Leukocytes as a Potential Biomarker for Osteoarthritis

Thesis

Submitted for Fulfilment of Master Degree in Orthopedic Surgery

By

Hesham Mohamed Asaad Elkhodary

M.B., B.Ch.,

Faculty of Medicine - Ain Shams University

Dr. Ahmed Sami Kamel

Assistant Professor of Orthopedic Surgery

Faculty of Medicine

Ain Shams University

Dr. Ahmed Mohamed Mohasseb

Lecturer of Orthopedic Surgery

Faculty of Medicine

Ain Shams University

Faculty of Medicine

Ain Shams University

2019

سورة البقرة الآية: ٣٢

Acknowledgment

First and foremost, I feel always indebted to ***ALLAH,*** *the Most Kind and Most Merciful.*

I'd like to express my respectful thanks and profound gratitude to ***Dr. Ahmed Sami Kamel****, Assistant Professor of Orthopedic Surgery, Faculty of Medicine- Ain Shams University for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

I am also delighted to express my deepest gratitude and thanks to ***Dr. Ahmed Mohamed Mohasseb,*** *Lecturer of Orthopedic Surgery, Faculty of Medicine, Ain Shams University, for his kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Last but not least my sincere thanks and appreciation to all patients participated in this study.

Hesham Mohamed Asaad Elkhodary

List of Contents

List of Tables

List of Figures

List of Figures (Cont...)

List of Abbreviations

Abb.	Full term
ADL	*Activities of daily living*
AIMS	*Arthritis Impact Measures Scale*
BC	*Bicompartmental*
BIPED	*Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic*
BMI	*Body mass index*
COX-2	*Cyclooxygenase-2*
DM	*Diabetes mellitus*
FDA	*Food and Drug Administration*
FGF	*Fibroblast growth factor*
HA	*Hyaluronic acid*
HE	*Hematoxylin and eosin*
HETE	*Hydroxyeicosatetraenoic acid*
HLA	*Human leukocytic antigen*
IA	*Intra-articular*
ICRS	*International Cartilage Repair Society*
IGF	*Insulin derived growth factor*
IKDC	*International knee documentation committee*
IL	*Interleukin*
IL1RN	*Interleukin 1 receptor antagonist*
KL scores	*The Kellgren and Lawrence scores*
KOOS	*Knee injury and Osteoarthritis Outcome Score*
NSAIDs	*Non-steroidal anti-inflammatory drugs*
OA	*Osteoarthritis*
OAIL	*Osteoarthritis Interlukin-1*
PBL	*Peripheral blood leukocytes*
PBMC	*Peripheral blood mononuclear cells*
PCR	*Polymerase chain of reaction*

List of Abbreviations (Cont...)

Abb.	Full term
PDGF	*Platelet derived growth factor*
PGE2	*Prostaglandin E2*
PRP	*Platelet-rich plasma*
QOL	*Quality of life*
RA	*Rheumatoid arthritis*
SDF	*Stromal derived growth factor*
SF	*Synovial fluid*
SKOA	*Symptomatic knee osteoarthritis*
SM	*Synovial Membrane*
Sport/Rec	*Sport and recreation*
TGF	*Transforming growth factor*
Th1	*T helper type 1*
TKA	*Total knee arthroplasty*
TNF	*Tumor necrosis factor*
UC	*Unicompartmental*
VAS	*Visual analogue scale*
VEGF	*Vascular endothelial growth factor*
WCC	*White cell count*
WOMAC	*Western Ontario and McMaster Universities Osteoarthritis Index*

ABSTRACT

Objectives: To figure out the role of leukocytes in prediction, diagnoses and prognosis of osteoarthritis. Hypothesizing whether leukocytes may serve as biomarkers for both early diagnosis and, perhaps more importantly, identification of osteoarthritis patients at higher risk for disease progression.

Patients and methods: 200 primary OA Patients were aspirated for synovial fluid samples, samples collected were sent to cytology for WBC count analysis and a Questionnaire filling of KOOS.

Results: As regard There was statistically significant relation found between severity of osteoarthritis and age of the studied cases with p-value = 0.015 while no statistically significant relation found with sex or BMI of the studied cases with p-value = 0.766 and 0.601 respectively. Also side of knee examined and appearance of synovial fluid aspiration samples did not show significant relation with severity of osteoarthritis with p-value = 0.840 and 0.597 respectively. Moreover there was highly statistically significant increase in synovial fluid white cell count with the increase of severity among the studied cases with p-value < 0.001 and there was highly statistically significant inverse relation found between severity of osteoarthritis and KOOS score. Yet that there was no statistically significant correlation found between age of the studied cases and KOOS score or synovial fluid white cell count.

Conclusion: There is a role for leukocytes in prediction, diagnoses and prognosis of osteoarthritis. Serving as a biomarkers for both early diagnosis and, perhaps more importantly, identification of osteoarthritis patients at higher risk for disease progression and severity.

Key Words: Osteoarthritis, white blood cell Count**,** Knee injury and osteoarthritis outcome score, leukocytosis**,** synovial fluid, age, sex, side, body mass index.

