Expression of Osteopontin in Synovial Tissue of Rheumatoid Arthritis: Its Role in Joint Destruction

Thesis

Submitted for Fulfillment of the Requirements for the MD Degree in Physical Medicine and Rehabilitation

By

Nermin Farouk Labib

M.B., B. Ch., M. Sc.

Under supervision of

Professor Doctor/ Nahed Mounir Sherif

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

Professor Doctor/ Hala Abdul-Hady Saleh

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

Professor Doctor/ Omar Hussein Omar

Professor of Radiodiagnosis
Faculty of Medicine, Ain Shams University

Professor Doctor/ Nedal Ahmed Hegazy

Professor of Pathology
Faculty of Medicine, Ain Shams University

Doctor/ Iman Mahmoud Ghanima

Assistant professor of Physical Medicine and Rehabilitation
Faculty of Medicine, Ain Shams University

Faculty of Medicine,
Ain Shams University
٢٠٠٨

Acknowledgement

First and foremost, thanks to ***ALLAH***, the most beneficent and most merciful.

I find no words by which I can express my sincere thanks, supreme gratitude to ***Prof. Dr. Nahed Mounir Sherif***, 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 her continuous guidance, patience, encouragement and expert supervision.

I would like also to express my gratitude to ***Prof. Dr. Hala Abdul Hady Saleh***, Professor of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University. For her unlimited help, precise instructions, kind encouragement and directions throughout this work.

I would like also to express my gratitude to ***Prof. Dr. Omar Hussein Omar***, Professor of Radiodiagnosis, Faculty of Medicine, Ain Shams University, for his instructions, unlimited help throughout this work.

I am deeply grateful to ***Prof. Dr. Nedal Ahmed Hegazy***, Professor of Pathology, Faculty of Medicine, Ain Shams University, who devoted her time, effort and experience most generously throughout this work.

I am greatly indebted to ***Dr. Iman Mahmoud Ghanima***, Assistant Professor of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, for her great help and guidance throughout this work.

I am grateful to the orthopedic surgery professors for their help which was essential for accomplishment of this work.

I would like to thank all our patients who were involved in this study for their cooperation and understanding of the importance of our research work and for their friendly attitude.

Also, I would like to thank all my Professors and colleagues in Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, for their help and support.

Finally, I wish to thank everyone who offered me help during the achievement of this research work especially my parents and husband whom with their continuous enthusiastic encouragement and everlasting help made the completion of this work possible.

Nermin Farouk Labib

List of Contents

Page

- List of abbreviations ii
- List of Figures vi
- List of Tables x
- Introduction & aim of the work ١
- Review of Literature:
  - Rheumatoid arthritis ٤
  - Osteopontin ٩٧
- Patients and methods ١٢٩
- Results ١٤٢
- Discussion ١٨٣
- Summary and conclusion ٢٠٩
- Recommendations ٢١٣
- References ٢١٤
- Arabic summary

List of Abbreviations

ACR ..... American college of rheumatology
AFA ..... antifilaggrin antibodies
AIMs ..... Arthritis impact measurements Scales
AKA ..... Antikeratin antibody
ALP ..... Alkaline phosphatase
ANA ..... Antinuclear antibodies
ANCA ..... Antineutrophil cytoplasmic antibodies
Ang-١ ..... Angiopoietin-١
Anti-CII ... Anticollagen type II
APF ..... Antiperinclear factor
Bcl-٢ ..... B cell lymphoma-٢
BiP ..... Binding protein
BMD ..... Bone mineral density
C١-٢ ..... Cervical ١-٢
CAM ..... Chorioallantoic membrane
CCPs ..... Cyclic citrullinated peptides
CCR ..... chemokine receptor (٢ adjacent cysteines)
CD ..... Cluster of differentiation
cDNA ..... Complementary deoxy ribonucleic acid
CFD ..... Color flow doppler
COX ..... Cyclooxygenase
CPPD ..... Calcium pyrophosphate dehydrate
CR ..... Conventional radiography
CRP ..... C-reactive protein
CT ..... Computed tomography
CTLA-٤ ... Cytotoxic T-lymphocyte associated antigen ٤
CXC ..... Chemokine (٢ cysteines separated by X amino acid)
DAB ..... Diaminobenzidine
DAS ..... Disease activity score
DEXA ..... Dual Energy X ray Absorptiometry
DIP ..... Distal interphalangeal
DMARDs.. Disease modified antirheumatic drugs
ds-DNA .... Double stranded deoxyribonucleic acid
DTH ..... Delayed type hypersensitivity
EAE ..... Experimental autoimmune encephalomyelitis
EBV ..... Epstein bar virus
ECM ..... Extracellular matrix

