ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN PATHOGENESIS OF POSTMENOPAUSAL OSTEOPOROSIS

Thesis Submitted In Partial Fulfillment of the Requirements of M.D in Physical Medicine, Rheumatology and Rehabilitation

By

MARY ATEF NASSIF SHAROBEEM

M.B, B.Ch, M.Sc. in Physical Medicine, Rheumatology and Rehabilitation

Faculty of Medicine – Ain Shams University

Under Supervision Of

Prof. Dr. MAHMOUD EL- TAYEB NASSER

Professor of Physical Medicine, Rheumatology and Rehabilitation Faculty of Medicine – Ain Shams University

Prof. Dr. HENAZ FAROUK KHALED

Professor of Physical Medicine, Rheumatology and Rehabilitation Faculty of Medicine – Ain Shams University

Asst. Prof. Dr. EMAN ABD EL HAMID KADDAH

Assistant Professor of Physical Medicine, Rheumatology and Rehabilitation

Faculty of Medicine - Ain Shams University

Asst. Prof. Dr. AHMED MOHAMED EL BADRAWY

Assistant Professor of Orthopedic Surgery Faculty of Medicine – Ain Shams University

Asst. Prof. Dr. SAHAR MOHAMED MAHDI

Assistant Professor of Histology Faculty of Medicine – Ain Shams University

> Faculty of Medicine Ain Shams University 2012

دور وجود عامل النمو البطانى الوعائى في منشأ مرض هشاشة العظام بعد انقطاع الطمث

رسالة مقدمة توطئة للحصول على درجة الدكتوراة فى الطب الطبيعى و الروماتيزم و التأهيل

مقدمة من

الطبيبة/ مارى عاطف نصيف شاروبيم

بكالوريوس الطب و الجراحة و ماجستير الطب الطبيعى و الروماتيزم و التأهيل

كلية الطب - جامعة عين شمس

تحت إشراف

أ. د. محمود الطيب ناصر

أستاذ الطب الطبيعى و الروماتيزم و التأهيل

كلية الطب – جامعة عين شمس

أ. د. هيناز فاروق خالد

أستاذ الطب الطبيعى و الروماتيزم و التأهيل كلية الطب – جامعة عين شمس

أ. م. إيمان عبد الحميد قداح

أستاذ مساعد الطب الطبيعي و الروماتيزم و التأهيل

كلية الطب – جامعة عين شمس

أ. م. أحمد محمد البدراوي

أستاذ مساعد جراحة العظام

كلية الطب - جامعة عين شمس

أ. م. سحر محمد مهدى

أستاذ مساعد الهستولوجي

كلية الطب – جامعة عين شمس

كلية الطب

جامعة عين شمس

7.17

<u>Acknowledgments</u>

First of all, thanks to GOD the most merciful and the most graceful for giving me the strength to carry out this work.

I would like to express my profound gratitude and great respect to Prof. Dr. Mahmoud EL- Tayeb Nasser, Professor of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University. It was an honor to me to carry out this work under his continuous guidance, encouragement and expert supervision.

I am greatly indebted and grateful to Prof. Dr. Henaz Farouk Khaled, Professor of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, for her great help, constructive remarks and guidance throughout this work.

I would like to express my deepest gratitude to Asst. Prof. Dr. Eman Abd El Hamid Kaddah, Assistant Professor of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, for her continuous help, precious advice and her valuable observation all through this work.

I am greatly appreciative to Asst. Prof. Dr. Ahmed Mohamed El Badrawy, Assistant Professor of Orthopedic Surgery, Faculty of Medicine, Ain Shams University, without whom the accomplishment of this work would have been impossible.

I would like to express my deep gratitude to Asst. Prof. Dr. Sahar Mohamed Mahdi, Assistant Professor of Histology, Faculty of Medicine, Ain Shams University, for her great help, valuable advice and support.

I am greatly honored to express my endless gratitude to Asst. Prof. Dr. Nermin Salah Youssef, Assistant Professor of Pathology, Faculty of Medicine, Ain Shams University, for her unlimited help. Her valuable cooperation helped me to accomplish this work.