INTRODUCTION

Osteoarthritis is the most common type of adult joint disease. A recent report from the Centers for Disease Control estimates that 52.5 million US adults had arthritis in 2010–2012, an increase from 2007–2009 (50 million); given its prevalence, osteoarthritis likely accounts for at least half of this total. While formerly considered a non-inflammatory joint disease, it now is well-appreciated that inflammation has been implicated in the pathogenesis of osteoarthritis.[1]

Osteoarthritis is increasing in frequency and severity in all aging populations with an estimated U.S. prevalence of over 50 million affected adults. Disease progression is associated with cartilage degradation, joint space narrowing, synovial membrane, and loss of function. Although traditionally considered a non-inflammatory joint disease, it is now well appreciated that inflammatory mediators are produced by articular tissues in osteoarthritis and have been implicated in disease pathogenesis. There is currently great interest in the field of osteoarthritis to identify biomarkers that provide a method for earlier diagnosis and identify patients at higher risk for disease progression. We hypothesized leukocytes, which traffic through the tissues of the inflamed joint, are activated and other locally-produced inflammatory mediators. We assessed the gene expression of leukocytes as potential biomarkers in osteoarthritis. Our data indicate leukocytes that

may serve as biomarkers for both diagnosis and, perhaps more importantly, identification of osteoarthritis patients at higher risk for disease progression.[2]

Although osteoarthritis is commonly described as non-inflammatory joint disease, synovial inflammation is increasingly recognized as contributing to the symptoms and progression of osteoarthritis. Synovial histological changes include synovial hypertrophy and hyperplasia with a big number of lining cells often accompanied by infiltration with lymphocytes.[3]

Osteoarthritis pathophysiology is complex and far from being understood. Although, clinically, subtypes can be defined by their etiology, clinical presentation and radiographic evaluation, it remains unknown how this translates into the cellular and molecular pathways of joint degradation. While it is understood that the pathophysiology includes biomechanical, hereditary and molecular factors, none of these mechanisms have provided enough information to halt disease progression. Although osteoarthritis has long been interpreted as a 'non-inflammatory' disease, a clinically relevant number of osteoarthritis patients present with signs of inflammation, e.g. joint swelling and effusion. Osteoarthritis has long been interpreted as a non-inflammatory disease.[4]

However, these inflammatory processes were interpreted mainly as a bystander, and not as a driving force in osteoarthritis

pathogenesis. A set of new studies has raised interest in this topic and aimed to map these inflammatory processes more precisely. Magnetic resonance imaging studies have shown that patients with inflammation show faster osteoarthritis progression, confirming the hypothesis that inflammation has an impact on disease progression. Understanding the biology of synovial inflammation and the disturbed homeostasis in osteoarthritis may ultimately reveal directions for new therapies. Further, it is pivotal to combine clinical and cellular parameters when trying to resolve the pathophysiology of this heterogeneous disease. To date, however, our knowledge of these inflammatory processes is insufficient and lacks answers to very basic questions, such as: which cell types are responsible for maintaining synovial inflammation in osteoarthritis joints, and does the inflammatory pattern differ between osteoarthritis subtypes? Studies by Sakkas *et al*. suggest that T cells are the predominant cell type in osteoarthritis synovium and especially underline the role of T cells with an activated phenotype, as well as T helper type 1 (Th1) polarized cells. Conversely, the study by Bondeson *et al*. favored the role of synovial macrophages and their main proinflammatory cytokines [interleukin (IL)-1, tumor necrosis factor (TNF)-α] in driving osteoarthritis synovitis. The synovial membrane is the main site of inflammation where cell–cell interaction takes place. Thus, it is of utmost interest to analyze this tissue in osteoarthritis pathology. Clinical data of disease subtypes have received little attention when analyzing osteoarthritis joint samples for cellular and molecular parameters.[4]

A major problem in cartilage repair is the lack of chondrogenic cells migrating from healthy tissue into defects and being essentially avascular.[5]

Microscopic examination in early osteoarthritis revealed for more than half of patients with synovial biopsy through arthroscopic technique having synovitis lesions with mononuclear infiltrates, diffuse fibrosis, thickening of the lining layer, macrophages appearance and vessels neoformation as well.[3]

The over expression of mediators of inflammation and the increased mononuclear cell infiltration were seen in early osteoarthritis, compared with late osteoarthritis.[3]

Currently, the US Food and Drug Administration (FDA) considers slowing of joint space narrowing as an outcome for trials of disease-modifying osteoarthritis drugs, but to date, no drugs are approved for this indication in the US or Europe. Radiographic changes occur well after histological and biochemical changes in joint tissues. Thus, future development of disease-modifying osteoarthritis drugs would be facilitated by validated prognostic biomarkers that identify subsets of patients at risk for progressive disease. Additionally, early diagnosis of osteoarthritis, ideally at a time that allows effective intervention and before radiographic damage has occurred, will require improved diagnostic imaging and biomarkers.[1]

Aim of the Work

The aim of this study is to figure out the role of leukocytes in prediction, diagnoses and prognosis of osteoarthritis. Hypothesizing whether leukocytes may serve as biomarkers for both early diagnosis and, perhaps more importantly, identification of osteoarthritis patients at higher risk for disease progression. So respectively aids in updating the treatment modalities of osteoarthritis.