INTRODUCTION

Osteoarthritis (OA) is the most common joint disease and cause of physical disability.[1] It is one of the most frequent causes of pain and loss of function in adults second to ischemic heart disease.[2] It is a joint disease resulting in cartilage attrition, subchondral bone remodeling, osteophyte formation, and synovial inflammation.[3-5] It was classified as degenerative arthritis, however, joint inflammation was found in some patients with OA.

Many reports have demonstrated that some cytokines, in particular TNF-alpha and Interleukin-1 beta (IL-1β), Interleukin-6 and Interleukin-8, so called proinflammatory, may have a major role in the pathogenesis of joint diseases.[6]

Proinflammatory cytokines are important mediators of acute and chronic inflammation, being associated with joint destruction in OA and Rheumatoid arthritis (RA).[7-9] These proinflammatory cytokines can cause strong stimulatory effects on bone resorption and inhibitory effects on bone formation and its elevated levels have been correlated with diseases that reflect inflammatory states.[10,11]

Interleukin-I (IL-1) is one of the most potent cytokines that cause deterioration of the functional properties of articular cartilage. IL-1 stimulates the synthesis of metalloproteinases (aggrecanase, collagenases, gelatinases, and stromelysins) and plasminogen activators in cartilage and decreases the synthesis of extracellular matrix macromolecules.[12-19] It was implicated that IL-1 has a role in the degeneration of cartilage due to its induction of proteoglycan loss and matrix degradation.[20] Elevated levels of IL-1 occur in the synovial fluid and cartilage tissue of patients with OA compared to healthy individuals,[21] implying a role in disease pathogenesis.

Interleukin-1 beta is localized to chondrocytes in the superficial zone of human OA cartilage, where degenerative changes have been identified on histological examination. Genomic studies have also provided some support for the involvement of IL-1b in OA.[22] Thus, IL-1b may be a fairly attractive therapeutic target in OA.[22] Clinical trials have also generated optimism for anti-IL-1b therapy as a new stratagem in OA treatment.[22]

Diacerein (4,5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid), a semi-synthetic anthraquinone derivative extracted from certain plants, has IL-1b inhibitory properties in vitro,[23,24] It has shown promise in OA treatment and has been in clinical use in Europe for several years. It directly inhibits IL-1 synthesis and release in vitro and down modulate IL-1 induced activities and have been shown to possess disease modifying effect in human subjects with finger joint and knee OA.[25]

Further study is needed to establish the short and long-term safety and effectiveness of diacerein in OA.[26]

In addition, no previous study was done in Egypt on the efficacy of diacerein in OA.

REVIEW OF LITERATURE

Osteoarthritis is a slowly progressive, degenerative and disabling joint disease that is characterized by focal degradation of articular cartilage with secondary components of synovial membrane inflammation.[27,28] In addition to the degeneration of articular cartilage, attempts at repair are found in the affected tissue. Cartilage cells (chondrocytes) play a key role, not only in the destructive process, but also in the repair response in the form of sclerosis of the subchondral bone and the formation of the bony outgrowths at the cartilage margins (osteophytes).

It has become apparent that anabolic and catabolic mediators, released from chondrocytes themselves or from other joint cells, drive both destructive and repair activities in the osteoarthritic joint. The cartilage degeneration is probably caused by enhanced activity of proteases, in combination with an imbalance with their natural inhibitors. As a response to the damaged extracellular matrix; chondrocytes that are normally nondividing proliferate, forming multicellular chondrocyte clones, and show enhanced production of collagen and proteoglycans during the early stages of the disease[29]. Pathophysiologic changes occur in OA cartilage due to the excessive expression of cartilage degrading proteinases, with resultant progressive breakdown of collagen fibers, and the degradation of proteoglycan, mainly aggrecan. Matrix metalloproteinases (MMPs) are considered to be important in the processes that contribute to the degenerative changes in OA cartilage.

Epidemiology

OA is one of the most prevalent conditions in the elderly population and one of the leading causes of disability. OA is the most common articular disease of the developed world and a leading cause of chronic disability mostly as a consequence of the knee OA and/or hip OA.[30]

Knee OA is more important not only for its high prevalence rate compared with other types of OA but also for its presentation at earlier age groups particularly in younger age groups of obese women. The incidence of knee OA increases by age and further increase with longer lifetime and higher average weight of the population[31]. The disease is painful and disabling and affects millions of patients.[32]

The prevalence rates of knee OA vary according to study population as well as the methods applied for diagnosis (data provided by plain radiographs, physical performance assessment, interviews and magnetic resonance imaging assessment).[30]

The prevalence rate was significantly higher among women than in men and increased significantly with age.

