Correlation between Ankylosing Spondylitis, Osteoarthritis and Metallothionein-1

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ABSTRACT

Background: Ankylosing spondylitis (AS) is a chronic progressive inflammatory arthritis characterized by inflammation of axial skeleton, peripheral joints, entheses and extraarticular systems such as eyes, bowel, lungs and heart. Ankylosing Spondylitis typically begins in early adulthood (20–40 years) and it is rare above the age of 40 years and below the age of 8 years with a prevalence ranging from 0.1% to 0.8% in the adult population. Osteoarthritis (OA) is a common degenerative joint disorder, wear and tear arthritis, in the elderly that often leads to joint pain and dysfunction, affecting people's quality of life. OA is usually featured by cartilage degradation, subchondral bone remolding, and synovium inflammation. The underlying etiology of OA is multifactorial in origin. These factors include family history, age, obesity, diabetes, synovitis, systemic inflammatory mediators, innate immunity, joint shape and dysplasia, trauma, and inflammation by metabolic syndromes. Metallothionein (MT) is a family of cysteine-rich, low molecular weight proteins (<10 Kilodalton) and is divided into four subfamilies in humans and designated as MT-1, MT-2, MT-3, and MT-4. The main function of MTs is to scavenge free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and combine both physiological (such as zinc, copper, and selenium) and xenobiotic (such as cadmium, mercury, silver, and arsenic) heavy metals through their thiol group of cysteine residues (represent nearly 30% of its constituent amino acid residue) to reduce oxidative stress and maintain the stability of intracellular heavy metal concentrations through which it participates in the regulation of metabolism and immunity. Since then, MTs have emerged as multiple effectors involved in immune homeostasis regulation. Accumulating experimental data from studies with MT-deficient human samples has demonstrated the critical immunoregulatory role of MT isoforms in cancer, infectious diseases, central nervous system diseases, autoimmune diseases. Isolated peripheral blood mononuclear cells (PBMCs) and synovial cells from erosive inflammatory osteoarthritis (OA) treated with human recombinant MT-1 significantly reduced the expression of proinflammatory cytokines TNF-α, IL-6, and IL-17 in the cells. Serum levels of TNF-α, IL-6, and IL-17 are significantly upregulated in patients with AS compared with Health controls. Importantly, the results also show that the serum level of MT-1 is positively correlated with the levels of proinflammatory cytokines TNF-α, IL-6, and IL-17 in patients with AS. S.MT-1 shows potential as an effective biological marker for AS diagnosis and activity.

Keywords: Ankylosing spondylitis, Osteoarthritis, Metallothionine-1

1. INTRODUCTION

Ankylosing spondylitis (AS) is a chronic, seronegative spondyloarthropathy, autoimmune, multisystem inflammatory disorder primarily involving the joints of the spine, the sacroiliac joints

(SIJs), and the surrounding tendons and ligaments. Common clinical manifestations of AS include back discomfort, increased spinal stiffness, and inflammation of the hips, shoulders, peripheral arthritis, enthesitis and extra-articular manifestations (EAM) include acute anterior uveitis and inflammatory bowel disease (IBD). [1].

The incidence and prevalence of AS vary widely in population demographics, socioeconomic factors, and certain ethnic populations, such as Northern European countries and seen least in people of Afro-Caribbean descent. It typically begins in early adulthood (20–40 years) and it is rare above the age of 40 years and below the age of 8 years with a prevalence ranging from 0.1% to 0.8% in the adult population. [2].

As an autoimmune disease, AS develops through complex interactions between genetic background and environmental factors. Although significant progress has been achieved in the past decades, the etiology of AS remains unclear to some extent. studies have revealed some factors that may be related to the occurrence of AS, including genetic background, immune reaction, microbial infection, and endocrinal abnormity. Important environmental influences include gut microbial dysbiosis and entheseal stress or trauma. [3].

Previously, genetic markers for prevalent susceptibility genes associated with immune regulation, including immunological synapse and T cell activation, have been studied. It is believed that AS is a hereditary disease, with HLA-B27 being the primary genetic risk factor as Arthritogenic peptide hypothesis**.** [4].

The arthritogenic peptide hypothesis postulates that structurally exclusive peptide-MHC complexes can directly initiate HLA-B27- specific autoimmune responses and can activate the response of certain HLA-B27-specific CD8+ T lymphocytes. The T lymphocytes react with these HLA-B27-peptide complexes, leading to autoreactivity and autoimmune [5]

The free heavy chain hypothesis is based on the observation that HLA-B27 molecules can exist on the cell surface. These free heavy chains exist as stable dimers and are capable of engaging allele-specific receptors on NK cells and T lymphocytes, the free heavy chain hypothesis postulates that engagement of these receptors will generate arthritis-causing events [6]

The HLA-B27 unfolded protein hypothesis postulates that HLA-B27 induces an unfolded protein response, which, in conjunction with activation by pattern recognition receptors (PRRs) such as those for lipopolysaccharide, would generate proinflammatory cytokines to such a degree as to cause arthritis. [7]

HLA-B27 is not the only HLA-B allele associated with AS, also HLA-B60 and HLA-B61 increases AS risk by approximately 1.5 fold. HLA-B40, HLA-A, HLA-DRB1, HLADQA1, HLA-DPB1 interact with TCRs and killer cell immunoglobulin (KIRs) expressed on NK cells and certain lymphocytes and participate in antigen presentation and other inflammatory processes in AS. [8]

Non-MHC Gene **as** Endoplasmic reticulum aminopeptidase ERAP1, ERAP2 and NPEPPS, three genes of the family of metallopeptidases have been shown to be associated with AS. [9]

Diagnosis, Disease Activity and Measurement:

1. Diagnostic Criteria:

The diagnosis of ankylosing spondylitis (AS) is generally made by combining clinical criteria of inflammatory back pain and enthesitis or arthritis with radiologic findings. Modified New York Criteria which was introduced in 1984. [Table 1]. To be classified as definite AS, the fourth or fifth criterion mentioned presents with any clinical criteria. [10].