My gratitude and thanks to all professors, staff and colleagues of Physical Medicine, Rheumatology and Rehabilitation department for their cooperative help.

I would like to thank all patients who participated in this work for their understanding and great cooperation.

I would like to express my endless gratitude and appreciation to my family for their patience, support and encouragement during the whole period of research.

CONTENTS

•	Introduction and Aim of the Work	1
•	Review of Literature	5
	o Bone Histology	5
	o Bone Modeling and Remodeling	30
	 Pathogenesis of Postmenopausal Osteoporosis 	62
	 Management of Osteoporosis 	72
	o Vascular Endothelial Growth Factor	92
•	Patients and Methods	110
•	Results	120
•	Discussion	154
•	Summary and Conclusion	178
•	Recommendations	183
•	References	184
•	Arabic Summary	

LIST OF ABBREVIATIONS

ActRIIA	ActDIIA	Activin Pacantor II A
B.V. Bone Volume BM Bone Marrow BMC Bone Mineral Content BMD Bone Mineral Density BMI Body Mass Index BMPs Bone Morphogenetic Proteins BMU Basic Multicellular Unit BSAP Bone Specific Alkaline Phosphatase BSP Bone sialoprotein Ca Calcium CBC Complete Blood Count c-fms Macrophage colony stimulating factor receptor. CIITA Class II Transactivator CI Chloride ion COX2 Cyclooxygenase 2 Ctsk Cathepsin k CTX Carboxy-Terminal Telopeptide of Type I Collagen DAB Diaminobenzidine DAP12 DNAX-Activating Protein DBD DNA-Binding Domain DBP Vitamin D Binding Protein DCS Dendritic Cells DEXA Dual-Energy X-Ray Absorptiometry DKK Dickkopf DXR Digital X-ray Radiogrammetry E.S. Eroded Surface E1 Estrone E2 Estradiol E3 Estriol ECS Endothelial Cells EDTA Ethylene-Diaminetetracetic Acid		<u>=</u>
BM	_	
BMC Bone Mineral Content BMD Bone Mineral Density BMI Body Mass Index BMPs Bone Morphogenetic Proteins BMU Basic Multicellular Unit BSAP Bone Specific Alkaline Phosphatase BSP Bone sialoprotein Ca Calcium CBC Complete Blood Count c-fms Macrophage colony stimulating factor receptor. CIITA Class II Transactivator CI Chloride ion COX2 Cyclooxygenase 2 Ctsk Cathepsin k CTX Carboxy-Terminal Telopeptide of Type I Collagen DAB Diaminobenzidine DAP12 DNAX-Activating Protein DBD DNA-Binding Domain DBP Vitamin D Binding Protein DCs Dendritic Cells DEXA Dual-Energy X-Ray Absorptiometry DKK Dickkopf DXR Digital X-ray Radiogrammetry E.S. Eroded Surface E1 Estrone E2 Estradiol E3 Estriol ECs Endothelial Cells EDTA Ethylene-Diaminetetracetic Acid		
BMD Bone Mineral Density BMI Body Mass Index BMPs Bone Morphogenetic Proteins BMU Basic Multicellular Unit BSAP Bone Specific Alkaline Phosphatase BSP Bone sialoprotein Ca Calcium CBC Complete Blood Count c-fms Macrophage colony stimulating factor receptor. CIITA Class II Transactivator CT Chloride ion COX2 Cyclooxygenase 2 Ctsk Cathepsin k CTX Carboxy-Terminal Telopeptide of Type I Collagen DAB Diaminobenzidine DAP12 DNAX-Activating Protein DBD DNA-Binding Domain DBP Vitamin D Binding Protein DCs Dendritic Cells DEXA Dual-Energy X-Ray Absorptiometry DKK Dickkopf DXR Digital X-ray Radiogrammetry E.S. Eroded Surface E1 Estrone E2 Estradiol E3 Estriol ECs Endothelial Cells EDTA Ethylene-Diaminetetracetic Acid		
BMI Body Mass Index BMPs Bone Morphogenetic Proteins BMU Basic Multicellular Unit BSAP Bone Specific Alkaline Phosphatase BSP Bone sialoprotein Ca Calcium CBC Complete Blood Count c-fms Macrophage colony stimulating factor receptor. CIITA Class II Transactivator CI Chloride ion COX2 Cyclooxygenase 2 Ctsk Cathepsin k CTX Carboxy-Terminal Telopeptide of Type I Collagen DAB Diaminobenzidine DAP12 DNAX-Activating Protein DBD DNA-Binding Domain DBP Vitamin D Binding Protein DCs Dendritic Cells DEXA Dual-Energy X-Ray Absorptiometry DKK Dickkopf DXR Digital X-ray Radiogrammetry E.S. Eroded Surface E1 Estrone E2 Estradiol E3 Estriol ECs Endothelial Cells EDTA Ethylene-Diaminetetracetic Acid		