Symptomatic knee OA was significantly more common in rural compared with urban populations.

About 13% of women and 10% of men aged 60 years and older have symptomatic knee OA. The proportions of people affected with symptomatic knee OA is likely to increase due to the aging of the population and the rate of obesity or overweight in the

general population.[33] During a one year period, 25% of people over 55 years may demonstrate persistent episode of knee pain, in whom about one in six have to consult their general practitioner about it in the same time period. About10% of people aged over 55 years have painful disabling knee OA of whom one quarter are severely disabled[34]. Prevalence of knee OA in men is lower compared with women. This was shown in a meta analysis of males and females in which the incidence of knee OA in males aged <55 years was lower than females.

Females, particularly those ≥55 years, tended to have more severe OA in the knee but not in other sites. The results of this study demonstrated sex differences incidence of knee OA particularly after menopausal age.[35] In a prospective study in which data were provided by radiographs, physical performance assessment, and interviews in 1996 and again (with the addition of magnetic resonance imaging assessment) in the follow-up visit during the years 2007, 2008. The prevalence of moderate-to-severe knee OA changed from 3.7% at the baseline assessment to 26.7% in the follow-up visit eleven years later. Middle-aged women had a high prevalence of moderate-to-severe knee OA.[36]

The knee joint shows relatively high incidence and prevalence of OA. This is to be expected because the knee joint is the largest articulation in the body and is one of the most complex joints in the human body consisting of not only single compartment but three compartments.[37,38] The patellofemoral compartment exhibits the highest prevalence of OA in the knee joint complex both in males and females. OA starts in the femora and proceeds to the tibia based on the mildness of OA observed on the tibia. The higher rate of patellofemoral compartment OA is indicative of less axial loading pressure and more overuse of the patella going over the femora during extension. Other researchers who investigated archaeological data found high levels of knee OA in individuals who participated in farming activities. This is explained by biomechanical stress on a daily basis.[39,40] They have found also that age is a significant etiological factor for males and females and that either sex is about equally susceptible to knee OA. Earlier female development of OA may be attributed to the endocrine system.[41]

The Prevalence of OA within the knee joint complex:

Of the three compartments, the most commonly affected during OA is the patellofemoral compartment.[42] Interestingly, some researchers support the idea that patellofemoral OA is the result of bipedal evolution.[39] Their findings indicate while modern and ancient humans all have high rates of patellofemoral OA, that quadrapedal non-human primates actually exhibit quite low levels of patellofemoral OA.[39] These evolutionary perspectives are interesting and biomechanically make some sense. The patella is a giant sesamoid bone, and it has a wide range of movement which is the most probable cause for such high prevalence of OA in bipedal humans.

The next most common compartment affected by osteoarthritic changes is the medial tibiofemoral compartment[37] which may be the result of specific mechanical loading of the knee produced by the morphology of the valgus angle (7°) between the femur and the tibia observed in the human leg. In congruence with the high frequency of osteoarthritic changes seen in the medial tibiofemoral compartment, there seems to be a very low frequency of this development in the lateral tibiofemoral comparetment.[39] Based on the biomechanics of the valgus angle, the sites with the highest frequency of OA do correspond to locations

of maximum biomechanical stress in the knee, supporting the role of this type of mechanical stress as a factor in OA.[37] Patellofemoral OA is three times as common as tibiofemoral OA.[42]

Etiology and risk factors of knee OA:

OA has a multifactorial etiologies, which occurs due to interplay between systemic and local factors.[30] OA affects all ages. In this debilitating disease several responsible genes are linked for its occurrence. Prolonged participation in high demanding sports, injury to the joint, obesity, and genetic susceptibility predispose adolescent athletes to the development of premature OA. Previous knee trauma increases the risk of knee OA 3.86 times.[43] Old age, female gender, overweight and obesity, knee injury, repetitive use of joints, bone density, muscle weakness, and joint laxity all play roles in the development of joint OA.

