

BIOCHEMICAL STUDY OF SOLUBLE ADHESION MOLECULES AS MARKERS IN BREAST CANCER PATIENTS

Thesis

Submitted by

Mohammed Mohammed Amin Ahmed

B.Sc. (Biochemistry – 2003)

Ain Shams University

For the fulfillment of master degree of science in
(Biochemistry)

Under Supervisors

Prof. Dr. Sanaa Osman Abdullah

Prof. of Organic Chemistry
Faculty of science
Cairo University

Dr. Ahmed Ebrahim Amin

Lecturer of Biochemistry
Faculty of Science
Cairo University

**Faculty of science
Cairo University**

2010

دراسة كيميائية حيوية للجزيئات الذائبة الاصقة كدلالات لمرضى سرطان الثدي

مقدمة من الطالب

محمد محمد امين احمد

بكالوريوس علوم (كيمياء حيوية-2003)

جامعه عين شمس

للحصول على درجة الماجستير فى

الكيمياء الحيوية

تحت اشراف

أ.د. سناء عثمان عبد الله

أستاذ الكيمياء العضوية

كلية العلوم – جامعة القاهرة

د. أحمد إبراهيم أمين

مدرس الكيمياء الحيوية

كلية العلوم – جامعة القاهرة

كلية العلوم
جامعة القاهرة

2010

APPROVAL SHEET FOR SUBMISSION

Title of thesis: Biochemical study of soluble adhesion molecules as markers in breast cancer patients

Name: Mohamed Mohamed Amin Ahmed

This thesis has been approved for submission by the supervisor:

1. **Prof. Dr. Sanaa Osman Abdullah**

Signature:

2. **Dr. Ahmed Ebrahim Amin**

Signature:

Prof. Dr. Ahmed Mohamed El-Badawy

Chairman of Chemistry Department

Faculty of Science

Cairo University

LIST OF ABBREVIATIONS

BM40	Osteonectin
BRCA1	breast cancer type 1 susceptibility genes
BRCA2	Breast Cancer Type 2 susceptibility genes
BSE	Breast self examination
CAMs	Cell adhesion molecules
CBE	Clinical breast examination
CCL3	C-C motif chemokine 3 or Macrophage inflammatory protein-1
CD26	Dipeptidyl peptidase IV
CD44	is a receptor for hyaluronic acid
cDNA	complementary DNA
CIA	Collagen- induced arthritis
COS cells	the cells being CV-1 (simian) in Origin, and carrying the SV40 genetic material
CRD	Carbo-hydrate-recognition domain
CRP	Complement-regulatory proteins
CTL	C-type lectins or Ca ⁺² -dependent
DCIS	Ductal carcinoma in situ
EGF	Epidermal growth factor
ELAM-1	Endothelial leukocyte adhesion molecule-1
ER	Oestrogen receptors
	Erythroblastic leukemia viral oncogene
ERBB2	homolog 2
ERT	Estrogen replacement therapy
ESL-1	E-selectin ligand-1
	a glycoprotein identified in the cyst fluid of
GCDFP15	cystic breast disease

HCELL	Hematopoietic cell E- and L-selectin ligand
HIV	Human immunodeficiency virus
HL-60 cells	Human promyelocytic leukemia cells
HRT	Hormone replacement therapy
HUVEC	Human umbilical vein endothelial cells
ICAM-1	Inter-Cellular Adhesion Molecule 1
IG	Immunoglobulin
IL-1	Interlukin-1
LCIS	Lobular carcinoma in situ
LDLR	Low-density lipoprotein receptor mutant
LFA-1	Lymphocyte function-associated antigen-1
NK cells	Natural killer cells
PECAM-1	Platelet endothelial adhesion molecules
PR	Progesterone receptors
PSGL-1	P-selectin glycoprotein ligand-1
QIDSPL	Integrin -binding motif
RA	Rheumatoid arthritis
RPTP	Receptor protein tyrosine phosphates
SLE	Systemic lupus erythematosus
SLex	Sialyl lewisx
SPARC	Secreted protein acidic and rich in cysteine
TDLU	Terminal ductal lobular units
TGN	Trans Golgi network
TNF	Tumor necrotic factor
U937 cells	is a human monocytic cell line
VCAM-1	Vascular cell adhesion molecules

