Aspartate Beta-Hydroxylase (ASPH) level of expression and clinical significance in Acute Myeloid Leukemia

Thesis

Submitted for partial fulfillment of Master degree in Clinical hematology

By

Fadwa Said Abdelazim Mohamed

MD in clinical pathology**,** Faculty of Medicine, Cairo University

Supervisors

Prof. Dr. Mohamed Osman Azzazi

Professor **of** Internal Medicine and Clinical Hematology

Faculty of Medicine – Ain Shams University

Dr. Amro Mohamed Sedky El-Ghammaz

Assisstant Professor of Internal Medicine and Clinical Hematology

Faculty of Medicine – Ain Shams University.

Dr. Mohammad Abd-Allah Shazly Hafez

Lecturer of Internal Medicine and Clinical Hematology

Faculty of Medicine – Ain Shams University

Ain shams university

Faculty of medicine

2020

ACKNOWLEDGMENT

First and foremost "Thanks to Great Allah", the most merciful and kind

I am very much indebted to Prof. Dr. ***Mohamed Osman Azzazi,*** *Professor of Internal Medicine and clinical hematology, faculty of medicine, Ain Shams University, I would like to express my deepest gratitude, great thanks, and appreciation to him for his precious remarks and great experience and effort that were very valuable in completing this work.*

I am very grateful to Dr. ***Amro Mohamed Sedky El-Ghammaz,*** *Assistant professor of Internal Medicine and clinical hematology, Ain Shams University, from him I received invaluable insights and learned the process of conducting and reporting scientific research by his precious remarks, kind guidance and valuable advices.*

I would like to sincerely thank Dr. ***Mohammad Abd-Allah Shazly Hafez,*** *Lecture of Internal medicine and clinical hematology, Faculty of Medicine, Ain shams University, for her great help in this work.*

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2OG	:	2-oxoglutarate
allo-HSCT	:	Allogeneic hematopoietic stem cell transplant
alloSCT	:	Allogeneic stem cell transplantation
AML	:	Acute myeloid leukemia
APL	:	Acute promyelocytic leukemia
ASPH	:	Aspartate β-hydroxylase
ATO	:	Arsenic trioxide
ATP	:	Adenosine triphosphate
BTG1	:	B-cell translocation gene-1
CBF	:	Core binding factor
CBF-AML	:	Core-binding factor Acute myeloid leukemia
CCND1	:	Cyclin D1
CI	:	Confidence interval
CR1	:	First complete remission
CTLs	:	Cytotoxic T-cells
D-loop	:	Displacement loop
ECD	:	Extracellular domain
EFS	:	Event-free survival
EGF	:	Epidermal growth factor
EGFDs	:	Epidermal growth factor-like domains
ELISA	:	Enzyme-linked immunosorbent assay
ELN	:	European leukemia net
ER	:	Endoplasmic reticulum
FAB	:	French–American–British
FLT3	:	FMS-like tyrosine kinase 3
FZD	:	Frizzled
GBM	:	Glioblastoma multiforme
H2AX	:	Histone family, member X
HCC	:	Human hepatocellular carcinoma
HIF-1α	:	Hypoxia-inducible factor 1-alpha

HNRH1	:	Heterogeneous nuclear ribonucleoprotein H
HSP	:	Heat shock proteins
ICC	:	Intrahepatic cholangiocarcinoma
IDHs	:	Isocitrate dehydrogenases
IFN-γ	:	Interferon-γ
IGF1R	:	IGF-1 receptor
IR	:	Insulin receptor
IRS-1	:	Insulin-receptor substrate type 1
ITD	:	Internal tandem duplication
LDL	:	Low density lipoprotein
LRP5 and LRP6	:	Receptor-related proteins 5 and 6
mAb	:	Monoclonal antibody
MAML1	:	Mastermind-like 1
MAPKK	:	Mitogen-activated protein kinase kinase
MFC	:	Multiparameter flow cytometry
MFI	:	Mean fluorescent intensity
MHC	:	Mostly major histocompatibility complex
MMP9	:	Matrix metalloproteinase-9
MRD	:	Monitoring of minimal residual disease
mtDNA	:	Somatic mitochondrial DNA
mtTFA	:	Mitochondrial transcription factor A
NADH	:	Nicotinamide adenine dinucleotide
NICD	:	Notch intracellular domain
OS	:	Overall survival
PBMCs	:	Peripheral blood mononuclear cells
PDAC	:	Pancreatic ductal adenocarcinoma
PNET2	:	Primitive neuroectodermal tumor 2
PTGS2	:	Prostaglandin-endoperoxide synthase 2
RFS	:	Relapse-free survival
ROA2	:	Ribonucleoprotein A2/B1
ROS	:	Reactive oxygen species
RQ-PCR	:	Real-time quantitative polymerase chain reaction

siRNAs	:	Small interfering RNAs
SMI	:	Small molecule inhibitor
STAT3	:	Signal transducer and activator of transcription 3
TCF/LEF	:	T-cell factor/lymphoid enhancer-binding factor
TK	:	Tyrosine kinase
TKD	:	Tyrosine kinase domain
TPR	:	Tetratricopeptide repeat
TRM	:	Treatment-related mortality
UBA1	:	Ubiquitin-like modifier activating enzyme 1
US	:	United states
WHO	:	The world health organization

Introduction

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disorder characterized by clonal expansion of myeloid progenitors (blasts) in the bone marrow and peripheral blood. Formerly, AML had a very poor prognosis. Due to improvements in therapeutic regimens and supportive care (e.g. anti-infective agents, and transfusion support), AML is now cured in approximately 35-40% of patients younger than 60 years (Döhner et al., 2015).

