DETECTION OF RECEPTOR FOR ALFA FETO-PROTEIN (RECAF) IN B- NON-HODGKIN'S LYMPHOMA BY IMMUNOHISTOCHEMICAL TECHNIQUES; ITS CORRELATION TO INFILTRATION BY HISTOPATHOLOGY

Thesis
Submitted for Partial Fulfillment of the Master
Degree in Clinical Pathology

By
Heba Fawzy Houssein Ibrahim
M.B., B.Ch.

Supervisors

Professor/ Hebatallah Adel Sedky

Prof. of Clinical Pathology, Faculty of Medicine, Ain Shams University

Professor/ Soha Raouf Youssef

Prof. of Clinical Pathology, Faculty of Medicine, Ain Shams University

Doctor/ Doaa Ahmed Gamal Eissa

Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University

> Faculty of Medicine, Ain Shams University 2011

اكتشاف مستقبلات الألفافيتوبروتين (ريكاف) في نخاع العظام في حالات أورام الغدد الليمفاوية الغير هودجكيني النوع (ب) باستخدام التحليل المناعي الكيميائي وعلاقته بالطرق التقليدية في اكتشاف الخلايا السرطانية

رسالة توطئة للحصول على درجة الماجستير فى الباثولوجيا الاكلينكية

مقدمة من الطبيبة/ هبه فوزي حسين ابراهيم

تحت إشراف: الدكتور/ هبة الله عادل صدقي أستاذ الباثولوجيا الإكلينكية كلية الطب جامعة عين شمس

الاستاذ الدكتور/سها رؤوف يوسف أستاذ الباثولوجيا الاكلينكية كلية الطب-جامعة عين شمس

الدكتور/دعاء أحمد جمال عيسى مدرس الباثولوجيا الاكلينكية كلية الطب-جامعة عين شمس

> كلية الطب جامعة عين شمس 2011

SUMMARY

Lymphomas constitute a broad group of tumors of the immune system that continue to challenge oncologists. The dynamics of lymphoma entails that it must be handled through a multi-disciplinary approach. The last decade has witnessed marked change in the profile of malignant lymphoma (*Holte et al.*, 2009).

Lymphomas are divided into Non-Hodgkin's Lymphoma (NHL) and Hodgkin's disease (HD). Non-Hodgkin's Lymphoma occurs roughly three times as frequent as Hodgkin's disease (HD) (*Friedberg et al.*, 2008).

In Egypt, malignant lymphomas are relatively common. The exact national incidence is not precisely known due to absence of a National Cancer Registry and the only data available are from hospital registries. At Ain Shams Radiation Oncology and Nuclear Medicine Department (RONMD), the total number of cancer cases from 2004 to 2006) was 4046 cases, with 413 lymphoma cases constituting 10.2% of cases. Relative predominance of NHL over HD was at a rate of 2: 1 (*Ibrahim et al.*, 2008).

ACKNOWLEDGMENT

IŅI IŅI

III)

順

Wij

III)

IIII)

IIII)

Wij

IIII

軸

IMI.

IIII

IIII

Wil

W

Wil

Щij.

Mil

Wi

Wij

Щij.

Mil.

Mil.

iiii

Wil

Wij

işir Diri

Щij

棚

Wil

棚

嘶响

軸

Щij

Щij

Mil

棚

Щį

W

嘶嘶

嘶嘶

Mil

軸

Mili

棚

Mil

幱

懶

咖啡

軸

棚

欄

棚

棚

幱

First of all, thanks to GOD for helping me to complete this work.

I would like to express my deep gratitude and appreciation to **Professor Hebatallah Adel Sedky,** Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her valuable knowledge, meticulous supervision and faithful assistance during this work. Words of thanks are little to express my gratefulness for her.

I am also deeply grateful to **Professor. Soha Raouf Youssef,** Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her sincere guidance and continuous encouragement during this work.

I am also grateful to **Dr. Doaa Ahmed Gamal Eissa,** Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her sincere guidance and continuous encouragement during this work.

Heba fawzy Houssein Ibrahim

LIST OF CONTENTS

Subjects	Page
* Introduction & Aim of the Work	1
* B-Non-Hodgkin's Lymphoma	5
* Diagnosis of B-Non-Hodgkin's Lymphoma	29
* Alpha Fetoprotein	70
* RECAF	76
* Subjects and Methods	88
* Results	100
* Discussion	142
* Summary	152
* References	158
* Arabic Summary.	

LIST OF TABLES

Tabl	e Title	
1	Known Risk Factors of NHL	16
2	WHO Classification of mature B-Cell Lymphoid	
	Neoplasms, 2008	18
3	WHO Classification of mature T-and NK-Cell	
	Lymphoid Neoplasms, 2008	19
4	Cotswolds modification of Ann Arbor Staging	
	System	26
5	Revised Response Criteria for Lymphoma	28
6	Types of Tissue Fixatives	49
7	Differential Diagnosis of B-NHL by IHC	57
8	Descriptive Data of Normal Control group included in	
	the study	112
9	Descriptive Data of Pathological Control group included	
	in the study	112
10	Descriptive Data of Cases group included in the study	
	(total).	113
11	Descriptive Data of Cases subgroups included in the	
	study	114
12	comparative Data of Main groups included in the study	115

LIST OF TABLES (CONT.)

