

Assessment of the Prognostic Role of Serum Angiogenin Level in Patients with Acute Myeloid Leukemia

Thesis

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

By

Mona Mohamed Abd Al-Aty
M.B.,B.Ch.Ain Shams University

Supervised By

Professor/ Hala Mahmoud Hamdy Abaza
Professor of Clinical Hematology
Faculty of Medicine - Ain Shams University

Doctor/ Mona Ahmed Ismail
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Doctor/ Deena Samir Mohamed
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
2011

تقييم الدور التنبؤى لمستوى الأنجيوجنين فى مصل مرضى سرطان الدم النقوى الحاد

رسالة

توطئه للحصول على درجة الماجستير

فى الباثولوجيا الاكلينيكية والكيميائية

مقدمه من

الطبيبة / منى محمد عبد العاطى

بكالوريوس الطب والجراحة العامة – كلية الطب جامعة عين شمس

تحت اشراف

الأستاذ الدكتور / هاله محمود حمدى أباطه

أستاذ أمراض الدم الاكلينيكية

كلية الطب - جامعة عين شمس

الدكتور / منى أحمد إسماعيل

أستاذ مساعد الباثولوجيا الاكلينيكية والكيميائية

كلية الطب - جامعة عين شمس

الدكتور / دينا سمير محمد

مدرس الباثولوجيا الاكلينيكية والكيميائية

كلية الطب – جامعة عين شمس

كلية الطب

جامعة عين شمس

٢٠١١

SUMMARY AND CONCLUSION

AML describes a heterogenous group of hematological disorders characterized by block in the terminal differentiation and unchecked proliferation of myeloid hemopoietic cell lineage. The accurate diagnosis of AML is important for appropriate treatment and management.

Angiogenesis is well known to play an essential role in the growth, dissemination and metastasis of hematological malignancies like AML. Among several angiogenic factors recognized, ANG has been identified as a specific potent positive regulator.

ANG, a 14 KDa monomeric protein, belongs to the pancreatic ribonuclease superfamily and possesses ribonucleolytic activity, which is critically involved in the induction of neovascularization by this protein.

High levels of soluble ANG have been observed in AML and they were correlated with advanced disease stage and poor prognosis.

Hence, the aim of the present study was to determine the serum level of ANG using ELISA technique, to investigate its prognostic role and its impact on patients' survival and to correlate its level with known clinical and laboratory prognostic factors in egyptian AML patients.

The current study was carried out on 30 newly diagnosed AML patients and 10 age and sex matched controls. All patients were subjected to complete history taking, thorough clinical examination and laboratory investigations including:

Acknowledgment

First of all, great thanks to “GOD”.







My sincere gratitude to Prof. Dr. Hala Mahmoud Hamdy Abaza, Professor of Clinical Hematology, Faculty of Medicine, Ain Shams University, for her kind assistance to follow closely every step in this work, sincere encouragement and valuable criticism. It was a great honour for me to work under her supervision.









I am deeply indebted to Dr. Mona Ahmed Esmael, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her most generous help, valuable advice and precious comments.

I would like to express deep appreciation to Dr. Deena Samir Mohamed, Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her valuable time, guidance, precious suggestions and considerable effort throughout this work.

Lastly, but not the least, I would like to record my greatest thanks and gratitude to my family for their actual help and support, and to everyone who helped me throughout this work.

List of Contents

	Page
 List of Abbreviations.....	i
 List of Figures.....	v
 List of Tables	viii
 Introduction.....	1
 Aim of the Work.....	2
 Review of Literature.....	3
<u>Chapter 1:</u> ACUTE MYELOID LEUKEMIA.....	3
A. DEFINITION.....	3
B. EPIDEMIOLOGY.....	3
C. PREDISPOSING FACTORS.....	4
D. MOLECULAR PATHOGENESIS.....	6
E. CLASSIFICATION.....	8
F. DIAGNOSIS.....	25
G . PROGNOSIS.....	43
H . TREATMENT.....	47
<u>Chapter 2:</u> ANGIOGENIN.....	51
A. INTRODUCTION.....	51
B. PHISICO-CHEMICAL PROPERITIES.....	51
C. ANG RECEPTORS.....	53
D. MECHANISM OF ANG ACTION.....	54
E. CONSEQUENCES OF ANG.....	59
F.PHYSIOLOGICAL ROLE OF ANG.....	60
G. CLINICAL SIGNIFICANCE OF ANG.....	61
H. METHODS OF ANG ASSAY.....	70