Table (1): Modified New York Criteria. [10].

New York Criteria
•Low back pain with inflammatory characteristics
•Limitation of lumbar spine motion in sagittal and frontal planes
•Decreased chest expansion
•Bilateral sacroiliitis grade 2 or higher
•Unilateral sacroiliitis grade 3 or higher
Definite ankylosing spondylitis when the fourth or fifth criterion mentioned presents with any clinical criteria.

A novel AS classification criteria jointly supported by the Assessment of SpondyloArthritis International Society (ASAS) was introduced in 2009 [Table 2], based on presence of low back pain for 3 months or more and sacroiliitis on imaging plus one or more of SpA features or HLA B-27 positive plus 2 or more SpA featuers with sensitivity of 82.9% and Specificity 84.4% [11].

Table(2): The 2009 ASAS classification criteria for AS. [11].

<table>
<tr><td colspan="2">In patients >= 3 months of low back pain and age of onset <45 years</td></tr>
<tr><td>Sacroiliitis on imaging
And
>= 1 SpA features</td><td>HLA B-27
And
>= 2 other SpA features</td></tr>
<tr><td>SpA features:-
Inflammatory back pain
Arthritis
Enthesitis
Uveitis
Dactylitis
Psoriasis
Crohn’s/ colitis
Good response to NSAIDs
Family history of SpA
HLA B-27
Elevated CRP</td><td>Sacroiliitis on imaging:-
-Active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA
-Definite radiographic sacroiliitis according to Modified New York criteria</td></tr>
</table>

2. Disease Activity:

1) The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI):

The Bath Ankylosing Spondylitis Disease Activity Index developed in Bath (UK), is an index designed to detect the inflammatory burden of active disease. The BASDAI can help to establish a diagnosis of AS in the presence of other factors such as HLA-B27 positivity, persistent buttock pain which resolves with exercise, and X-ray or MRI-evident involvement of the sacroiliac joints. It can be easily calculated and accurately assesses the need for additional therapy; a person with AS with a score of four out of a possible 10 points while on adequate NSAID therapy is usually considered a good candidate for biologic therapy as in (Figure 1). BASDAI Score interpretation.ranges from 0 (no disease activity) to 10 (maximal disease activity). A cut off of 4 is used to define active disease. The scores for questions 5 and 6 (severity and duration of morning stiffness) are averaged, the result is then averaged with the remaining 4 question scores to give a final score out of 10.

Questions
(1) VAS overall level of fatigue/tiredness past week (2) VAS overall level of AS neck, back, or hip pain past week (3) VAS overall level of pain/swelling in joints other then neck, back, or hips past week (4) VAS overall discomfort from any areas tender to touch or pressure past week (5) VAS overall level of morning stiffness from time of awakening past week (6) Duration of morning stiffness from time of awakening (up to 120 minutes)
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; VAS, visual analogue scale.

< 1.4 – < 2 Remission, < 2.8 – < 4 Low disease activity, > 5.9 High disease activity. [12].

Figure (1) BASDAI Qurstionnaire. [12].

2) The Ankylosing Spondylitis Disease Activity Score (ASDAS):

The Ankylosing Spondylitis Disease Activity Score is an index to assess disease activity in AS. the preferred score uses CRP, rather than ESR.

-ASDAS-CRP =0.12 x Back Pain + 0.06 x Duration of Morning Stiffness + 0.11 x Patient Global + 0.07 x Peripheral Pain/Swelling + 0.58 x Ln (CRP+1)

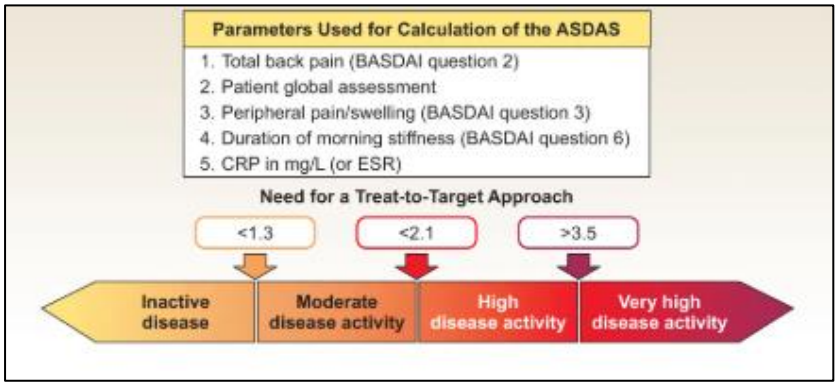
-ASDAS-ESR = 0.08 x Back Pain + 0.07 x Duration of Morning Stiffness + 0.11 x Patient Global + 0.09 x Peripheral Pain/Swelling + 0.29 x √ (ESR)

-Back pain, patient global assessment, duration of morning stiffness and peripheral pain/swelling are all assessed on a numerical rating scale (from 0 to 10).