For those over 60 years old, the prognosis has improved but remain poor (Dombret et al., 2015). Chromosomal abnormalities (deletions and translocations) are identified in approximately 50% of all adult patients with primary AML and have long been recognized as the genetic events that cause and promote this disease (Meyer and Levine, 2014). Certain cytogenetic abnormalities, including t(8;21)(q22;q22),t(15;17)(q22;q12) and inv(16)(p13.1;q22), are associated with longer remission and survival, while alterations of chromosomes 5, 7, complex karyotype (described as >3 chromosomal abnormalities) and 11q23 are associated with poor response to therapy and shorter overall survival (Döhner et al., 2015). In contrast, about 40-50% of all AML cases are cytogenetically normal (CN-AML) (Meyer and Levine, 2014). Although, this group has an intermediate risk of relapse, a substantial heterogeneity is found in this population regarding clinical outcome. Molecular screening of this AML category is important in risk stratification and therapy decisions (Döhner et al., 2010). The identification

of new mutations in AML has raised prognostic and probably therapeutic implications for patients with AML.

Despite recent advances in treatment, acute myeloid leukemia (AML) remains difficult to cure with high rates of relapse. Relapsed/refractory AML patients who are elderly or unfit for cytotoxic chemotherapy and whose disease fails to respond to hypomethylating agents represent an unmet need, and new safe and effective treatment options are needed for this patient population (Holtzman et al., 2018).

Aspartate β-hydroxylase (ASPH) is a transmembrane protein that hydroxylates aspartyl and asparaginyl residues of epidermal growth factor (EGF)-like protein domains, and promotes cellular motility, migration, and adhesion. ASPH is highly expressed during fetal development and in placental trophoblasts, but not in any other healthy adult human tissue (Holtzman et al., 2018).

As early as 1996, the over-expression of ASPH was recognized as an indicator of carcinoma in humans. Further research has correlated elevated ASPH levels (variously in affected tissue or blood serum) with hepatocellular carcinoma (Ince et al., 2000; Xue et al., 2009), adenocarcinoma (pancreatic cancer) (Palumbo et al., 2002), prostate cancer (Xue et al., 2009), and lung cancer (Hampton, 2007). The pancreatic study (Palumbo et al., 2002) showed elevated ASPH only in diseased tissue, but not in adjacent normal and inflamed tissue.

In a recent study, ASPH was reported to be overexpressed in approximately 40% of patients with AML and serves as a promising therapeutic target. An ASPH nanoparticle vaccine is currently under clinical investigation and has shown promising results in solid tumors. Several plans to expand clinical testing of targeting ASPH to AML are ongoing (Holtzman et al., 2018).

AIM OF THE WORK

The aim of the present study is to assess the level of expression of Aspartate Beta-Hydroxylase (ASPH) in serum of Egyptian AML patients and study its clinical significance.

Review of Literature

ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia (AML) is an uncommon, yet commonly-fatal myeloid malignancy whose incidence appears to be increasing. It is defined by the malignant clonal expansion of a progenitor cells coupled with a differentiation arrest (De Kouchkovsky and Abdul-Hay, 2016). Biologically-distinct subtypes of AML have variable prognoses, and sociodemographic and healthcare factors influence many aspects of the care of AML patients and consequently their survival. Better understanding of AML pathogenesis and predictors of response will ultimately guide therapeutic investigation and hopefully improve the clinical outcomes of AML patients (Shallis et al., 2019).

EPIDEMIOLOGY

Incidence

Among adults in the United States (US), it has been estimated that approximately 21,450 adults to be diagnosed with AML in 2019 (Siegel et al., 2019). Of all subtypes of leukemia, AML accounts for the highest percentage (62%) of leukemic deaths. In 2016 the age-adjusted incidence of AML in Surveillance, Epidemiology, and End Results (SEER0 was 4.3 per 100,000 person-years (SEER, 2019). Estimated age-adjusted AML incidence rates in the United Kingdom (UK), Canada and Australia mirror that of the US population (Gangatharan et al., 2013; Shysh et al., 2017). A Danish registry-based analysis reported an overall age-adjusted AML incidence rate of 5.4 per 100,000 person-years (95% confidence interval (CI), 4.99–5.74)