Tab No.	le Title	Title Page	
13	Comparison between DLBCL, FL and MCL		
	Subgroups.	115	
14	Comparison between CLL Subgroup and each of		
	DLBCL, FL and MCL.	116	
15	Comparison between HCL Subgroup and the Main		
	subgroups (DLBCL, FL and MCL Subgroups)	116	
16	Comparison between CLL and HCL Subgroups	117	
17	Comparison between Non-Quantitative Data in		
	Subgroups of Cases Group.	117	
18	Comparison between Quantitative Data in Subgroups of		
	Cases Group	118	
19	Comparison between different subgroups included in the		
	study as regards ROC curve	119	
20	comparison between NC, PC and cases as regards		
	percent of RECAF positive cases	119	
21	comparison between B-NHL subgroups as regards		
	percent of RECAF positive cases	120	
22	Comparison between RECAF negative and RECAF		
	positive in the cases involved in the study as regards		
	clinical findings:	120	
23	Comparison between RECAF negative and RECAF		
	positive in the cases involved in the study as regards		
	laboratory findings:	121	
24	Comparison between routine histopathology and		
	RECAF as regards BM infiltration	121	

LIST OF FIGURES

	igure Title No.	Page	
1	The Anatomy of a Lymph Node	•••	6
2	The Anatomy of Spleen	•••	7
3	Hypothetical scheme of lymphocyte differentiation	•••	9
4	Differentiation scheme, showing nomenclature for variou	IS	
	types of T cells (top) and B cells (bottom)	•••	11
5	Differentiation scheme, showing postulated normal		
	counterpart of many of the T- and B-cell neoplasms	•••	14
6	B-Lymphoblastic lymphoma	•••	33
7	Small lymphocytic lymphoma in Lymph Node by Low		
	Power showing diffuse effacement of Nodal		
	Architecture	•••	33
8	Small lymphocytic lymphoma by High power	•••	34
9	Lymphoplasmacytic lymphoma in Bone Marrow Biopsy.	•••	35
10) Hairy Cell Leukemia		35
1.	l Hairy Cell Leukemia in stained smear	•••	36
12	2 Spleen; marginal zone lymphoma		37
13	3 Follicular lymphoma with aggregates of lymphoma cells		
	throughout the lymph node		39

Fig No		age
14	Follicular lymphoma at high magnification showing small	
	lymphoid cells with condensed chromatin and irregular	
	or cleaved nuclear outlines	39
15	Mantle cell lymphoma, low power showing neoplastic	
	lymphoid cells surrounding small atrophic germinal	
	center producing mantle zone pattern of growth	40
16	Mantle cell lymphoma, high power showing homogenous	
	population of small lymphoid cells with somewhat	
	irregular nuclear outlines resembling prolymphocytes	40
17	Diffuse large B-cell lymphoma; cells have large nuclei,	
	open chromatin and large nucleoli	42
18	Burkitt's lymphoma; low power showing starry sky	
	appearance	44
19	Burkitt's lymphoma; high power with cells having	
	multiple small nucleoli and high mitotic index	45
20	Direct Immunoperoxidase Technique	53
21	Indirect Immunoperoxidase Technique	53
22	Placental Alkaline Phosphatase (PLAP): There is positive	
	cytoplasmic staining for PLAP	56
23	S100 Protein: positive nuclear staining for S100	56

Figure No.	Title 1	Page
24 Lymph	noblastic lymphoma, TdT- positive Immunopheno-	
typin	ng protein	58
25 Small 1	lymphocytic lymphoma, CD23- positive (IP)	59
26 Bcl-2 I	Expression in Reactive and Neoplastic Follicles, Ag	, ,
Bccl-	-2 is Present in Mantle Zone Cells but not Follicula	ır
Cente	er B-cells, B; Follicular lymphoma show strong	
centr	ral bcl-2 Staining	60
27 Mantle	e cell lymphoma, Cyclin D1-positive (IP)	61
28 Diffuse	e large B-cell lymphoma, CD20-positive (IP)	62
29 Burkitt	t's lymphoma, EBV-positive (In situ hybridization)	63
30 Molecu	ular configurations of human AFP (HAFP) (left),	
huma	an albumin (ALB) (center), and human Gc vitamin	-
D bir	nding protein	72
31 Diagra	mmatic representation of the proposed tri-domain	
struc	ture of HAFP transitioning from the native to the	
Molt	en Globule (MG) state	72
32 Histo-I	RECAF on breast tumors. (A) Fibroadenoma	
(nega	ative), 100X; (B) Ductal carcinoma, 200X; (C)	
Lobu	ılar carcinoma, 100X; (D) Axillary Lymph node	
meta	stasized by a breast carcinoma, 100X	79
33 Frozen	sections of two different breast ductal carcinomas	stained
with	RECAF	. 80