EDTA ..... Ethylenediaminetetraacetic acid
ELISA ..... Enzyme linked immunosorbent assay
ELR ..... Glycine-leucine-arginine amino acid sequences
ENA ..... Epithelial neutrophil activating protein
ERM ..... Ezrin, radixin, moesin proteins
ESR ..... Erythrocyte sedimentation rate
Eta-١ ..... Early T-cell activation gene ١
Fc ..... Fragment-crystalline
FGF ..... Fibroblast growth factor
FLS ..... Fibroblast like synoviocytes
fMLP ..... Formyl methionyl-leucyl-phenylalanine
Gly ..... Glycolysation
GM-CSF .. Granulocyte macrophage colony stimulating factor
GPI ..... Glucose-٦-phosphate isomerase
GRO ..... Growth related oncogene
HAQ ..... Heath assessment questionnaire
Hb ..... Hemoglobin
HIF ..... Hypoxia inducible transcription factor
HLA ..... Human leukocytic antigen
HMG ..... High mobility group protein
HPF ..... High power field
HRUS ..... High resolution ultrasound
IAP ..... Inhibitor of apoptosis
ICAM ..... Intercellular adhesion molecule
IFN-γ ..... Interferon γ
Ig ..... Immunoglobulin
IL ..... Interleukin
iNOS ..... Inducible nitric oxide synthetase
IP ..... Inducible protein
I-TAC ..... Inducible T cell alpha chemoattractant
JAK-STAT Janus kinases, signal transducers and activators of transcription
JIA ..... Juvenile idiopathic arthritis
kDa ..... Killodalton
KO mice .. Knock out mice
LPS ..... Lipopolysaccharides
Lt ..... Left
MACTAR McMaster Toronto arthritis
MAdCAM Mucosal addressin cell adhesion molecule

Max ..... Maximum
MCP ..... Metacarpophalangeal
MCP-١ ..... Monocyte chemoattractant protein-١
MDHAQ .. Multidimensional heath assessment questionnaire
Mg ..... Milligram
Mg٢+ ..... Magnesium
MHAQ ..... Modified heath assessment questionnaire
MHC ..... Major histocompatibility complex
MHz ..... Megahertz
Mig ..... Monokine induced by interferon gamma
Min ..... Minimum
MLS ..... Macrophage like synoviocyte
Mm ..... Millimeter
MMP ..... Matrix metalloproteinase
Mn٢+ ..... Manganese
MRI ..... Magnetic resonance imaging
mRNA ..... Messenger ribonucleic acid
MSUS ..... Musculoskeletal ultrasound
MTP ..... Metatarsophalangeal
MTX ..... Methotrexate
NF-$_k$B ..... Nuclear factor kappa beta
NHP ..... Nottingham health profile
NO ..... Nitric oxide
NS ..... Non significant
NSAIDs ... Nonsteroidal anti-inflammatory drugs
OA ..... Osteoarthritis
OPN ..... Osteopontin
PBMC ..... Peripheral blood mononuclear cell
PBS ..... Phosphate buffered saline
PDGF ..... Platelet derived growth factor
PDUS ..... Power doppler ultrasonography
PECAM ... Platelet-endothelial cellular adhesion molecule
PF ..... Platelet factor
PGA ..... Patient's global assessment
PGE٢ ..... Prostaglandin E٢
PIP ..... Proximal interphalangeal
Plt ..... Platelets
PMNLs .... Polymorphnuclear leukocytes
PVP ..... Polyvinyl pyrrolidone
PXR ..... Plain X ray