BMPs		
BMU Basic Multicellular Unit BSAP Bone Specific Alkaline Phosphatase BSP Bone sialoprotein Ca Calcium CBC Complete Blood Count c-fms Macrophage colony stimulating factor receptor. CIITA Class II Transactivator CI Chloride ion COX2 Cyclooxygenase 2 Ctsk Cathepsin k CTX Carboxy-Terminal Telopeptide of Type I Collagen DAB Diaminobenzidine DAP12 DNAX-Activating Protein DBD DNA-Binding Domain DBP Vitamin D Binding Protein DCs Dendritic Cells DEXA Dual-Energy X-Ray Absorptiometry DKK Dickkopf DXR Digital X-ray Radiogrammetry E.S. Eroded Surface E1 Estrone E2 Estradiol E3 Estriol ECs Endothelial Cells EDTA Ethylene-Diaminetetracetic Acid		
BSAP Bone Specific Alkaline Phosphatase BSP Bone sialoprotein Ca Calcium CBC Complete Blood Count c-fms Macrophage colony stimulating factor receptor. CIITA Class II Transactivator CI Chloride ion COX2 Cyclooxygenase 2 Ctsk Cathepsin k CTX Carboxy-Terminal Telopeptide of Type I Collagen DAB Diaminobenzidine DAP12 DNAX-Activating Protein DBD DNA-Binding Domain DBP Vitamin D Binding Protein DCs Dendritic Cells DEXA Dual-Energy X-Ray Absorptiometry DKK Dickkopf DXR Digital X-ray Radiogrammetry E.S. Eroded Surface E1 Estrone E2 Estradiol E3 Estriol ECs Endothelial Cells EDTA Ethylene-Diaminetetracetic Acid		ž •
BSP Bone sialoprotein Ca Calcium CBC Complete Blood Count c-fms Macrophage colony stimulating factor receptor. CIITA Class II Transactivator CI Chloride ion COX2 Cyclooxygenase 2 Ctsk Cathepsin k CTX Carboxy-Terminal Telopeptide of Type I Collagen DAB Diaminobenzidine DAP12 DNAX-Activating Protein DBD DNA-Binding Domain DBP Vitamin D Binding Protein DCs Dendritic Cells DEXA Dual-Energy X-Ray Absorptiometry DKK Dickkopf DXR Digital X-ray Radiogrammetry E.S. Eroded Surface E1 Estrone E2 Estradiol E3 Estriol ECs Endothelial Cells EDTA Ethylene-Diaminetetracetic Acid		
Ca		
CBC		=
c-fms		
CIITA		-
CIT		
COX2Cyclooxygenase 2 CtskCathepsin k CTXCarboxy-Terminal Telopeptide of Type I Collagen DABDiaminobenzidine DAP12DNAX-Activating Protein DBDDNA-Binding Domain DBPVitamin D Binding Protein DCsDendritic Cells DEXADual-Energy X-Ray Absorptiometry DKKDickkopf DXRDigital X-ray Radiogrammetry E.SEroded Surface E1Estrone E2Estradiol E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid		
Ctsk		
CTX		· · · · · · · · · · · · · · · · · · ·
DAB		
DAP12		
DBD		
DBP		_
DCsDendritic Cells DEXADual-Energy X-Ray Absorptiometry DKKDickkopf DXRDigital X-ray Radiogrammetry E.SEroded Surface E1Estrone E2Estradiol E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid		
DKKDickkopf DXRDigital X-ray Radiogrammetry E.SEroded Surface E1Estrone E2Estradiol E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid		
DXRDigital X-ray Radiogrammetry E.SEroded Surface E1Estrone E2Estradiol E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid	DEXA	Dual-Energy X-Ray Absorptiometry
DXRDigital X-ray Radiogrammetry E.SEroded Surface E1Estrone E2Estradiol E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid	DKK	Dickkopf
E1Estrone E2Estradiol E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid		
E2Estradiol E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid	E.S.	Eroded Surface
E3Estriol ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid	E1	Estrone
ECsEndothelial Cells EDTAEthylene-Diaminetetracetic Acid	E2	Estradiol
EDTAEthylene-Diaminetetracetic Acid	E3	Estriol
	ECs	Endothelial Cells
EGFEpidermal Growth Factor		-
1	EGF	Epidermal Growth Factor

