EVALUATION OF CD49d IN B-CHRONIC LYMPHOCYTIC LEUKEMIA BY FLOW CYTOMETRY

Thesis

Submitted for Partial Fulfillment of M.Sc. Degree
In Clinical and Chemical Pathology

By

Noha Mohamed Abd El Rahman

M.B., B.Ch

Ain Shams University

Supervised by

Professor Doctor / Salwa Saad Moustafa

Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Professor Doctor / Manal A. Shams Eldin El Telbany

Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Doctor / Hanan Mohamed Mahmoud

Lecturer of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
2009

تقييم أهمية السي دي٤٩د في مرض سرطان الدم الليمفاوي المزمن (ب) باستخدام جهاز التدفق الخلوي

رسالة

توطئة للحصول علي درجة الماجستير
في الباثولوجيا الإكلينيكية والكيميائية

مقدمة من

طبيبة /نهي محمد عبد الرحمن
بكالوريوس الطب والجراحة العامة
كلية الطب – جامعة عين شمس

تحت إشراف

الأستاذ الدكتور/ سلوى سعد مصطفي
أستاذ الباثولوجيا الإكلينيكية والكيميائية
كلية الطب – جامعة عين شمس

الأستاذ الدكتور/ منال أحمد شمس الدين التلباني
أستاذ الباثولوجيا الإكلينيكية والكيميائية
كلية الطب – جامعة عين شمس

الدكتور/ حنان محمد محمود
مدرس الباثولوجيا الإكلينيكية والكيميائية
كلية الطب – جامعة عين شمس

كلية الطب
جامعة عين شمس
٢٠٠٩

Acknowledgement

*First, I thank "**God**" for granting me the power to proceed and to accomplish this work.*

*I would like to express my endless gratitude and appreciation to **Prof. Dr. Salwa Saad Mostafa**, Professor of Clinical and Chemical Pathology, Ain Shams University, for giving me the honor of working under her supervision and providing me a lot of encouragement throughout this work.*

*I would like to express my sincere gratitude and deepest appreciation to **Prof. Dr. Manal Ahmed Shams el din El Telbany**, Professor of Clinical and Chemical Pathology, Ain Shams University, for her valuable advices, precise comments and constructive remarks throughout this work.*

*My deepest thanks to **Dr. Hanan Mohamed Mahmoud**, Lecturer of Clinical and Chemical Pathology, Ain Shams University, for her valuable suggestions and advices that enlightened my way in creating such a work.*

*Last, but not least, I would like to thank **My Dad, Mum, My Dear Husband and My Son** for their cooperation and encouragement.*

INTRODUCTION

B- Cell Chronic lymphocytic leukemia (B- CLL) is a hematopoietic neoplasm of B- lymphocytes found in the peripheral blood, bone marrow, and /or lymph nodes. The morphology reveals a monomorphic small round cell population of B- lymphocytes. It is the most common adult leukemia, with a variable clinical course. Some experience an aggressive disease course that lead to premature death while others live for decades and never require therapy ***(Glassman and Hayes, 2005; Shanafelt et al., 2008).***

Traditional risk parameters of CLL including Rai classification; which is a clinical staging system based on the presence of lymphadenopathy, organomegaly, and cytopenias; serum β2 - microglobulin level and marrow infiltration patterns were the initial prognostic tools in CLL ***(Kay and Shanafelt, 2007).***

Recent risk parameters were defined as prognostic markers that appear to be clearly related to the biology of B- CLL including defects detectable on fluorescence in situ hybridization, immunoglobulin heavy-chain variable region (Ig VH) mutational status, surface marker CD38, and intracytoplasmic ZAP-70 levels **(Méhes,2005; Kay and Shanafelt, 2007).**

CD49d plays a critical role in leucocyte trafficking, activation and survival, also facilitates interactions between leucocytes and stromal cells found in the marrow or germinal center of lymphoid follicles via VCAM-1 and fibronectin. Notably, in addition to these adhesion functions, CD49d can also serve as a signaling receptor that influences B-cell survival via up regulation of Bcl-2 family members **(Shanafelt et al., 2008).**

Studies suggest that CD49d expression on CLL B-cells is lower than normal B-cells, differs from other low-grade B-cell malignancies, demonstrates intra-patient variation, and is associated with disease stage and the presence of lymphadenopathy. While several investigators have demonstrated that signaling via CD49d in CLL B cells reduces both spontaneous and drug-induced apoptosis in vitro, little is known about the prognostic importance of CD49d in patients with CLL ***(Shanafelt et al., 2008).***

AIM OF WORK

The aim of this study is to evaluate CD49d expression in B-Cell Chronic lymphocytic leukemia patients and to test its impact as prognostic marker. Also, to correlate it with CD38 (prognostic marker) and markers of tumor burden as clinical staging and CD23.

