Expression of DNMT3A Gene in Adult Acute Myeloid Leukemia Patients

Thesis

Submitted for Fulfillment of Master Degree

in Internal Medicine

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2018

سورة البقرة الآية: ٣٢

Acknowledgments

*First of all, I am deeply indebted to **Allah**, the Most Merciful and the most Gracious for the unlimited help, guidance, support, faith, blessings, and gifts and for everything.*

I would like to express my deepest appreciation and gratitude to **Prof. Dr. Inas Ahmed Hussein Asfour,** *Professor of Internal Medicine and Clinical Hematology, Faculty of Medicine-Ain Shams University, for her great support, close supervision and continuous encouragement throughout the whole work, it was a great honor to work under her supervision.*

I stand in a great debt to **Dr. Hany Mohammed Abd-Allah Hegab,** *Professor of Internal Medicine and Clinical Hematology, Faculty of Medicine-Ain Shams University, for his guidance, supervision through the whole study.*

Special thanks and appreciation to **Dr. Walaa Ali El-Salakawy,** *Assistant Professor of Internal Medicine and Clinical Hematology, Faculty of Medicine-Ain Shams University, for her active guidance and keen supervision which were of great help throughout this work.*

Special thanks and tribute to **Dr. Mohamed Tarif Hamza,** *Assistant Professor of Internal Medicine and Clinical Hematology, Faculty of Medicine-Ain Shams University, for the efforts and time he has devoted to accomplish this work.*

My deepest appreciation to **Dr. Alia Mohammed Saeed,** *Lecturer of Clinical Pathology, Faculty of Medicine-Ain Shams University, for her great support, close supervision and continuous encouragement throughout the whole work.*

Finally special thanks to all member of my Family, for their care and support throughout my life.

Dina Mohamed Abdou Mansour

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List of Abbreviations

Abbrev.	Full-term
AML	: Acute myeloid leukemia
APL	: Acute promyelocytic leukemia
Asxl1	: Additional sex combs like-1
BCOR	: BCL6 Corepressor
biCEBPA	: Biallelic CEBPA mutation
CBF	: Core binding factor
CBF	: Core binding factor;
CD33	: Cluster of differentiation
CK-AML	: Complex karyotype
CN-AML	: Cytogenetically normal acute myeloid leukemia
CR	: Complete remission
DMRGM	: DNA-methylation regulatory gene mutations
DNMT3A	: DNA methyltransferase-3-alpha gene
DNMT3A^{WT}	: DNA methyltransferase-3-alpha gene wild type
EFS	: Event-free survival
ELN	: European Leukemia Net
FISH	: Fluorescence in situ hybridization
FLT3	: fms-related tyrosine kinase 3
Hb	: Hemoglobin
HDAC	: Histone deacetylase
HMA	: Hypomethylating agents
HSCT	: Hematopoietic stem cell transplantation
HSPC	: Hematopoietic stem and progenitor cells

IDH	: Isocitrate dehydrogenase
kmt2a	: Lysine methyltransferase 2A.
MK-AML	: Monosomal karyotype
MRD	: Minimal residual disease
ncRNA	: Non-coding RNA
NGS	: Next generation sequencing
NO	: Nitric oxide
OS	: Overall survival
PFS	: Progression-free survival
PLT	: Platelets
RNA-seq	: RNA Sequencing
RNS	: Reactive nitrogen species
ROC curve	: Receiver-operating characteristic curve
ROS	: Reactive oxygen species
RUNX1	: Runt-related transcription factor 1
SNP	: Single nucleotide polymorphism
TCGA	: The Cancer Genome Atlas
TET2	: Ten-Eleven Translocation 2
TLC	: Total leucocytic count
WGS	: Whole genome sequencing
α-KG	: Alpha ketoglutarate
2-HG	: 2-hydroxyglutarate
5-hmC	: 5-hydroxymethyl-cytosine
5-mC	: 5-methyl-cytosine

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Abstract

Background

Acute Myeloid Leukemia (AML) is one of the most lethal hematological malignancies in the whole world, so continuous searching for modalities of diagnosis, treatment and prognosis is one of the continuous challenges in modern science. DNMT3A gene (DNA methyl transferase 3a) a member of epigenetic modifiers family which have such a significant role in DNA methylation and the cellular apoptosis and leukomogensis.