r	Correlation coefficient
RA	Rheumatoid arthritis
RANA	Rheumatoid arthritis associated nuclear antigen
RANK	Receptor of activation of nuclear factor
RANTES .	Regulated on activation normally T cell expressed and secreted
RF	Rheumatoid factor
RGD	Arginine-glycine-aspartic amino acid sequences
rOPN	Recombinant OPN
Rt	Right
RT-PCR ..	Reverse transcriptase polymerase chain reaction
S	Significant
SD	Standard deviation
SDF-١	Stromal cell derived factor-١
SE	Shared epitope
SFMC	Synovial fluid mononuclear cell
SFs	Synovial fibroblasts
Sig.	Significance
SLAYGLR	Ser-Leu-Ala-Tyr-Gly-Leu-Arg amino acid sequences
SLE	Systemic lupus erythematosus
SLEDAI ...	Systemic lupus erythematosus disease activity index
SSI	Spread Severity Index
SVVYGLR	Ser-Val-Val-Tyr-Gly-Leu-Arg amino acid sequences
TGF-B	Transforming growth factor beta
Th	T helper
Tie	Receptor tyrosine kinase
TIMPs	Tissue inhibitor matrix metalloproteinase
TMJ	Tempromandibular joint
TNF	Tumor necrosis factor
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UC	Ulcerative colitis
US	Ultrasound
VAS	Visual analogue scale for pain
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VLA	Very late antigen

List of Figures

Figure NO.	Figure title	Page NO.
Figure (١):	Outline of the management of rheumatoid arthritis	٨٤
Figure (٢):	Some of the features of the OPN molecule	٩٨
Figure (٣):	Pro- and anti-inflammatory actions of OPN in cell injury	١١٣
Figure (٤):	Shows HRUS apparatus	١٣٥
Figure (٥):	Shows suprapatellar longitudinal application in moderate knee flexion	١٣٧
Figure (٦):	Shows sex distribution among RA patients	١٤٣
Figure (٧):	Shows modified disease activity score (DAS) among RA patients	١٤٤
Figure (٨):	Shows the distribution of different extra-articular manifestations of RA patients	١٤٦
Figure (٩):	A comparison between patient and control groups regarding the laboratory data	١٤٨
Figure (١٠):	Shows the frequency of the radiological grades among patients under study	١٤٩
Figure (١١):	Longitudinal sonographic scan of the right knee joint in a RA patient showing, thickened irregular synovium situated between the femur (F) and the quadriceps tendon (T) and the thickness of the synovium was determined	١٥١
Figure (١٢):	Longitudinal sonographic scan of the right knee joint in a RA patient showing decreased in cartilage thickness	١٥١

Figure (١٣): Longitudinal sonographic scan of the right knee joint in a RA patient showing, bone erosions ١٥٢

Figure (١٤): Power Doppler sonographic image of the left knee joint in a RA patient showing no increase in synovial blood flow (power Doppler grade ٠) ١٥٢

Figure (١٥): Longitudinal sonographic scan of the right knee joint in a RA patient showing increased synovial blood flow (power Doppler grade ١). ١٥٣

Figure (١٦): Longitudinal sonographic scan of the left knee joint in a RA patient showing increased synovial blood flow (power Dopple grade ٢). ١٥٣

Figure (١٧): Power Doppler sonographic scan of the right knee joint in a RA patient showing, hypertrophic synovium and its vascularization. The flow pattern of the synovial blood flow was determined and the resistive index (RI) was calculated automatically ١٥٤

Figure (١٨): Shows comparison between RA patients group and controls group regarding synovial blood flow grades ١٥٥

Figure (١٩): Shows comparison of high resolution ultrasound data between RA patients group and control group under study ١٥٦

Figure (٢٠): Control synovial tissue showing weak minimal immunoreactivity for OPN in synovial lining and weak scattered

perivascular staining in the sublinining layer (X١٠٠) ١٦١

Figure (٢١): Control synovial tissue showing weak minimal immunostaining for OPN in synovial lining cells (X٤٠٠) ١٦٢

Figure (٢٢): Showing expression of OPN in RA synovium: OPN staining is located predominantly in synovial lining rather than the sublining (X١٠٠) ١٦٢

Figure (٢٣): Showing expression of OPN in RA synovium: synovial lining cells stain strongly for OPN while no staining is detected in the lymphoid aggregates (X٤٠٠) ١٦٣

Figure (٢٤): RA synovial sublining tissue showing less remarkable OPN expression, mainly located in the perivascular extracellular tissue and some scattered inflammatory cells. It is absent in the lymphoid aggregates (X٢٠٠) ١٦٣

Figure (٢٥): RA synovial tissue showing strong cytoplasmic staining for OPN (+++) in lining synoviocytes (X٤٠٠) ١٦٤

Figure (٢٦): RA synovial tissue showing strong cytoplasmic staining for OPN (+++) in lining synoviocytes more than the sublining layer (X٤٠٠) ١٦٤