-The 3 cut-offs selected to separate these states were:

- Low disease activity is defined as $1.3 \leq ASDAS \leq 2.1$
- Moderate as $<2.1< ASDAS \leq 3.5$
- High as ASDAS >3.5

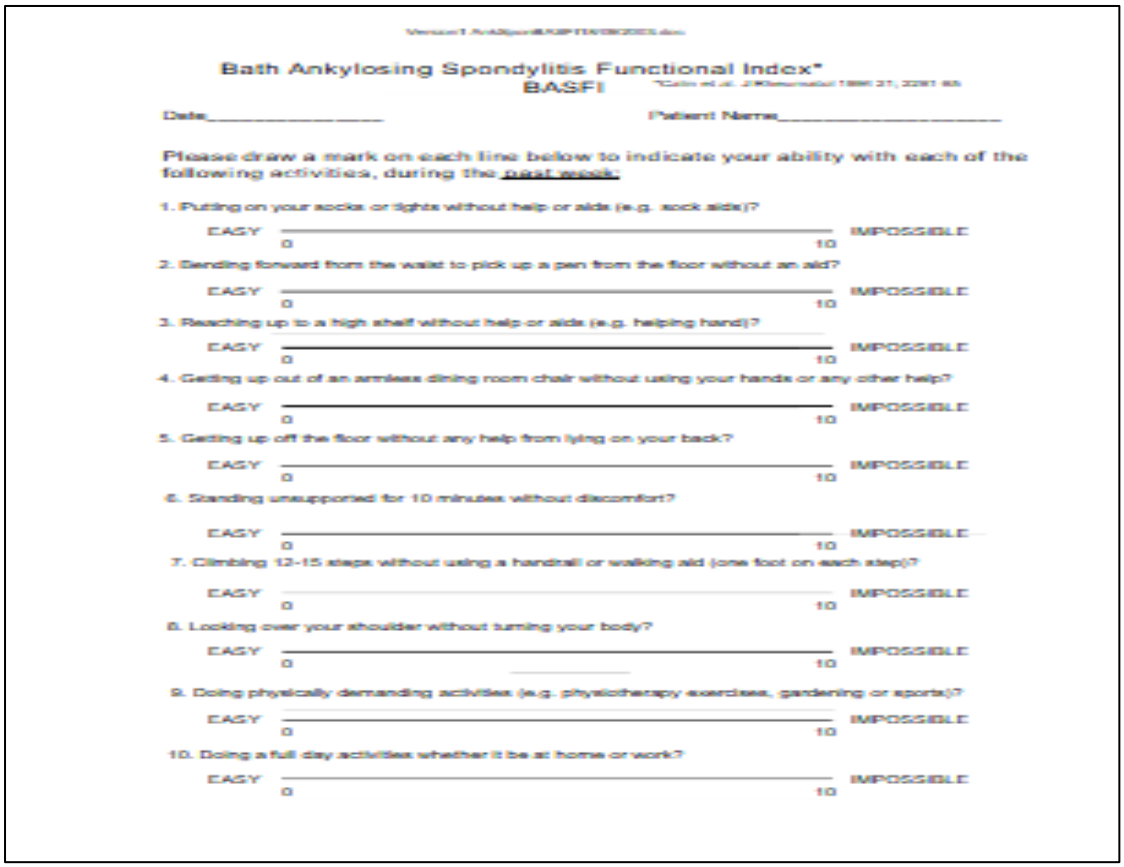
-Cut-offs for improvement scores were: change ≥1.1 units for "clinically important improvement" and change ≥2.0 units for "major improvement". [13].

Figure (2): 

ASDAS-CRP Questionnaire and interpretation. [13].

3) The Bath Ankylosing Spondylitis Functional Index (BASFI):

The Bath Ankylosing Spondylitis Functional Index (figure 3) is a functional index which can accurately assess functional impairment due to the disease, as well as improvements following therapy. The BASFI is not usually used as a diagnostic tool, but rather as a tool to establish a current baseline and subsequent response to therapy. [14].



Bath Ankylosing Spondylitis Functional Index*
BASFI

Date_____ Patient Name_____

Please draw a mark on each line below to indicate your ability with each of the following activities, during the past week:

1. Putting on your socks or tights without help or aids (e.g. sock aids)?
EASY 0 — 10 IMPOSSIBLE
2. Bending forward from the waist to pick up a pen from the floor without an aid?
EASY 0 — 10 IMPOSSIBLE
3. Reaching up to a high shelf without help or aids (e.g. helping hand)?
EASY 0 — 10 IMPOSSIBLE
4. Getting up out of an armless dining room chair without using your hands or any other help?
EASY 0 — 10 IMPOSSIBLE
5. Getting up off the floor without any help from lying on your back?
EASY 0 — 10 IMPOSSIBLE
6. Standing unsupported for 10 minutes without discomfort?
EASY 0 — 10 IMPOSSIBLE
7. Climbing 12-15 steps without using a handrail or walking aid (one foot on each step)?
EASY 0 — 10 IMPOSSIBLE
8. Looking over your shoulder without turning your body?
EASY 0 — 10 IMPOSSIBLE
9. Doing physically demanding activities (e.g. physiotherapy exercises, gardening or sports)?
EASY 0 — 10 IMPOSSIBLE
10. Doing a full day activities whether it be at home or work?
EASY 0 — 10 IMPOSSIBLE

Figure (3): BASFI Questionnaire.