Figure No.	Title	Page	
34 Breast fine need	le biopsies stained for RECAF: (A and l	B)	
Fibroadenoma	s	8	30
35 Different tissues	stained with Histo-RECAF	8	31
36 Expression of Al	FP-R in gastric tissues	8	2
37 Positively Charg	ed Slides.	9	1
38 Xylene Solution		9	1
39 Primary Antibod	ly (AFP Receptor Mouse Monoclonal		
Antibody)		9	2
40 Peroxidase Block	k	9	2
41 Secondary Antib	ody	9	3
42 Sterptavidin Pred	oxidase	9	4
43 DAB Plus Chror	nogen	9	5
44 DAB Plus Subst	rate	9	5
45 Placental tissue s	stained with RECAF (positive control)	9	7
46 Subgroups of B-	NHL	12	22
47 LDH level in dif	ferent B-NHL subgroups	12	22
48 Frepuency of B-	symptoms in different B-NHL subgroup	ps. 12	23
49 Frepuency of ly	mphadenopathy in different B-NHL		
subgroups		12	23
50 Comparison bety	ween the different stages in DLBCL	12	24

Figure No.	Title	Page	
51 Comparison be	etween the different stages in FL	1	24
52 Frequency of I	HSM in different subgroups of B-NHL	1:	25
53 Frequency of I	BM infiltration in different B-NHL		
subgroups		1	25
54 BM infiltration	n by RECAF in different B-NHL subgrou	ups 1	26
55 Comparison be	etween cases and PC groups as regards		
RECAF posi	tive cases.	1:	26
56 Comparison be	etween non leukemic subgroups as regar	ds	
RECA F pos	itive cases	1	27
57 Comparison be	etween histopathology and RECAF as		
regards BM	infiltration in DLBCL	1	28
58 Comparison be	etween histopathology and RECAF as		
regards BM	infiltration in FL	1	28
59 Comparison be	etween histopathology and RECAF as		
regards BM	infiltration in MCL	1	28
60 ROC curve bet	tween patients and controls as regard		
RECAF		1	29
61 ROC curve be	etween DLBCL and controls as regard	d	
RECAE		12	9

Figure No.	Title	Page
62 ROC curve	between FL and controls as	regard
RECAF		130
63 ROC curve b	between MCL and controls as re	egard
RECAF		130
64 ROC curve b	between CLL and controls as reg	gard
RECAF		
65 ROC curve b	between HCL and controls as re	gard
RECAF		

LIST OF ABBREVIATIONS

α : Alpha.

Ab : Antibody.

AEC : Amino Ethyl Carbazole.

AFT : Alpha Fetoprotein.

ALCL : Anaplastic Large Cell Lymphoma.

ALK : Anaplastic Lymphoma Kinase.

ALL : Acute Lymphoblastic Lymphoma/Leukemia.

APC : Antigen Presenting Cell.

 β : Beta.

Bcl-2 : Surface Antigen. Bcl-6 : Surface Antigen.

BL : Burkitt's lymphoma.

BM : Bone Marrow.

CBC : Complete Blood Count.CD : Cluster of Differentiation.

CD-40L : CD-40 ligand.

CLL: Chronic Lymphocytic Leukemia.

CML : Chronic Myeloid Leukemia.

C-myc : Oncogene.

CNS : Central Nervous System.

CR : Complete Response.

CRu : Complete Response unconfirmed.

CSF : Cerebro-Spinal Fluid.

CT : Computed Tomography.

 δ : Delta.

DAB : Diamino Benzedine tetrachloride.

DBP : Vitamin-D Binding Protein.

LIST OF ABBREVIATIONS (Cont.)

DLBCL : Diffuse Large B-Cell Lymphoma.

dl : Deciliter.

DNA : Deoxy Ribonucleic Acid.

DPX : Mounting Solution. EBV : Epstein-Barr Virus.

EH : Enzyme histochemistry.EM : Electron Microscopy.

ESR : Erythrocyte Sedimentation Rate.

FCM : Flow Cytometry.

FDC : Follicular denderitic cells.

FG : 18-Flourodeoxy Glucose.

FISH : Fluorescent in situ Hybridization.

FL : Follicular Lymphoma.FNA : Fine Needle Aspirate.

g : Gram.

 γ : Gamma.

GIP : Growth Inhibitory Peptide.

GIT : Gastro-Intestinal Tract.

Hb : Hemoglobin.

HCC : Hepato-Cellular Carcinoma.

HCL : Hairy Cell Leukemia.HD : Hodgkin's Disease.

HIV : Human Immunodeficiency Virus.

HHV-8 : Human Herpes Virus-8.

HL: Hodgkin's Lymphoma.

HLA-DR : Human Leukocyte Antigen Domain Receptor.

HSM : Hepatosplenomegaly.