antigen presenting cells to remove the stimulus for the ongoing, but ineffective activation of cytotoxic cells, it should be emphasized that it is usually not sufficient to treat an identified organism to control hyperinflammation with the possible exception of leishmania-induced HLH, which in most patients can be treated successfully with liposomal amphotericin only. In genetic cases the ultimate aim is stem cell transplantation to exchange the defective immune system by normally functioning cells. Treatment should be guided primarily by the

severity of signs and symptoms, but also known familiarity of the disease, age of the patient and underlying conditions have to be considered ***(Janka and Schneider, 2004).***

Initial therapy in patients with hemophagocytic lymphohistiocytosis (HLH) consists of etoposide and dexamethasone for 8 weeks in varying doses. In the HLH-2004 protocol, cyclosporine is added in the beginning. Intrathecal methotrexate is used only with persistently abnormal CSF or progressive neurologic symptoms. Resolved nonfamilial hemophagocytic lymphohistiocytosis does not require continuation of the therapy regimen unless disease reactivation occurs after completion of the initial therapy or unless patients are undergoing bone marrow transplantation (BMT). For the remaining children with persistent nonfamilial disease or familial disease, continuation therapy with etoposide IV infusions, dexamethasone pulses, and cyclosporine PO is instituted at week 9 from the start of initial treatment ***(Henter et al., 2002).***

Pre-B-cell colony-enhancing factor (PBEF) is an inflammatory cytokine involved in several inflammatory diseases ***(Gao et al., 2015).*** It was first identified as a cytokine that acted synergistically with interleukin (IL)-7 and stem cell factor to stimulate early stage B cell formation ***(Samal et al., 1994).*** PBEF has been identified to react with several cytokines involved in HLH ***(Osugi et al., 1997).***

AIM OF THE WORK

Evaluation of the role of pre-B-cell colony- enhancing factor (PBEF) as a diagnostic and prognostic marker in children with hemophagocytic lymphohistiocytosis.

The study also aimed to study the clinical epidemiological characteristics of hemophagocytic lymphohistiocytosis.

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH)

Hemophagocytic lymphohistiocytosis (HLH) is a severe inflammatory disorder characterized by a significant accumulation of activated $CD8^+$ T lymphocytes and histiocytes in the bone marrow (BM) and lymphoid tissues. The cytokine storm resulting from the accumulation of activated immune cells leads to fever, hepatosplenomegaly, impaired liver function and other clinical and laboratory manifestations of HLH ***(Filipovich, 2009)***. Classically, HLH is divided into primary or familial HLH (FHL) and secondary HLH (sHLH). FHL, which is an autosomal recessive disease, is caused by mutations in the genes encoding the molecules involved in the granule exocytosis machinery of cytotoxic T lymphocytes and natural killer (NK) cells. Although the pathogenesis of sHLH is largely unknown, the two forms of HLH, or sHLH and FHL, have similar clinical characteristics ***(Janka, 2007).***

Epidemiology:

Defining the true incidence is an impossible task as HLH is a condition that some consider a faith-based diagnosis, making the phenotype of the provider as important as the patient to identify and report "HLH" versus other conditions characterized by inflammation ***(Castillo and Carcillo, 2009).*** The Swedish national registry provides the a rigorous estimate

the incidence of primary HLH with 1.5 cases/million live births in Sweden 2007-2011, up slightly from 1.2 cases/million in previous studies (1987-1996, 1997-2006) ***(Meeths et al, 2014).***

In North America, frequency of specific gene defects varies significantly with ethnicity/race ***(Jordan et al., 2011).***

The disease is seen in all ages and has no predilection for race or sex ***(Janka, 2012).***

Pathophysiology:

In the normal physiological context, granule-mediated cytotoxic function of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) is required for clearance of viral infection as well as regulation and termination of the inflammatory response ***(Lykens et al., 2011).***

Thus, defects in NK cell and CTL granule-mediated cytotoxicity result in ineffective clearance of infection and defective suppression of antigen presentation, leading to persistent antigen exposure and prolonged cytotoxic T-cell activation ***(Lykens et al., 2011).*** Until recently, the pathophysiology of secondary HLH was not well understood. However, the finding of an HLH/MAS like condition from repeated Toll-like receptor 9 stimulation in a murine model could explain the potential mechanism of HLH in inflammatory conditions with normal T-cell cytotoxicity ***(Behrens et al., 2011).*** IFN-γ has been shown to play a critical role in macrophage activation and hemophagocytosis ***(Zoller et al., 2011)***.