	Subjects and Methods	75
	Results.....	84
	Discussion	97
	Summary and Conclusion	103
	Recommendations	105
	References	106
	Tables after references.....	I
	Arabic Summary	1

LIST OF ABBREVIATIONS

α	Alpha
~	Approximately
β	Beta
%	Percent
°C	Centigrade
ACP	Acid phosphatase
aFGF	Acidic fibroblast growth factor
AML	Acute myeloid leukemia
ANAE	Alpha-naphthyl acetate esterase
ANG	Angiogenin
ANLL	Acute nonlymphocytic leukemia
ADH	Anti diuretic hormone
APMF	Acute panmyelosis with myelofibrosis
APL	Acute promyelocytic leukemia
BM	Bone marrow
bFGF	Basic fibroblast growth factor
C	Cytosine
c	Cytoplasmic
CAE	Chloro-acetate esterase
CBC	Complete blood count
CBF	Core binding factor
CD	Cluster of differentiation
CEBPA	CCAAT/enhancer-binding protein alpha
CGH	Comparative genomic hybridization
CML	Chronic myeloid leukemia
CR	Complete remission
CSF	Colony stimulating factor
del	Deletion
DIC	Dissiminated intravascular coaglopathy
DNA	Deoxyribonucleic acid
DW	Distilled water
ECM	Extra cellular matrix
EDTA	Ethylene Diamine Tetracetic Acid
EGIL	European group of immunological classification of leukemia
ELISA	Enzyme-linked immunosorbent assay

EM	Electron microscope
ErK	Extracellular signal related kinase
ETO	Eight-twenty-one
FAB	French-American-British
FCM	Flowcytometry
FDP	Fibrin degradation products
FISH	Fluorescence in-situ hybridization
FLT3	FMS-like tyrosine kinase 3
FLT3-	Tyrosine kinase domain of FLT3
FN	False negative
FP	False positive
g	gram
G	Guanine
GLn	Glutamine
G1	Pre-synthetic phase of cell cycle
GVL	Graft versus leukemia
GVHD	Graft versus host disease
Hb	Hemoglobin
HLA	Human leucocyte antigens
HGF	Hematopoietic growth factors
HM	Hepatomegally
HSCT	Hematopoietic stem-cell transplantation
HS	Highly sensitive
IBD	Inflammatory bowel disease
IL	Interleukins
Inv	Inversion
IPT	Immunophenotyping
ISH	In situ hybridization
kDa	kilo Dalton
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LN	Lymphadenopathy
MDR-1	Multidrug resistant gene
MDS	Myelodysplastic syndromes
MIC	Morphology, immunophenotyping & cytogenetics
MPD	Myeloproliferative disorders.
MPO	Myeloperoxidase
MLL	Myeloid/ lymphoid or Mixed lineage

MRC	Myelodysplasia related changes
MRD	Minimal Residual Disease
mRNA	Massenger ribonucleic acid
MYHII	Smooth muscle myosin heavy chain
NAP	Neutrophil alkaline phosphatase
NPM1	Nucleophosmin, member 1 gene
NS	Non significant
NSE	Non-specific esterase
OA	Osteoarthritis
OS	Overall survival
P	Short arm of the chromosome
P53	Protein 53 kDa
PAS	Periodic acid Schiff
PB	peripheral blood
PCR	Polymerase chain reaction
PDEGF	Platelet derived endothelial cell growth factor
PH	Pleckstrin homology
PLT	Platelet
PML	Promyelocytic leukemia
PT	Prothrombin time
RA	Rheumatoid arthritis
RAR	Retinoic acid receptor
Rb	Retinoblastoma
RNA	Ribonucleic acid
RNase	Ribonuclease
RT-PCR	Reverse transcription-polymerase chain reaction
SAPK	Stress associated protein kinase
SBB	Sudan black-B
SE	Specific esterase
t	Translocation
t-PA	Tissue plasminogen activator
TGF-β	Transforming growth factor β
TLC	Total leukocyte count
TSG	Tumer suppressor gene
VEGF	Vascular endothelial growth factor
μg	microgram