Osteoarthritis (OA)

Osteoarthritis (OA) is a common degenerative joint disease affecting the elderly, causing joint pain and deformity, and may even lead to disability in severe cases. Usually, the symptoms progress slowly over years. Other symptoms may include joint swelling and decreased range of motion.[15].

Osteoarthritis (OA) is a prevalent joint disease and one of the most common symptomatic health conditions with a prevalence ranging from 12.3 % to 21.6 %. It is included among the most common joint diseases in the world and a major cause of disability in the aging population. The disease also affects young athletes, many middle-aged people and particularly in older people, it can cause severe pain and physical disability. Knee osteoarthritis (KOA) is a prevalent disease, affecting an estimated 32.5 million adults in the US, with 14% of the American population experiencing symptomatic KOA.[16].

Damage from mechanical stress with inadequate self-repair by joints is believed to be the primary cause of osteoarthritis. Sources of this stress may include misalignment of bones congenitally or due to pathogenic causes; mechanical injury; excess body weight; loss of strength in the muscles supporting a joint; and impairment of peripheral nerves, leading to sudden and uncoordinated movements.[17].

And of secondary causes of OA: Congenital disorders as in hip or dysplasias, Trauma, Metabolic diseases and Endocrine disorders.[18].

Among inflammatory mediators, the role of cytokines has been studied the most, and many cytokines have been found in OA joints in correlation with the severity of inflammation, and these play various roles in disrupting the balance of catabolic and anabolic activity in joint tissues. IL-1β, IL-6, and TNF-α cytokines play the most important roles in pathogenesis and disease severity of OA, while IL-15, IL-17, IL-18, IL-21, and other chemokines have also been implicated. IL-1β is produced by several cell types in joints, including chondrocytes, immune cells infiltrating the synovium, osteoblasts, adipocytes, and synoviocytes; its expression is elevated in OA synovial fluid and membranes. IL-1β strongly induces the expression and release of proteolytic enzymes, such as matrix metalloproteinases (MMPs) and aggrecanases, and suppresses the expression of ECM components, including type II collagen and aggrecan. It also acts synergistically with other cytokines, IL-6 and chemokines including IL-8, MCP-1, and CCL5, to further increase inflammation.[19].

Diagnosis, Disease Activity and Measurement:

1. Diagnostic Criteria:

Diagnosis is made with reasonable certainty based on history, clinical examination and radiological modalities. The most commonly applied criteria for knee OA are those described by the European League Against Rheumatism (EULAR) based on hard tissue enlargement and swelling of certain joints. These criteria were found to be of high sensitivity and specificity for osteoarthritis, the American

College of Rheumatology (ACR), and the National Institute for Health and Care Excellence (NICE). [20].

EULAR criteria

Proposition number five of the EULAR criteria was used. According to these criteria, patients older than 40 years of age with movement-related joint pain, morning knee stiffness of less than 30 min, and functional limitations have knee OA if they in addition have one or more of these examination findings: Crepitus, restricted range of motion, and bony enlargement. [21].

ACR criteria

According to the decision tree, patients with knee pain have OA if they fulfill one of the following groups of criteria:

1) Crepitus, morning knee stiffness of 30 min or less, and age of 38 years or above

2) Crepitus, morning stiffness of longer than 30 min, and bony enlargement

3) No crepitus, but bony enlargement

knee pain was defined as movement-related knee pain. [22].

NICE criteria

According to the criteria from NICE, patients can be diagnosed with knee OA if they are 45 years or older, have movement-related joint pain and either no morning knee stiffness or stiffness of 30 min or less.[23].

2. Assessment of OA:

A number of classification systems are used for gradation of osteoarthritis:

- **Visual Analogue Scale (VAS "0-10cm")**

It is used for assessment of the knee pain. VAS is a horizontal line, 100 mm (10 cm) in length, anchored by word descriptors at each end .The patient marks on the line the point that he/she feels to represent his/her perception of current state. The VAS score is determined by measuring in millimeters from the left hand end of the line to the point that the patient marks as in (Figure 4). [24].

Categorization of Pain:

- 0 means: No pain
- 1-4 means: Mild pain.
- 5-6 means: Moderate pain.
- 10 means: Severe pain.