Figure (٢٧): RA synovial tissue showing intermediate cytoplasmic staining for OPN (++) in lining synoviocytes (X٤٠٠) ١٦٥

Figure (٢٨): The correlation between synovial thickness and bone erosion ١٦٩
Figure (٢٩): The correlation between synovial thickness and cartilage thickness ١٦٩
Figure (٣٠): The correlation between synovial thickness and spread severity index ١٧٠
Figure (٣١): The correlation between synovial thickness and resistive index (RI) ١٧٠
Figure (٣٢): The correlation between resistive index and disease activity score ١٧٤
Figure (٣٣): The correlation between resistive index and spread severity index ١٧٤
Figure (٣٤): The correlation between OPN positive cells and DAS ١٧٦
Figure (٣٥): The correlation between OPN positive cells and spread severity index ١٧٦
Figure (٣٦): The correlation between OPN positive cells and synovial thickness ١٧٧
Figure (٣٧): The correlation between OPN positive cells and bone erosion ١٧٧
Figure (٣٨): The correlation between OPN positive cells and cartilage thickness ١٧٨
Figure (٣٩): The correlation between OPN positive cells and RI ١٧٨

List of Tables

Table No.	Table Title	Page No.
Table (١):	Shows major adhesion molecule interactions with rheumatoid arthritis synovial endothelium	٢٧
Table (٢):	Shows RA autoantigens with relatively high specificity	٥٣
Table (٣):	Shows the benefits of MSUS for rheumatologists	٦٥
Table (٤):	Shows the demographic data of both groups under study	١٤٢
Table (٥):	Shows the clinical data of RA patients	١٤٣
Table (٦):	Shows the classification of RA patients according to their disease activity	١٤٤
Table (٧):	Shows modified index of synovitis activity of the knee joint among RA patients under study	١٤٥
Table (٨):	Shows the frequency of different extra-articular manifestations of RA patients	١٤٦
Table (٩):	Shows laboratory data among patient group	١٤٧
Table (١٠):	Shows laboratory data among control group	١٤٧
Table (١١):	Shows comparison of laboratory data between RA patients group and control group under study	١٤٨
Table (١٢):	Shows the radiological data among patients under study	١٤٩

Table (١٣): Shows comparison between RA patients group and control group regarding synovial blood flow grades ١٥٥

Table (١٤): Shows comparison between RA patients group and control group regarding other HRUS findings ١٥٦

Table (١٥): Shows the comparison between RA patients with positive RF and those with negative RF as regards HRUS findings ١٥٧

Table (١٦): Shows the comparison between RA patients with moderate modified DAS and those with severe modified DAS as regards HRUS findings ١٥٨

Table (١٧): Shows grades of modified index of knee synovitis activity in relation to HRUS findings of RA patients group ١٥٩

Table (١٨): Shows the different radiological grades according to Larsen's score in relation to HRUS results of RA patients group ١٦٠

Table (١٩): Shows a comparison between patients and controls regarding the immuno-histochemical findings ١٦٥

Table (٢٠): Shows the comparison between RA patients with positive rheumatoid factor (RF) and those with negative RF as regards the immunohistochemical data ١٦٦

Table (٢١): Shows a comparison between grades of modified DAS and the immuno-histochemical findings ١٦٦

Table (٢٢): Shows the modified index of synovitis activity grades of the knee joint in relation

to the immunohistochemical data among RA patient group ١٦٧

Table (٢٣): Shows the different radiological grades according to Larsen's score in relation to immunohistochemical data among RA patient group ١٦٧

Table (٢٤): Shows correlation between synovial thickness and various clinical, laboratory, radiological and immunohistochemical parameter of RA patients group ١٦٨

Table (٢٥): Shows correlation between cartilage thickness and various clinical, laboratory, radiological and immunohistochemical parameter of RA patients group ١٧١

Table (٢٦): Shows correlation between bone erosion and various clinical, laboratory, radiological and immunohistochemical parameter of RA patients group ١٧٢

Table (٢٧): Shows correlation between resistive index (RI) and various clinical, laboratory, radiological and immunohistochemical parameter of RA patients group ١٧٣

Table (٢٨): Shows correlation between percentage of OPN positive cells and various clinical, laboratory and radiological parameters of RA patients group ١٧٥

Table (٢٩): Shows synovial blood flow grades in relation to various clinical data of RA patient ١٧٩