μl	microliter
UC	Ulcerative colitis
WCPP	Whole chromosome painting probe
WHO	World health organization
WT1	Wilms'tumor1

List of Figures

Figure No.	Figure Title	Page No.
1	The AML/ETO t(8;21)(q21;q22)	15
2	inv(16) diagram	16
3	PML-RAR-alpha gene involvement in t (15;17)(q22;q12)	17
4	FEL-MLL gene involvement in 11q23, MLL	18
5	Large numbers of immature basophilic precursors in the PB of patient with acute basophilic leukemia	22
6	APMF (a) BM biopsy	23&24
7	Granulocytic sarcoma in lymph node	25
8	granulocytic sarcoma of the gums	26
9	Leukemia cutis	26
10	Pyoderma gangrenosum of legs	27
11	Pyoderma gangrenosum of knee	27
12	Erythema multiforme lesions of the hand	27
13	BM aspirate smear of a case of AML minimally differentiated (AML M0)	30
14	BM aspirate smear of a case of AML without maturation (AML M1)	30
15	BM aspirate smear of a case of AML with maturation (AML M2)	31
16	BM aspirate smear of a case of APL (AML M3)	31
17	BM aspirate smear of a case of AMMoL (AML M4)	32
18	BM aspirate smear of a case of M4Eo AML with atypical young eosinophils (AML M4Eo)	32
19	BM aspirate smear of a case of (AML M5a)	33

Figure No.	Figure Title	Page No.
20	BM aspirate smear of a case of (AML M5b)	33
21	Bone marrow aspirate smear of a case of AML M4 (AMMoL), MPO stain	36
22	Bone marrow aspirate smear of a case of AML M5 (AMoL) NSE stain	36
23	Crystal structure of human angiogenin	52
24	Hypothetical scheme for the mechanism of action of ANG	59
25	A sandwich ELISA	72
26	The principle of immunohistochemistry	73
27	The principle of ISH	74
28	Pie chart showing distribution of cases according to sex	92
29	Pie chart showing distribution of cases according to hepatomegaly	92
30	Pie chart showing distribution of cases according to splenomegaly	93
31	Pie chart showing distribution of cases according to lymphadenopathy	93
32	Pie chart showing distribution of cases according to Hb level	94
33	Pie chart showing distribution of cases according to TLC count	94

Figure No.	Figure Title	Page No.
34	Pie chart showing distribution of cases according to Plt count	95
35	Pie chart showing distribution of cases according to outcome	95
36	Comparison between patient and control groups regarding mean values of angiogenin	96
37	Mean values of ANG in relation to patient outcome	96

List of Tables

Table No.	Table Title	Page No.
1	Conditions predisposing to development of AML	5
2	FAB classification for acute myeloid leukemia	10
3	The association of morphology (FAB), cytogenetic and immunophenotype in AML	11
4	The WHO classification system of AML(2008)	13
5	Cytochemistry of AML	35
6	Scoring of biphenotypic leukemia	38
7	Associations between specific chromosome aberrations and FAB subtypes of AML	39
8	Prognostic factors in AML	44
9	Cytogenetic classification of AML	46
10	Descriptive statistics of studied parameters	88
11	Comparative study between patient and control groups regarding studied parameters	88
12	Correlation between ANG concentration and other parameters of studied patients	89
13	Multivariate analysis between patients' outcome and other parameters of studied patients	90
14	Diagnostic performance of serum ANG	91
I	Clinical features and outcome of studied patients	I
II	Laboratory data of studied patients	III