VEGF	Vascular endothelial growing factor
VLA4	Very Late Antigen-4

LIST OF TABLES

No.		Page
1	Staging of breast cancer	26
2	Immunoglobulin-like adhesion molecules	40
3	Major integrin pairings	43
4	Comparison between soluble VCAM-1 in Control group and Breast cancer group.	87
5	Comparison between soluble VCAM-1 in Control group and Benign group	88
6	Comparison between soluble VCAM-1 in Benign group and Breast cancer group.	89
7	Comparison between soluble VCAM-1 in Control group and Lymph node Negative group	91
8	Comparison between soluble VCAM-1 in Control group and Lymph node Positive group.	92
9	Comparison between soluble VCAM-1 in Benign group and Lymph node Negative group.	93
10	Comparison between soluble VCAM-1 in Benign group and Lymph node Positive group.	94
11	Comparison between soluble VCAM-1 in Lymph node Negative group and Lymph node Positive group.	95
12	Comparison between soluble VCAM-1 in Benign group and stage I group	98

No.		Page
13	Comparison between soluble VCAM-1 in stage I group and stage II group	99
14	Comparison between soluble VCAM-1 in stage I group and stage III group	100
15	Comparison between soluble VCAM-1 in stage II group and stage III group	101
16	Comparison between soluble E-selectin in Control group and Breast cancer group	103
17	Comparison between soluble E-selectin in control group and Benign group	104
18	Comparison between soluble E-selectin in Benign group and Breast cancer group.	105
19	Comparison between soluble E-selectin in Control group and Lymph node Negative group	107
20	Comparison between soluble E-selectin in Control group and Lymph node Positive group.	108
21	Comparison between E-selectin in Benign group and Lymph node Negative group	109
22	Comparison between soluble E-selectin in Benign group and Lymph node Positive group.	110
23	Comparison between soluble E-selectin in Lymph node Negative group and Lymph node Positive group	112
24	Comparison between soluble E-Selectin in benign group and stage I group	114

No.		Page
25	Comparison between soluble E-Selectin in stage I group and stage II group	115
26	Comparison between soluble E-Selectin in stage I group and stage III group	116
27	Comparison between soluble E-Selectin in stage II group and stage III group	117
28	95% Confidence Interval of soluble VCAM-1 in Individual group	119
29	95% Confidence Interval of soluble E-Selectin in Individual group	121
30	Summary Table of the different comparative values	122

LIST OF FIGURES

No		Page
1	The major classes of adhesion receptors, shown embedded in a putative plasma membrane	39
2	VCAM-1/ICAM-1/CD31 (PECAM-1) structural comparison	42
3	Domain structures of P-selectin, E-selectin, L-selectin, and PSGL-1.	46
4	Schematic illustration of different forms of VCAM-1 generated by alternative RNA splicing.	60
5	Sera VCAM-1 mean level of Control group and Breast cancer group	87
6	Sera VCAM-1 mean level in Control group and Benign group	88
7	Sera VCAM-1 mean level in Benign group and Breast cancer group	89
8	Sera VCAM-1 mean level in Control group and Lymph node Negative group	91
9	Sera VCAM-1 mean level in Control group and Lymph node Positive group	92
10	Sera VCAM-1 mean level in Benign group and Lymph node Negative group	93
11	Sera VCAM-1 mean level in Benign group and Lymph node Positive group	94

