FLUORESCENT INSITU HYBRIDIZATION ANALYSIS OF ALPHA/ DELTA RECEPTOR GENE REARRANGEMENT (14q11) IN ACUTE LYMPHOBLASTIC LEUKEMIA

Thesis

Submitted for Partial Fulfillment of M.D. Degree in Clinical and Chemical Pathology

Presented By

Rasha Mohammad El -Demerdash Sarhan

M.B., B.CH. (Clinical and Chemical Pathology)
Ain Shams University

Under Supervision of

Professor / Afaf Abd-El Aziz AbdelGhaffar

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

Professor / Dahlia Ahmed El Sewefy

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

Professor / Amal Abd-Elhamid Mohammad

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

Doctor/ Reem Abd-El khalek Khattab

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

> Faculty of Medicine Ain Shams University 2011



Acknowledgement

First and foremost, I feel always indebted to **Allah** the most merciful. Who gives me the power to accomplish this work.

The accomplish of this thesis attributed to the extensive support and assistance from my academic advisor Prof. Dr. Afaf Abd El-Aziz Abdel Ghaffar Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University.

I would like to express my deepest thanks and gratitude and sincerest appreciation to **Dr. Dahlia Ahmed El Sewefy**, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her patience, help, constructive criticism, valuable support and direction.

I also express my deepest thanks to Dr. Amal Abd El Hamid Mohammad, Assistant Professor of Clinical and Chemical Pathology; for her helpful advice and continuous encouragements which were quiet valuable in accomplishing this work.

I wish to thank Dr. Reem Abd El Khalek Khattab, Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her kind supervision and helpful advice.

I wish to thank them all for her cooperation, assistance and guidance.

Many thanks and appreciation to all those who generously gave me time to fill in the forms used in this work. Without their help and cooperation, this study would have never been completed.

Finally, I am grateful to my **family** and especially my father **Dr.**Mohammed El-Demerdash Sarhan for his special support, entirely care, and endless love.

Rasha El-Demerdash

List of Contents

Title	Page No.
Introduction	1
Aim of the work	4
Review of Literature	
Chapter I (Acute Lymphoblastic leukemia)	5
Epidemiology	6
Risk factors of ALL	7
Classification of ALL	10
Diagnosis	14
Clinical manifestation	24
Prognosis	25
Differential diagnosis of ALL	32
Treatment	33
Minimal residual disease in childhood ALL	39

Chapter II (T cell receptor rearrangement in Acute
lymphoblastic leukemia)
••••••
44
Origin of cells of the immune system
Anatomy and development of thymus and thymic
microenvironment
45
T-lymphocyte development and differentiation47
Mechanism of T cell selection in the thymus50
T cell receptor gene families53
Translocations which frequently result from errors of
the recombinase system
55
T-cell receptor rearrangement
Generation of diversity in B and T lymphocytes64
B-lymphocyte development and differentiation68
Immunoglobulin structure71
Diagnostic role of T cell receptor gene rearrangement .82
Chapter III(Methods od detection of TCR
rearrangement in Acute lymphoblastic
leukemia)

Introduction

•••••	•••••
86	
Methods of t cell receptor gene rearrangement	87
Polymerase chain reaction	87
FISH	96
Flow cytometry	99
Southern Blot analysis	101
DNA micro-array Platforms	103
Single stranded confirmational polymorphism	103
Applications of molecular techniques	106
Subjects and methods	110
Results	125
Discussion	151
Summary and conclusion	164
Recommendations	167
References	168
Arabic Summary	

List of Tables

Title							Page No.
Table	(1):	The	FAB (cation	of	ALL
	10	•••••	•••••••	•••••	•	••••••	•••••
Table	(2):	Special	morpholo	ogical	variants	s of	ALL
	11	•••••	••••••	•••••	,	••••••	•••••
Table	(3): C	lassifica	tion of AL	L acco	rding to	o the	WHO
	classifi	ication				(mod	lified)
	•••••	•••••	• • • • • • • • • • • • • • • • • • • •				•••••
	12						
Table	(4): In	nmunolo	gic classif	ication	of child	dhood	acute
	lympho	oblastic	leukemia	immu	nopheno	otypes	and
	FAB				cl	lassifi	cation
		•••••				•••••	•••••
	13						
Table	(5): M	Iorpholo	gical, cytoo	chemica	al, and b	oioche	mical
	charact	teristics	helpful in	disting	uishing	ALL	from
	AML						
		•••••				•••••	
	17						