List of Contents

List of Abbreviations

α ..... Alpha
β ..... Beta
κ ..... Kappa
λ ..... Lambda
μ ..... Mu
μL..... Microlitre
ALL ..... Acute lymphoblastic leukaemia
AML ..... Acute myeloblastic leukaemia
ATM ..... Ataxia-telangiectasia mutated gene
Bag-1..... Bcl-2 associated athanogene 1
Bax..... Bcl-2-associated factor X
Bcl.....B cell leukemia/lymphoma
Bcl-2.....B cell leukemia/lymphoma 2
Bcl-x_L.....B cell leukemia/lymphoma X long
Bcl-x_S.....B cell leukemia/lymphoma X short
B-CLL .....B cell chronic lymphocytic leukemia
BCR .....B-cell Receptor
BM ..... Bone Marrow
BML..... Bone Marrow lymphocyte
B-PLL.....B-cell prolymphocytic leukemia
CBC ..... Complete blood count
CD..... Cluster of differentiation
CGH..... Comparative genomic hybridization
CLL ..... Chronic lymphocytic leukemia
CLL/PLL….. Chronic lymphocytic leukemia with increased prolymphocytes
del..... Deletion
dL..... Decilitre
DNA..... Deoxyribonucleic acid
DW..... Distilled water
ECOG..... Eastern co-operation oncology group

EDTA.....Ethylene diamine tetraacetic acid
F .....Female
FAB classification…. French American British Classification
FCM.....Flow cytometry
FISH .....Fluorescence in situ hybridization
FITC .....Fluorescein isothiocyanate
G 0.....Gap 0
g.....Gram
GM-CSF.....Granulocyte-macrophage colony-stimulating factor
H_2O .....water
Hb .....Hemoglobin
HCL .....Hairy cell leukemia
HLA .....Histocompatability leucocyte antigen
HM .....Hepatomegaly
HMS .....Hyporeactive malarial splenomegaly
HS .....Highly significant
IAP.....Inhibitors of apoptosis protein
Ig .....Immunoglobulin
IgVH .....Immunoglobulin variable region heavy gene
IL .....Interleukin
IPT .....Immunophenotyping
ITP.....Immune thrombocytopenic purpura
IU .....International unit
KD.....Kilodalton
$KHCO_3$ ..... Potassium bicarbonate
L .....Litre
LDH .....Lactate dehydrogenase
LDT ..... Lymphocyte doubling time
LN .....Lymph node enlargement
M.....Male
Mcl-1.....Myeloid cell leukemia sequence 1
mL.....Millilitre
MoAbs .....Monoclonal antibodies

NCI-WG.....USA National Cancer Institute Working Group
Na.....Sodium
NaCL .....Sodium chloride
NaH_2PO_4.....Sodium dihydrogen phosphate
NH_4CL.....Ammonium chloride
NHL .....Non-Hodgkin lymphoma
NS .....Non significant
p .....Short arm of the chromosome
P53 .....(P) means protein and the number after it represents the molecular weight of this protein
PB.....Peripheral blood
PBL.....Peripheral blood lymphocyte
PBS .....Phosphate buffer saline
PC .....Personal computer
PCD .....programmed cell death
PCNA.....Proliferating cell nuclear antigen
PCR .....Polymerase chain reaction
PE.....Phycoerythrin
PH .....Pleckstrin homology
PLT.....Platelets
PR .....Partial remission
q .....Long arm of the chromosome
rpm .....Revolution per minute
S .....Significant
sCD23 .....Soluble CD23
SCT.....Stem cell transplantation
SEM.....Standard error of mean
SD.....Standard deviation
sIg .....Surface immunoglobulin
SLVL .....Splenic lymphoma with villous lymphocytes
SM .....Splenomegaly
SPSS.....Statistical package for Social Science
TGF-β.....Transforming growth factor-β
TK.....Thymidine kinase

TLC ..... Total leukocytic count
TNF-α ..... Tumor necrosis factor alpha
VEGF..... Vascular endothelial growth factor
VEGFR1..... Vascular endothelial growth factor receptor-1
VEGFR2..... Vascular endothelial growth factor receptor-2
VLA .....very late antigen
VCAM-1..... Vascular cell adhesion molecule-1
MADCAM-1..mucosal addressin cellular adhesion molecule-1
WHO ..... World Health Organization
ZAP-70 ..... Zeta- associated protein of 70 KDa

List of Tables

List of Figures