Coexpression of c-KIT and *FLT3* Receptors in Patients with Acute Myeloid Leukemia

Thesis

Submitted for the Partial Fulfillment for the Master Degree in Clinical and Chemical Pathology

By

Youstina Melad Anwaar

(M.B.B.Ch)

Faculty of Medicine – Ain-Shams University

Supervised by

Prof. Dr. Afaf Abd El Aziz Abd El Ghafar

Professor of Clinical and Chemical Pathology

Faculty of Medicine -Ain Shams University

Dr. Rasha Abd El Rahman El Gamal

Lecturer of Clinical and Chemical Pathology

Faculty of Medicine -Ain Shams University

Faculty of Medicine

Ain Shams University

2015

Acknowledgement

First and above all my deepest gratitude and thanks to **ALLAH** *for achieving any work in my life*

I would like to express my endless gratitude and deepest appreciation to **Prof. Dr. Afaf Abd El Aziz Abd El Ghafar,** *Professor of Clinical and chemical Pathology, Faculty of Medicine, Ain Shams University, for her continuous guidance, valuable suggestions, encouragement and keen supervision throughout the work. It was a great honor for me to work under her supervision.*

Special thanks and gratitude to **Dr. Rasha Abd El Rahman El Gamal,** *Assistant professor of Clinical and chemical Pathology, Faculty of Medicine, Ain Shams University, for her active support, valuable comments, offering me much of her time and effort and guidance throughout the work.*

It is of great pleasure to dedicate this work to ***my family****, who supported me throughout my life and surrounded me with all warmth and love that have been of great help in presenting this work.*

Youstina Melad

List of Contents

List of Abbreviations

Abb.	Full term
AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
BM	Bone marrow
CBC	Complete blood count
CBF	Core binding factor
CD	Cluster of differentiation
CEBPA	CCAAT/enhancer-binding protein α
CLL	Chronic lymphoid leukemia
CML	Chronic myelocytic leukemia
CMV	Cytomegalovirus
CNS	Central nervous system
CR	Complete remission
DCs	Dendritic cells
EDTA	Ethylene diaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal- regulated kinase
ETO	Eight twenty one
FAB	French-American-British
FCM	Flow cytometry
FISH	Fluorescence in situ hybridization
FITC	Fluorescein isothiocyanate
FL	*FLT3* ligand
FLK2	Fetal liver kinase 2
FLT3	FMS-like tyrosine kinase 3
FMS	Feline McDonough Sarcoma
G-CSF	Granulocyte colony stimulating factor
GIT	Gastrointestinal tract
GM-CSF	Granulocyte–macrophage colony stimulating factor
GRB2	Growth factor receptor bound protein 2
HB	Hemoglobin

List of Abbreviations *(Cont...)*

Abb.	Full term
HLA	Human leukocyte antigen
HRMA	High resolution DNA melting analysis
HS	Highly significant
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
IPT	Immunophenotyping
ITD	Internal tandem duplication
JM	Juxtamembrane
Kb	Kilobase
KDa	Kilodalton
LDH	Lactate dehydrogenase
MAPK	Mitogen activated protein kinase
MDS	Myelodysplastic syndrome
MLL	Mixed lineage leukemia
MoAb	Monoclonal antibody
MPAL	Mixed phenotypic acute leukemia
MPN	Myeloproliferative neoplasm
MPO	Myeloperoxidase
MRD	Minimal residual disease
NCAML	Normal cytogenetics acute myeloid leukemia
NPM	Nucleophosmin
NS	Non significant
NSE	Non specific esterase
OS	Overall survival
P	Probability test
PAS	Periodic acid Schiff
PB	Peripheral blood
PBS	Phosphate buffered saline
PE	Phycoerythrin
PI3K	Phosphoinositol-3-kinase
PLZF	Promyelocytic leukemia zinc finger
RARA	Retinoic acid receptor alpha
RBCs	Red blood cells

List of Abbreviations *(Cont...)*

Abb.	Full term
RQ-PCR	Real–time quantitative polymerase chain reaction
RR	Risk of relapse
RT	Room temperature
RT_PCR	Reverse transcriptase polymerase chain reaction
RTK	Receptor tyrosine kinase class
S	Significant
s-AML	Secondary AML
SBB	Sudan Black B
sCD117	Soluble CD117
SCF	Stem cell factor
SD	Standard deviation
STAT5	Signal transducer and activator of transcription 5
STK1	Stem cell tyrosine kinase 1
t-AMl	Therapy-related AML
TK1	Tyrosine kinase one
TK2	Tyrosine kinase two
TKD	Tyrosine kinase domain
TLC	Total leukocytic count
TM	Transmembrane
WBC	White blood cells
WHO	World Health Organization
WT *FLT3*	Wild type FMS-like tyrosine kinase 3
X^2	Chi-square test

List of Tables

List of Tables *(Cont...)*

List of Figures

List of Figures *(Cont...)*

List of Figures *(Cont...)*

Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of acquired somatic genetic alterations in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation and differentiation **(Fröhling *et al.*, 2005)**. This would lead to disruption in hematopoiesis with accumulation of immature or blast cells in the bone marrow and the peripheral blood, that would be manifested clinically by bone marrow failure, severe cytopenias and death if left untreated **(Ho & Butera, 2011)**.

AML shows an age-related incidence, mainly increasing with age with the majority of patients older than age 60 **(Zander *et al.*, 2008)**. AML is relatively rare in children, accounting for 15–20% of pediatric leukemias, but causes a disproportionate number of childhood cancer deaths. For children less than 15 years of age overall survival rates are now approximately 60–70% **(Creutzig *et al.*, 2012)**.

Proliferation is considered to be one of the mechanisms for AML & receptor tyrosine kinases (RTK) are amongst the proliferative markers that have significant

contribution in leukemogenesis. The key proliferative RTKs for AML include c-KIT receptor (CD117) and FLT-3 receptor (CD135) **(Sharawat *et al.*, 2013)**. CD117, a member of class III RTK, is a diagnostic marker for AML and is expressed in >85% of patients with AML **(Bene *et al.*, 1998)**. CD117 might promote activation of pro-oncogenic regulatory molecules such as the signal transducer and activator of transcription **(Baumgartner *et al.*, 2009)**. There is variable data to suggest that CD117 overexpression may or may not be associated with outcome in AML **(Sharawat *et al.*, 2013)**.

The FLT-3 receptor (CD135), which is also a member of class III protein RTKs, is aberrantly expressed in most human leukemias, including more than 90% of cases of AML **(Brown *et al.*, 2004)**. It was reported that stimulation of FLT-3 receptors induces their proliferation and inhibits apoptosis by induction of BCL-2 **(Lisovsky *et al.*, 1996)**. Accordingly, it is considered to play an important role in the survival and expansion of primary leukemic blasts **(Bruserud *et al.*, 2003)**.

Flowcytometric immunophenotyping remains indispensable for proper identification of different AML subtypes **(Creutzig *et al.*, 2012)**. As it can evaluate the

simultaneous expression of several antigens on any given cell population, flow cytometry can be regarded as an appropriate preference for detecting the coexpression of CD117 & CD135 on myeloid blasts in AML cases.

Aim of the Work

The aim of the present work is to:

- Study the coexpression levels of CD135 and CD117 on myeloblasts in patients and controls.

- Assess impact of coexpression of CD135 and CD117 on outcome in AML patients.