ELISA	Enzyme-Linked Immunosorbant Assay
	Estrogen Response Element
	Estrogen Receptors
	Estrogen Replacement Therapy
	Estrogen Receptor Alpha
	Estrogen Receptor Beta
	Erythrocyte Sedimentation Rate
FasL	
	Fc Receptor gamma
	Food and Drug Administration
	Fibroblast Growth Factors
Flk-1	Fetal Liver Kinase-1
Flt-1	fms –like tyrosine kinase receptor 1
	Frizzled Receptor
	Follicle Stimulating Hormone
GM-CSF	Granulocyte/Monocyte Colony-Stimulating Factor
H&E	Hematoxylin and Eosin
H +	Hydrogen Ion
H2CO3	Carbonic Acid
HCO3 ⁻	Bicarbonate Ion
HIFs	Hypoxia-Inducible Factors
HRP	Horseradish Peroxidase
HRT	Hormone Replacement Therapy
	Interferon Gamma
IGFs	Insulin-Like Growth Factors
IHC	Immunohistochemistry
ILs	
ITAM	Immunoreceptor Tyrosine-Based Activation Motif
kDa	kiloDalton
KDR	Kinase insert Domain –containing Receptor
Kg/m ²	Kilogram/Square Meter
	Luteinizing Hormone
	LDL Receptor–Related Protein 5
	Mitogen Activated Protein
	Macrophage Colony-Stimulating Factor
MMP	Matrix Metalloproteinase

Maga	M 1 10 0 11
	Mesenchymal Stem Cells
	Neutral Buffered Formalin
	Nuclear Factor of Activated T cells 1
	Nuclear Factor – kappa B
	Natural Killer
Nrp	-
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
NTX	Amino-Terminal Telopeptide of Type I Collagen
Ob.S	Osteoblast Surface
OC	Osteocalcin
OPG	Osteoprotegrin
OPN	Osteopontin
	Osteoclast-Associated Receptor
Osx	
PBS	Phosphate-Buffered Saline
PDGF	Platelet Derived Growth Factor
PGE2	Prostaglandin E2
	Prostaglandins
	Phosphatidylinositol-3
	Paired Ig-Like Receptor-A
	Phospholipase C gamma
	Poly (lactic- co- glycolic acid)
	Placenta Growth Factor
PsA	Psoriatic Arthritis
PTH	Parathyroid Hormone
	Quantitative Computed Tomography
_	Quantitative Ultrasound
	Rheumatoid Arthritis
RANK	Receptor Activator of Nuclear Factor kappa B
	Receptor activator of nuclear factor kappa B ligand
	Rough Endoplasmic Reticulum
	Arginine, Glycine and Aspartic acid
	recombinant human Bone Morphogenetic Protein -2
	recombinant human VEGF-A
ROS	Reactive Oxygen Species
	recombinant Parathyroid Hormone