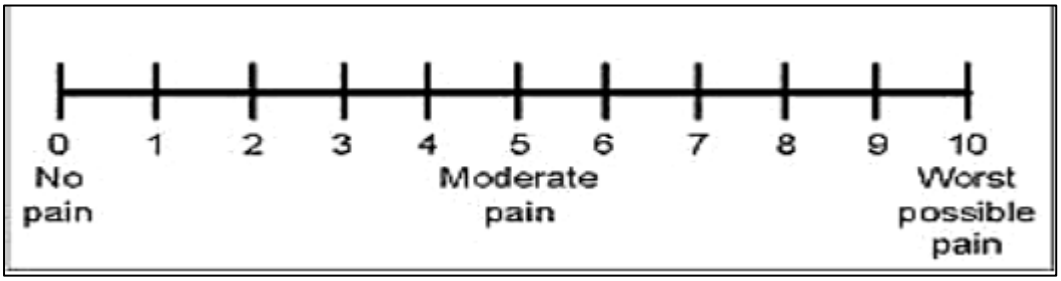
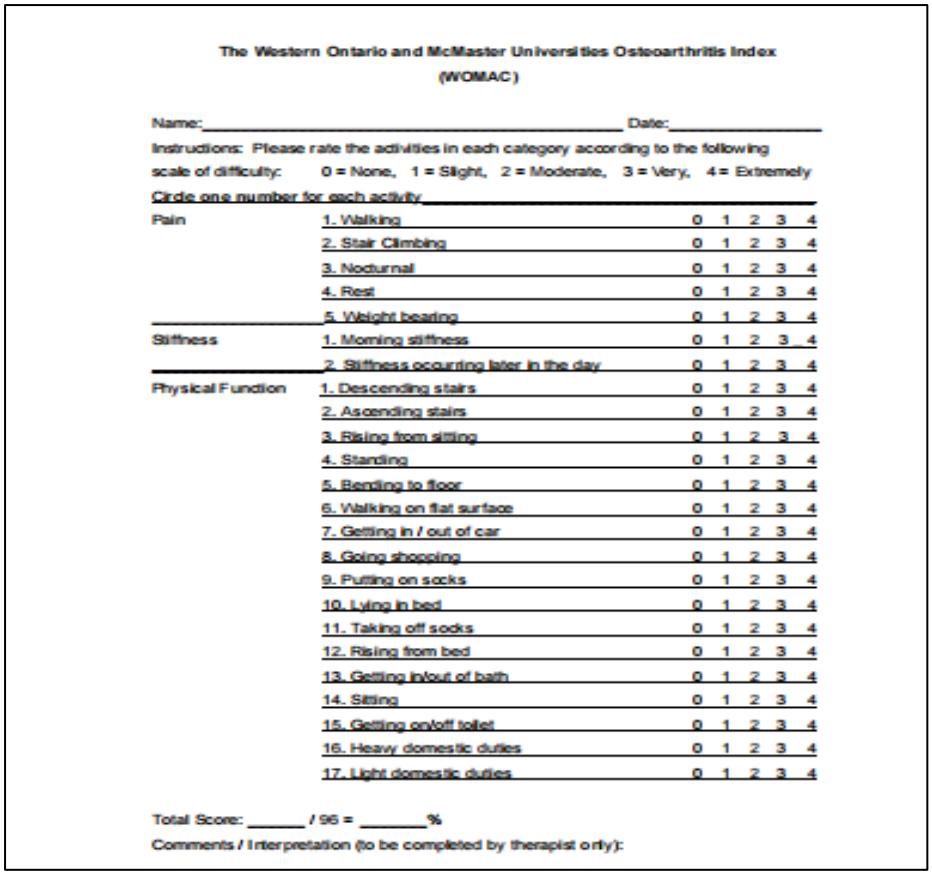


Figure (4): Visual analogue scale (VAS) for pain scoring.

- The Western Ontario and



The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)

Name:_____ Date:_____

Instructions: Please rate the activities in each category according to the following scale of difficulty: 0 = None, 1 = Slight, 2 = Moderate, 3 = Very, 4 = Extremely

Circle one number for each activity

Pain	1. Walking	0 1 2 3 4
	2. Stair Climbing	0 1 2 3 4
	3. Nocturnal	0 1 2 3 4
	4. Rest	0 1 2 3 4
	5. Weight bearing	0 1 2 3 4
Stiffness	1. Morning stiffness	0 1 2 3 4
	2. Stiffness occurring later in the day	0 1 2 3 4
Physical Function	1. Descending stairs	0 1 2 3 4
	2. Ascending stairs	0 1 2 3 4
	3. Rising from sitting	0 1 2 3 4
	4. Standing	0 1 2 3 4
	5. Bending to floor	0 1 2 3 4
	6. Walking on flat surface	0 1 2 3 4
	7. Getting in / out of car	0 1 2 3 4
	8. Going shopping	0 1 2 3 4
	9. Putting on socks	0 1 2 3 4
	10. Lying in bed	0 1 2 3 4
	11. Taking off socks	0 1 2 3 4
	12. Rising from bed	0 1 2 3 4
	13. Getting in/out of bath	0 1 2 3 4
	14. Sitting	0 1 2 3 4
	15. Getting on/off toilet	0 1 2 3 4
	16. Heavy domestic duties	0 1 2 3 4
	17. Light domestic duties	0 1 2 3 4

Total Score: _____ / 96 = _____%

Comments / Interpretation (to be completed by therapist only):

McMaster Universities Arthritis Index (WOMAC) scaleis widely used in the evaluation of Hip and Knee Osteoarthritis. It is a self-administered questionnaire consisting of 24 items divided into 3 subscales (Pain, Stiffness and Physical Function). [25].

Figure (5): WOMAC Index

- Kellgren-Lawrence grading scale for the presence and severity of knee or hip radiographic osteoarthritis (ROA) is commonly graded using the Kellgren/Lawrence (K/L) method. This semiquantitative approach primarily evaluates osteophytes and joint space narrowing to assign a score between 0 (no ROA) to 4 (severe ROA). ROA is typically defined as K/L grade ≥2. [26].