No		Page
12	Sera VCAM-1 mean level in Lymph node Negative group and Lymph node Positive group	96
13	Sera VCAM-1 mean level in benign group and stage I group	98
14	Sera VCAM-1 mean level in Stage I group and Stage II group	99
15	Sera VCAM-1 mean level in Stage I group and Stage III group	100
16	Sera VCAM-1 mean level in Stage II group and Stage III group	101
17	Sera E-Selectin mean level in Control group and Breast cancer group.	103
18	Sera E-Selectin mean level in Control group and Benign group	104
19	Sera E-Selectin mean level in Benign group and Breast cancer group	105
20	Sera E-Selectin mean level in Control group and Lymph node Negative group	107
21	Sera E-Selectin mean level in Control group and Lymph node Positive group	108
22	Sera E-Selectin mean level in Benign group and Lymph node Negative group	109
23	Sera E-Selectin mean level in Benign group and Lymph node Positive group	110
24	Sera E-Selectin mean level in Lymph node	112

No		Page
	Negative group and Lymph node Positive group	
25	Sera E-Selectin mean level in Benign group and Stage I group	114
26	Sera E-Selectin mean level in Stage I group and Stage II group	115
27	Sera E-Selectin mean level in Stage I group and Stage III group	116
28	Sera E-Selectin mean level in Stage II group and Stage III group	117
29	VCAM-1 of individual groups	118
30	E-Selectin of individual groups	120
31	Correlation between VCAM-1 and E-selectin in benign group.	123
32	Correlation between VCAM-1 and E-selectin in breast cancer	124
33	Correlation between VCAM-1 and E-selectin in Lymph node negative group	125
34	Correlation between VCAM-1 and E-selectin in Lymph node Positive group	126
35	Correlation between VCAM-1 and E-selectin in Stage III Group	127
36	Illustrate the relationship between tumor growth and VCAM-1	131

CONTENTS

	Page
Introduction and Aim Of the Work	1
Chapter 1. Review of Literature	5
1.1. Anatomy of normal breast	5
1.2. Benign breast diseases:	7
1.3. breast cancer	8
1.3.1. Epidemiology of breast cancer	9
1.3.2. RISK FACTORS IN BREAST CANCER	11
1.3.3. BREAST CANCER SCREENING GUIDELINE	16
1.3.4. HISTOLOGIC CLASSIFICATION OF BREAST CANCER	20
1.4. Adhesion Molecules in Biology and Oncology	38
1.4.1. Immunoglobulin-like superfamily	39
1.4.2. Integrins	42
1.4.3. Cadherins	44
1.4.4. Selectins	45
1.4.5. E-Selectin	47
1.4.6. vascular cell adhesion molecule 1	56

	Page
1.5. Function of cell adhesion molecules in development	62
1.5.1. motility and migration	62
1.5.2. Proliferation, neoplasia, malignancy	63
Chapter 2. Subjects and methods	65
Chapter 3. Results	86
Chapter 4. Discussion	128
Chapter 5. Summary and Conclusion	137
Chapter 6. References	142
Arabic Summary	

INTRODUCTION

Cancer is a major health problem world wide and the morbidity and mortality from cancer give rise to much suffering. Breast cancer is the most common malignancy in women worldwide (*Stewart, 2003*).

The causes of breast cancer are fundamentally unknown although we do know that there are some important predisposing factors, most of these are in some way related to hormonal factors. Positive family history is important risk factors for breast cancer (*Loman et al., 2001*).

Breast cancer spreads to local lymph nodes both in the axilla and the internal mammary chain, and then via the blood stream to produce distant metastases. A combination of genetic changes with the tumor allows for increased and chaotic cellular division, a reduction in apoptosis, invasion of local tissue and angiogenesis to support the tumor's continued growth (*Abeloff and Wolff et al., 2008*).

The improvement in breast cancer screening, >50-60% of tumors are detected early, before axillary lymph node involvement. Such patients are considered to have a better prognosis than node positive breast cancer patients, but 20-30% of them will relapse after surgery. There obviously is a need for good prognostic factors to accurately define subgroups of patients who would benefit from