RT-PCR	.Reverse Transcriptase Polymerase Chain Rection
Runx2	.Runt-Related Transcription Factor 2
SERMs	.Selective Estrogen Receptor Modulators
SFRP	.Secreted Frizzled Related Protein
SIRP-β1	.Signal Regulatory Protein β1
T.S	.Trabecular Separation
Tb. Th.	.Trabecular Thickness
TGF-β	.Transforming Growth Factor-Beta
Th-1	.T helper-1
TNFs	.Tumor Necrosis Factors
TRAF6	.TNF Receptor Associated Factor 6
TRAP	.Tartrate-Resistant Acid Phosphatase
TREM-2	.Triggering Receptor Expressed By Myeloid Cells-2
VDRs	.Vitamin D Receptors
VEGF	.Vascular Endothelial Growth Factor
VEGFR	.Vascular Endothelial Growth Factor Receptor
WHO	.World Health Organization
Wnt	.Wingless-Type and Integrase 1

LIST OF FIGURES

FIC	GURE PAG	GE
1.	Gross structure of bone.	6
2.	Structure of cortical bone and trabecular bone.	7
3.	Normal trabecular bone of the iliac crest by direct three- dimentional morphometric analysis.	8
4.	Woven bone: there is lack of any particular alignment of the collagen fibrils.	ne 10
5.	Lamellar bone: there is a well-delineated orientation of the collagen fibrils and coordinated arrangement of the cells.	
6.	Schematic illustration of collagen formation.	11
7.	Collagen fiber and fibril structure with locations of pores a hole zones.	nd 12
8.	Osteoblast differentiation.	15
9.	Osteoblasts by hematoxylin and eosin stain (H&E) stain.	16
10.	Diagram represents the process of differentiation from osteoblast precursors to osteocytes embedded in the mineralized matrix.	19
11.	Scanning electron micrograph shows osteocytes and their canalicular processes.	20
12.	Active osteoclast with ruffled border in lacuna (Giemsa stain).	21
13.	Osteoclast differentiation from a hematopoietic stem cell to mature multinucleated osteoclast.	o a 22
14.	Schematic representation of the structure and actions of the osteoclast.	e 24
15.	Osteoclast cell and mechanism of bone resorption.	25

16.	Regulation of Ca levels in blood and tissues.	28
17.	Differentiation of osteoblasts, osteoclasts and basic multicellular units (BMUs).	31
18.	Phases of bone remodeling.	32
19.	Pathways of vitamin D metabolism.	36
20.	Central nervous system participation via leptin in bone turnover.	38
21.	Genomic pathway of estrogen signal transduction.	40
22.	Nonnuclear estrogen-signaling pathway through cell surface receptors.	ee 41
23.	Role of RANKL in osteoclast differentiation and activity.	46
24.	The essential signaling pathway for normal osteoclastogenesis.	49
25.	The co-stimulatory signals cooperate with RANKL for normal osteoclastogenesis.	50
26.	Interaction of the Wnt and BMP pathways.	56
27.	Schematic representation of the main mechanisms and feedback interactions by which estrogen deficiency leads to bone loss.	o 69
28.	Quantitative Computed Tomography (QCT).	75
29.	Quantitative Ultrasound performed on the calcaneus.	75
30.	(A) Bone volume is calculated from measurements of cortical thickness of the metacarpals.(B) A computerized automated analysis has been develope for the radiogrammetry.	76 d 76
31.	Human intact PTH 1–84, the first 34 amino acids are highlighted.	85

32.	Mode of action of strontium ranelate.	87
33.	VEGF family members and their specific VEGF receptors	s. 94
34.	Dual-energy x-ray absorptiometry (DEXA) device.	113
35.	Image analysis system device.	115
36.	A bar chart showing comparison between the three group regarding serum E2 (pg/ml).	s 122
37.	A bar chart showing comparison between the three group regarding BMD at femoral neck, lumbar spine and forearm.	s 123
38.	A bar chart showing comparison between the three group regarding T–score at femoral neck, lumbar spine and forearm.	s 125
39.	A photomicrograph of a section from iliac crest bone of premenopausal female showing the cancellous bone trabeculae and bone marrow spaces (BM).	126
40.	A photomicrograph of a section from iliac crest bone of premenopausal female showing branching and anastomos thick trabeculae with BM spaces inbetween.	ing 127
41.	A photomicrograph of a section from iliac crest bone of perimenopausal female showing slightly discontinuous bout trabeculae which appeared relatively thin separated by widened BM spaces.	one 127
42.	 (A) A photomicrograph of a section from iliac crest bone postmenopausal female showing significantly discontinuous thin bone trabeculae separated by widened BM spaces with more abundant fat cells. (B) A photomicrograph of a section from iliac crest bone postmenopausal female showing an irregular eroded surface of bone trabeculae. 	ous th 128 of