- Grade (0): No radiographic features of OA are present.
- Grade (1): Doubtful joint space narrowing (JSN) and possible osteophyte lipping.
- Grade (2): The presence of definite osteophytes and possible JSN on AP weight-bearing radiograph.
- Grade (3): Multiple osteophytes, definite JSN, sclerosis and possible bony deformity.
- Grade (4): Large osteophytes, marked JSN, severe sclerosis and definitely bony deformity.

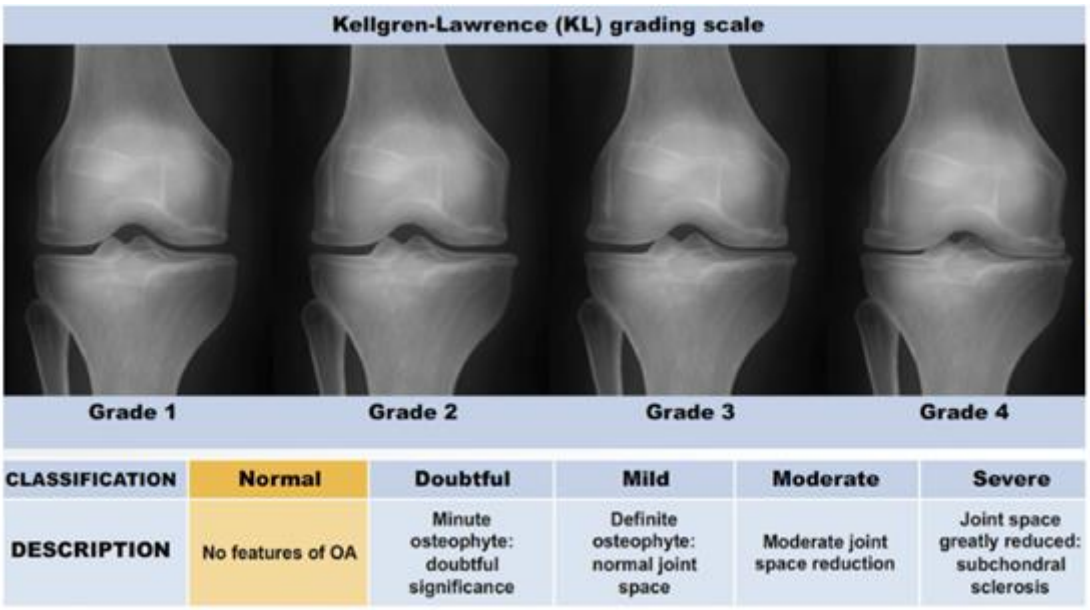


Figure (6): The Kellgren and Lawrence grading system to assess the severity of knee OA. [27].

- Tönnis classification for osteoarthritis of the hip joint, also using only projectional radiography features.

1. Grade 0: absent osteoarthritis.
2. Grade 1: slight narrowing of the joint space, slight lipping at the joint margin, and slight sclerosis of the femoral head or acetabulum; Grade 2: presence of small bony cysts, further narrowing of the joint space, and moderate loss of femoral head sphericity
3. Grade 3: the most severe and indicates large cysts, severe narrowing of the joint space, severe femoral head deformity, and avascular necrosis. [28].

- Knee injury and Osteoarthritis Outcome Score (KOOS) and Hip disability and Osteoarthritis Outcome Score (HOOS) surveys.

It was developed as an instrument to assess the patient's opinion about their knee and associated problems. KOOS is intended to be used for knee injury that can result in post-traumatic OA as anterior cruciate ligament (ACL) injury, meniscus injury, chondral injury, etc. KOOS is meant to be used over short and long time intervals; to assess changes from week to week induced by treatment or over years due to the primary injury or post-traumatic OA.

- 5 Subscales of KOOS:

- Pain
- Activities of daily living (ADL)
- Function in sport
- Function in recreation

- Knee-related quality Of life (QOL).

How to Calculate the Index Score:

The last week is taken into consideration when answering the questions. Standardized answer options are given (5 Likert boxes) and each question gets a score from (0 to 4). A normalized score (100) indicating no symptoms and (0) indicating extreme symptoms. The result can be plotted as an outcome profile. KOOS has high test-retest reproducibility. KOOS includes WOMAC osteoarthritis index. [29].

• The ZAGAZIG ultrasonographic score is a validated tool for ultrasonographic assessment of KOA which includes five domains: (*a) KOA severity (Grade (G) 0–4),* which depends on the shape of distal femoral osteophytes. (*b) Effusion (G 0–3)* in the form of abnormal anechoic or hypoechoic intra-articular material that is compressible and does not exhibit a Doppler signal. (*c) Synovitis (G 0–3)* in the form of abnormal hypoechoic or hyperechoic intra-articular tissue that is poorly compressible and may exhibit a Doppler signal. (*d) Pes anserine tendonitis/bursitis (G 0–2)*. (*e) Baker's cyst (G 0–2)* in the form of a thin hypoechoic space delimited by echoic borders corresponding to the tissue-fluid interface anatomically present between the medial head of the gastrocnemius and the semimembranosus muscles. [30].

3 Disease activity:

Algo-functional Indices for the Hip and Knee:- Lequesne developed an index of severity for knee OA (ISK). [31].

- **Sections of the Index:**

- Pain or discomfort.
- Maximum distance walked.
- Activities of daily living (ADL).

- **Interpretation:**

- Minimum points for each section: (0).
- Maximum points for each section: (8).
- Minimum index score: (0).

Maximum index score of the 3 score sections =8×3= (24).