Aim of the work

To Asses DNMT3A gene in newly diagnosed adult acute myeloid leukemia patient.

Subjects and Methods

A total of 45 patients with AML were included in the study. They were subjected to detailed medical, full clinical and hematological examination, and laboratory investigations including; CBC, CRP, peripheral blood smear for blasts, Bone marrow aspirate and cytogenetics.

Results

The most commonly found within the patient's pool was high prevalence of DNMT3A expression in AML M4-M5 subtype according to FAB classification (French and British classification). DNMT3A gene expression was significantly associated with lower response to treatment by conventional induction chemotherapy regemin (3+7), Lower overall survival (O.S) and progression free survival (P.F.S).

Conclusions/interpretation: DNMT3A gene should be included in the preliminary work up of AML patients as a prognostic factor.

Key words: DNMT3A, AML, FAB, CRP**,** O.S, P.F.S.

Introduction

Acute myeloid leukemia (AML) is an aggressive malignancy of the bone marrow characterized by uncontrolled proliferation of immature myeloid lineage cells**.** This type of cancer usually gets worse quickly if it is not treated. It is the most common type of acute leukemia in adults ***(American Cancer Society, 2015).***

AML is not a single disease. It is the name given to a group of leukemia that develops in the myeloid cell line in the bone marrow. Some years ago doctors from America, France and Britain decided to classify AML into eight different sub-types based on the appearance of the leukemic cells under the microscope. Each sub-type provides information on the type of blood cell involved and the point at which it stopped maturing properly in the bone marrow. This is known as the French-American-British (FAB) classification system ***(Alvi et al., 1990).***

The current World Health Organization classification system for AML uses additional information, obtained from more specialized laboratory techniques, like genetic studies, to classify AML more precisely. This information also provides more reliable information regarding the likely course (prognosis), of a particular subtype of AML, and the best way to treat it.

The most important factor in predicting prognosis in AML is the genetic make-up of the leukemic cells ***(Byrd et al., 2002).*** Certain cytogenetic changes are associated with a more favourable prognosis than others. This means that they are more likely to respond well to treatment, and may even be cured. Favourable cytogenetic changes include: a translocation between chromosome 8 and 21 t(8;21), inversion of chromosome 16; inv(16) and a translocation between chromosome 15 and 17; t(15;17) ***(Haferlach et al., 2003).*** Other cytogenetic changes are associated with an average or intermediate prognosis, while others still are associated with a poor, or unfavourable prognosis. It is important to note that in most cases of AML, neither 'good' or 'bad-risk' cytogenetic changes are found. People with 'normal' cytogenetics are also regarded as having an average prognosis ***(Renneville et al., 2013).***

Recent advances in the research of acute myeloid leukemia (AML), especially the identification of novel genetic mutations, have enabled hematologists to stratify this heterogeneous disease entity into distinct subtypes beyond the scopes of cyto-morphology and cytogenetics. The progress not only brings insight into the pathogenesis of AML but also helps refine the treatment strategies for this group of patients, Along with the development of whole genome sequencing, it is probable that the major genetic

aberrations have been almost completely identified ***(Gaidzik et al., 2013).*** The next stage is to clarify the consequence of these molecular alterations, especially for the newly identified molecules.Mutations in several newly identified genes, such as TET2 and IDH1/2, lead to the aberrant hyper-methylation signature in AML cells ***(Yin et al., 2004).*** Collectively, these recent findings strongly suggest a link between recurrent genetic alterations and aberrant epigenetic regulation, resulting in abnormal DNA methylation statuses in myeloid malignancies.

The DNA methyltransferase 3A (DNMT3A) gene mutation is identified by whole-genome sequencing in patients with acute myeloid leukemia (AML). DNMT3A encodes for the enzyme DNA (cytosine-5) methyltransferase 3A and belongs to the family of other methyltransferases. These enzymes are involved in adding methyl groups to the cytosine residue of CpG dinucleotides and thus play an important role in epigenetic regulation of genes ***(Cagnetta et al., 2014),*** but the mechanism of DNMT3A mutation - associated leukemogenesis was still unknown. Mutations in DNMT3A are quite common in AML, occurring in approximately 20% of patients ***(Hartmann et al., 2009).*** The most common mutation is a substitution of the amino acid arginine at position 882 (R882) ***(Shah, 2011).*** DNMT3A mutations often co-occur with FLT3 ITD, NPM1, IDH1, and