43.	A bar chart showing comparison between the three group regarding B.V., Tb.Th. and Ob.S.	s 130
44.	A bar chart showing comparison between the three group regarding T.S. and E.S.	s 130
45.	 (A) A photomicrograph of a section from iliac crest bone showing cytoplasmic reaction of the VEGF expression in osteoblasts. (B) A photomicrograph of a section from iliac crest bone premenopausal female showing a markedly increased VE expression. Osteoblasts appeared positively stained. (C) A photomicrograph of a section from BM spaces of premenopausal female showing VEGF positively stained cells. Their cytoplasm contains brownish VEGF positive granules. 	131 of GF 131
46.	(A) A photomicrograph of a section from iliac crest bone preimenopausal female showing a less VEGF expression fewer osteoblasts.(B) A photomicrograph of a section from BM spaces of perimenopausal female showing less VEGF expression in fewer positively stained BM cells.	in 132
47.	 (A) A photomicrograph of a section from iliac crest bone postmenopausal female showing a nearly absent VEGF expression in osteoblasts lining bone trabeculae. (B) A photomicrograph of a section from BM spaces of postmenopausal female showing minimal VEGF expression in scattered positively stained BM cells. 	133
48.	 (A) A photomicrograph of a section from iliac crest bone showing nuclear and cytoplasmic reactions of ERα in osteoblasts. (B) A photomicrograph of a section from iliac crest bone premenopausal female showing an enhanced ERα expression. Osteoblasts lining bone trabeculae had intens ERα positively stained nuclear and cytoplasmic reactions 	134 of ely

	(C) A photomicrograph of a section from BM spaces of premenopausal female showing $ER\alpha$ positively stained Bi cells.	M 135
49.	(A) A photomicrograph of a section from iliac crest bone perimenopausal female showing less $ER\alpha$ expression in fewer positively stained osteoblasts lining bone trabecular (B) A photomicrograph of a section from BM spaces of perimenopausal female showing less $ER\alpha$ expression in fewer BM stromal cells.	
50.	 (A) A photomicrograph of a section from iliac crest bone postmenopausal female showing much weaker ERα expression. Smaller number of osteoblasts lining bone trabeculae appeared positively stained. (B) A photomicrograph of a section from BM spaces of postmenopausal female showing weaker expression of EFS Smaller number of BM stromal cells appeared positively stained. 	137
51.	A bar chart showing comparison between the three group regarding VEGF and $\text{ER}\alpha$ expressions.	s 138
52.	A bar chart showing comparison between the normal, osteopenic and osteoporotic patients in the three groups regarding VEGF and $ER\alpha$ expressions.	139
53.	 (A) Positive correlation between VEGF expression percentage and E2 levels (pg/ml). (B) Positive correlation between ERα expression percentage and E2 levels (pg/ml). 	141 age 141
54.	(A) Negative correlation between VEGF expression and a of patients.(B) Negative correlation between ERα expression and against patients.	142
55.	Positive correlation between VEGF expression and $\text{ER}\alpha$ expression.	143

56.	(A) Positive correlation between VEGF expression and Bl of femur.	MD 144
	(B) Positive correlation between VEGF expression and BI	MD 144
	(C) Positive correlation between VEGF expression and BI of forearm.	MD 144
57.	(A) Positive correlation between ER α expression and T–score of femur.	145
	 (B) Positive correlation between ERα expression and T–score of lumbar spine. (C) Positive correlation between ERα expression and T– 	146
	• •	146
58.	(A) Positive correlation between E2 levels (pg/ml) and B.V.%.	147
	(B) Positive correlation between E2 levels (pg/ml) and Tb.Th. um.	147
	(C) Positive correlation between E2 levels (pg/ml) and Ob.S.%.	148
	(D) Negative correlation between E2 levels (pg/ml) and T. um.	S. 148
	(E) Negative correlation between E2 levels (pg/ml) and	148
	L.D. /0.	140