Metallothionein=1 (MT-1)

MTs constitute a superfamily which are intracellular, have low molecular weight (<7000 Dalton), of nonenzymatic polypeptides of 61–68 amino acids, characterized by high cysteine content (18–23 cysteine residues; 30%) , lack of aromatic amino acids, and few or no histidine residues but with abundant thiol groups to bind to heavy metals. [32].

The main function of MTs is to scavenge free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and combine both physiological (such as zinc, copper, and selenium) and xenobiotic (such as cadmium, mercury, silver, and arsenic) heavy metals through their thiol group of cysteine residues (represent nearly 30% of its constituent amino acid residue) to reduce oxidative stress and maintain the stability of intracellular heavy metal concentrations through which it participates in the regulation of metabolism and immunity. [33].

Since then, MTs have emerged as multiple effectors involved in immune homeostasis regulation. Accumulating experimental data from studies with MT-deficient human samples has demonstrated the critical immunoregulatory role of MT isoforms in cancer, infectious diseases, central nervous system diseases, autoimmune diseases, and inflammatory bowel diseases. Notably, MT1 is widely expressed in almost every organ to maintain homeostasis. Therefore, it has been most extensively investigated in recent decades.[34].

Under physiological conditions, MT1 is involved in regulating the steady-state of metal, alleviating heavy metal poisoning, and protecting the body against oxidative stress, inflammation, and other cell damage caused by stress reaction. Elucidating the immunoregulatory functions and mechanisms of MT1 in inflammation diseases will provide a potent immunotherapy target for these diseases.[35].

Antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, serve as links between activation of innate and adaptive immunity and play a crucial role in governing T-cell immunity. APC functional dysregulation has the potential to initiate diverse immune diseases. Previous research has found that MT1 has some immunosuppressive properties towards DCs. IL-10-expressing DCs showed high levels of MT1, which is essential for inducing the T regulatory cell phenotype and promoting the proliferation of $Foxp3^+$ T cells. Increased MT1 expression in IL-10-expressing DCs is dependent on dexamethasone or $ZnCl_2$ stimulation, whereas upregulated MT1 expression on the DC membrane is strongly dependent on $ZnCl_2$ but not dexamethasone stimulation.[36].

It has been suggested that histone deacetylase Sirt1 is involved in the degradation of FoxP3 in Tregs. However, the presence of Zn can turn around the Sirt1-mediated degradation of FoxP3 in Treg. Thus, at least one possible mechanism is that MT1 on the surface of DCs donates Zn to Tregs, which in turn inhibits Sirt1-induced degradation of FoxP3 and maintains the phenotype of Tregs. Since FoxP3+ Tregs exhibit tolerance functions that play a crucial role in controlling inflammatory responses.[37].

In addition to their role as an APC, macrophages perform a variety of other functions in the fight against microbial invasions. Lipopolysaccharide (LPS) stimulates macrophages to produce large amounts of proinflammatory cytokines such as TNF-α, IL-β, and IL-6, responsible for inflammatory damage and infection defense. MT1 deficient macrophages dampen the expression of LPS-induced inflammatory cytokines, including TNF-α, IL-β, and IL-6. The enhanced effects of MTs on LPS-induced inflammatory responses in macrophages are directly due to the regulation of nuclear factor

kappa B (NF-κB) activity. A recent report indicated that MT1 and MT2 were substantially elevated in granulocyte-macrophage colony-stimulating factor (GM-CSF)-activated macrophages in a STAT3- and STAT5-dependent manner during *H. capsulatum* infection. GM-CSF also alters Zn redistribution in *H. capsulatum*-infected macrophages by regulating transporters. Thus, in the absence of MT1 and MT2, a lack of Zn sequestration in macrophages would reduce the ability of GM-CSF to inhibit *H. capsulatum* growth. Overall, these findings appear to support the importance of MT1 and MT2 acting on macrophages in defense against infection and inflammation *via* involving multiple signaling pathways. [38].

Acute-phase inflammatory responses can induce MT1 expression. It has been demonstrated that bacterial endotoxin-lipopolysaccharide (LPS) has an acute inductive effect on MT1 expression in a range of tissues, including the liver, heart, kidney, and brain. However, MT1 induction by LPS is rapid but not sustained and alters zinc metabolism, indicating that MT1 is a vital component of acute-phase inflammation.[39].

MT-1 and RA:-

Rheumatoid arthritis (RA), one of the most common autoimmune diseases, is characterized by chronic inflammation of joints and surrounding tissues. Although the cause of RA is still unclear, it is believed to involve a disruption of immune homeostasis. As a stress response protein to sequester toxicants, MT has been shown to have functional effects on immune cells, indicating that MT1 may play a role in regulating autoimmune diseases such as RA**.** Previous studies found that MT1 expression is significantly upregulated in rheumatoid arthritis and is closely related to RA disease activity. Furthermore, the synovial inflammation and pathological symptoms in rheumatic patients were dramatically suppressed when MT1 was locally administrated.[40].

Further investigation revealed that MT1 inhibits RA pathogenesis by shifting the differentiation of $CD4^+$ T cells toward Treg cells and reducing the frequency of Th17 cells. MT1 modulated the balance of Th17/Treg cell immune homeostasis in RA pathogenesis, most likely dependent on the activation of the STAT3 signaling pathway. In addition, MT1 may also play a critical role in regulating Th1 immunity in autoimmune arthritis. According to these findings, MT1 may prove to be a potential therapeutic target for autoimmune diseases. [41].