Table		-		profiles			
Table	18 (7)	: Im	munophe	enotypic	type	es of	ALL
7D 11	21						
Table	(8): P ALL	rimary	chromo	somal abo	erratio	ns in B	-lineage
Table	23			ALL rel			
Table	28 (10):	Correl	ation of	prognosi	is with	n bone	marrow
Table	29	Risk o		ation syst			
Table				combina			•

Introduction

laboratory

data

regarding

patients

	141						
Table	(19):	Com	parison	betwe	een	different	patients
	outcome)	accordin	g	to	clinical	l data
	•••••	• • • • • • • • • • • • • • • • • • • •	•••••	• • • • • • • • • •	• • • • • • •		•••••
	142						
Table	(20):	Com	parison	betwe	een	patients	outcome
	accordin	ng	to		labo	oratory	data
	142						
Table		٦		4	۸ ـ1.	141	1: . 4:
Table		•	ırıson be			uits and	pediatrics
	regardin	g		clin	ical		data
	•••••	• • • • • • • • • • • • • • • • • • • •	•••••	•••••	• • • • • • •	•••••	•••••
	143						
Table	(22):	Comp	aring la	aborato	ory	data in	adults &
	pediatrio	es					
	143						
Toblo		CDas	1/a11	raarrar	aaam	ont in all	notionts
Table			-				patients,
	B-ALL		and		T-AI	LL	patients
	•••••	• • • • • • • • • • • • • • • • • • • •	•••••	•••••	• • • • • • •	••••••	•••••
	144						
Table	(24): (Compa	aring cli	nical	and	laborator	y data in
	Patients	with	1 TCR	αδ ([14q1	11) rearr	angement

Introduction

	positive		and		negative
Table	144 (25): R rearrangeme B-ALL	elation ent, Outcom	between	TCRαδ sk standar	(14q11)
Table	145 (26): Compregarding	parison bo TCRαδ	etween ad 14q11	ults and p	pediatrics angement
Table	145 (27): Logist dependable group)}	tic regress	ion model	with Outc	ome as a
	146 (28): Cor rearrangeme	mparison	between	TCR αδ	(14q11)
Table	146 (29): Compa		ween TCR	•) in ALL

Introduction	_
147	
Table (30): Comparison between TCR αδ (14q11) in T-	
ALL cases with co-expression of CD 10 or CD19	
147	

List of Figures

Title					Page No.
C			lymphol		
16		•••••	••••••	••••••	••••••
Figure	(2):	Cytogenetic	markers.	Figure	showing
cy	togenet	ic s	subsets	of	ALL
•••				•••••	
22	2				
Figure (3): Orig	gin of the ce	ells of the in	nmune sys	stem
Figure	(4):	Matu	ration	of T	cells
•••					•••••
45	5				
Figure	(5):	Developm	ent of	mature	T cells
•••		•••••	• • • • • • • • • • • • • • • • • • • •	•••••	•••••
50)				
Figure ((6): Med	chanism of	T cell selec	ction in tl	ne thymus
•••					
50)				
Figure_	(7-a):	Break p	oints in	14q11 (TCRA/D)
<u></u>		•••••			•••••
52	2				

Figure		(7-b):	V	'DJ	recoi	mbination
5.		••••••	••••••	••••••	•••••	••••••
Figure	(8-a):	T cell	recept	or ge	ene famili	es, TCR
re	earrange	ment, co	onfigura	tion (of T cell	receptor,
p	ossible	combin	ations	of	T cell	receptors
•	•••••	• • • • • • • • • • • • • • • • • • • •	•••••	•••••	•••••	•••••
6	7					
Figure	(8-b):	T ce	ll rece	eptor	alpha ai	nd delta
		•••••	•••••	•••••	•••••	•••••
6	8					
Figure	(9):	Develo	pment	of	B lyn	nphocytes
		••••••	•••••	•••••	•••••	•••••
7						
Figure	(1	.0):	Immu	nogloł	oulin	structure
		••••••	•••••	••••••	••••••	•••••
7:			.•	1		C
_		_			rearrange	
ir	nmunog	lobulin F	I genes,	, orgai	nization of	κ, λ light
cl	hain ge	nes and	immun	oglobu	ılin class	switching
• ·	•••••		•••••	•••••		
7	6					

Figure	e (12	2): "Po	lymerase	chain	reaction."
	88 e (13):				e PCR cycle
			escent	insitu	Hybridization
	97 e (15):		southern	blot	hybridization
Figure	101				Polymorphism
	105 e (17):	Relation	between	outcom	ne and LDH
	149 e (18):	Relation	between	Outcon	ne and TLC
Figure	150	Relation			(14q11) and