MT-1 and Gout:-

In a previous study showing: Compared with healthy controls, patients with active gout showed higher levels of MT-1 mRNA in peripheral blood mononuclear cells and protein in serum, particularly those with tophi. No significant difference in serum MT-1 levels was observed among patients with inactive gout, HCs, and patients with hyperuricemia without gout. Furthermore, no significant difference was observed between patients with gout with kidney damage and HCs. In addition, serum interleukin (IL)-1β, IL-6, and IL-8 levels were significantly increased in patients with active gout, particularly in those

with tophi. The serum MT-1 level was positively correlated with CRP, as well as with IL-1β, IL-6, and IL-18. Concluded that the higher levels of MT-1 were found in patients with gout, which were correlated with disease activity and gout related pro-inflammatory cytokines. Indicating MT-1 may serve as a new marker for predicting disease activity.[42].

MT-1 and Multiple sclerosis:-

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) with multifactorial pathogenesis. MT is thought to play an important role in the pathogenesis and progression of MS. Clinical data revealed that MT1 and MT2 expression levels were remarkably increased in the brain lesions of MS patients, and these two MT isoforms appear to be primarily present in macrophages/microglia and reactive astrocytes. Interestingly, inactive MS lesions had slightly higher MT expression than active MS lesions, indicating that MT may be involved in MS disease remission.[43].

Inflammatory cytokines such as IL-6 and TFN-α and oxidative stress found in MS lesions were most likely responsible for MT induction. These results are consistent with previous research on MT1 and MT2 expression during experimental autoimmune encephalomyelitis (EAE), a relevant preclinical phase of MS. Therefore, the expression profile of MT1 might be a useful predictor of clinical signs of MS. [44].

Accumulating research has stressed the beneficial effects of MT1 in EAE, preventing demyelination, enhancing neuroprotective capacities, and restraining inflammatory responses**.** In EAE, a case with MT1/2 deficiency showed increased macrophage and T-cell infiltration in the central nervous system (CNS) and elevated expression of proinflammatory cytokines IL-1β, IL-6, and TNF-α. These proinflammatory cytokines can further activate macrophages and lymphocytes to increase inflammation in the CNS. [45].

The loss of MT1 and MT2 also increases oxidative stress, which is a vital mediator of apoptotic cell death and myelin damage in EAE. Confirming these results, treatment with MT protein can ameliorate pathological progression in EAE. Overexpression of MT1 significantly reduces tissue loss and vascular edema while improving focal cerebral ischemia and reperfusion.[46].

MT-1 and Parkinson's disease:-

Parkinson's disease (PD) is one of the most common inflammatory neurodegenerative diseases. It results from the progressive degeneration of dopamine neurons that innervate the striatum.[47]. MTs are metal-binding proteins in the CNS that are released by astrocytes and associated with neuroprotection. There is a substantial increase in the expression of MT1 isoforms, MT1E, MT1F, MT1G, MT1H, MT1M, and MT1X in both PD nigra and frontal cortex. Astrocytes play a neuroprotective role by upregulating the expression of MT1, which indicates the importance of MT1 in the development of PD. [48].

The protective effect of MT1 in PD was recently identified through an artificial transducing experiment, in which human MT1A was transduced into mitochondria by a cell-penetrating artificial mitochondria-targeting peptide (CAMP). Treating a cell culture model of PD with CAMP-hMT1A restored tyrosine hydroxylase expression and mitochondrial activity and reduced ROS production. Therefore, delivery of MT1 into mitochondria might be therapeutic against PD by alleviating mitochondrial damage. [49].

MT-1 and Inflammatory bowel disease:-

The expression of MT1 was significantly higher in the inflamed colitis tissue from DSS-induced colitis cases. In the DSS-induced colitis cases, MT1 deficiency leads to disease exacerbation. It promotes the development of excessive intestinal inflammation by upregulating inflammatory cytokines, including TNF-α, IFN-γ, and IL-17. MT1 secretion is most likely derived from F4/80-positive macrophages in the intestinal mucosal, ensuring its anti-intestinal inflammation properties. Although MT1 signaling failed to influence the number of F4/80-positive cells, the proinflammatory function of these cells was effectively restrained following LPS stimulation. These findings indicate that MT1 plays a protective role against intestinal inflammation**.** MT1-expressing macrophages might be a therapeutic candidate in IBD. [50].

-MT-1 and Atopic Dermatitis:-

In response to topical contact of dinitrofluorobenzene (DNFB) stimulation, increased expression of MT1 was identified in the nucleus. Because nuclear MT1 localization improves protection against oxidative stress and genomic damage, the function of MT1 in the nucleus may be required to protect AD development.[51].

They found that MT1/2-deficient cases had more severe AD in comparison to normal ones. Moreover, MT1/2 deficiency had more $CD4^+$ T cells and decreased typical Th2-dominated inflammation. MT1/2-deficient AD-like cases also showed increased expression of superoxide dismutase (SOD) and NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 1 (NDUFAF1). Because both SOD and NDUFAF1 are required in cells to defend against reactive oxygen species (ROS), the protective role of MT1/2 in AD seems to rely on an antioxidant mechanism mediated by SOD and NDUFAF1. Despite the potential mechanisms of MT1 in AD being not yet clear, these findings indicate that MT1/2 plays a protective role against AD development. [52].

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