Determination of risk factors particularly in the weightbearing joints and their modification may reduce the risk of OA and prevent subsequent pain and disability.[33,44] Mechanical forces exerted on the joints are a significant cause of OA and one of the most modifiable risk factors as determined by body mass index (BMI). Female sex, lower educational levels, obesity, and poor muscular strength are associated with symptomatic disease and subsequent disability.[45]

In a review of literature 14 contributing variables including occupational (extrinsic) and personal (intrinsic) were considered as risk factors. Two factors of kneeling and squatting are considered the main primary risk factors in correlation with knee disorders.[44]

Frequent squatting predispose people to the development of knee OA. Approximately 40% of men and approximately 68% of women reported squatting≥1 hour per day at age 25. Prolonged squatting is a strong risk factor for tibiofemoral knee OA among elderly.[46] Occupations involving squatting or kneeling more than two hours daily were associated with two-fold significantly increased risk of moderate to severe radiographic knee OA. Obesity alone or in patients with metabolic syndrome increases the risk of radiographic knee OA but has a lesser effect progression of knee OA.[46,47] Relationship between BMI and OA of the knee is mainly linear, and duration of increased joint loading or gaining weight is also significant. Twenty seven percent of cases of hip arthroplasty and 69% knee arthroplasty may be attributed to obesity[49]. In a systematic literature search obesity was consistently the main factor with knee OA by OR=2.63.[43]

Obesity is also associated with the hip and hand OA. This indicates that excess adipose tissue produces humoral factors, altering articular cartilage metabolism. It has been postulated that the leptin system could be a link between metabolic abnormalities in obesity and increased risk of OA.[48] Meniscal surgery increases the risk of further knee OA by 2.6 times.[50] Patients undergoing partial meniscectomy and reconstruction surgery are significantly more likely to develop radiographic evidence of OA than those with normal menisci.[51] Inflammatory process has been shown to be associated with OA. Inflammation may have a contributive role in the development and progression of OA. In one study the median level of high sensitive CRP in progressive knee OA was higher than non-progressive disease.[52] The median value of CRP is significantly associated with

functional disability, joint tenderness, pain, fatigue, global severity, and depression in OA. Mean CRP level in OA is greater than healthy individuals.[53]

In a study of relationship between biochemical markers of arthritis and the radiographic grading of OA in knees, a significant relationship was found between the joint space width and radiographic knee OA. The joint space width decreased with increasing Kellgren-Lawrence grade. Pyridinoline and tissue inhibitor of metalloproteinases-1 (TIMP-1) exhibited a significant relationship to the Kellgren-Lawrence grade but only urinary pyridinoline had a significant correlation.[54] In another study, raised serum CRP at entry was predictor of Knee OA progression. Serum CRP at entry was not predictive of progression between entry and five years but serum CRP at-3 years was predictive of progression by OR=1.95. Female sex was a strong risk factor even in the subgroup without radiographic knee OA (KL=0/1).[55] For instance, the greater total body fat of the average adult female may partially account for the gender disparity toward OA, given that females theoretically demonstrate higher levels of adipose derived systemic leptin concentrations than their male counterparts. Female gender increases the risk of knee OA 1.84 times.[43] In patients with knee OA, particularly at early stage of the disease, levels of serum vitamin D is significantly lower than individuals without knee OA. Vitamin D deficiency increases the risk of knee OA by OR=2.63. Higher than 6 pregnancies increases the risk of knee OA by 1.95 times.[56]

Risk factors of knee osteoarthritis:

- Age
- Genetic susceptibility
- Obesity
- Female gender
- Repetitive knee trauma
- Muscle weakness
- Joint laxity
- Mechanical forces
- Kneeling
- Squatting
- Meniscal injuries

Pathogenesis of OA

The extracellular matrix structure plays an integral role in the function of cartilage. In this tissue, matrix homeostasis is controlled by the chondrocytes through a balanced regulation of synthesis and degradative events, with the rate of new matrix synthesis being equal to the rate of matrix degradation. Both processes are controlled by a variety of extracellular messenger proteins, termed growth factors and/or cytokines.[28] The term cytokine refers to the fact that disparate group of intercellular messenger molecules go from cell to cell ("cyto") and make the targeted cell do something ("kine," from the same root as kinetic).[57] Cytokines are a complex family of small regulatory proteins able to mediate intercellular communication and play a crucial role in immunologic and inflammatory reactions.[6] Disturbance or alteration in the net effects of multiple growth factors and cytokines may compromise the macromolecule synthesis and degradation

pattern, therefore being responsible for the development of pathological conditions such as OA.[28]

Even if articular tissue destruction characterizes the OA condition, synovial inflammation is of fundamental importance in the progression of cartilage lesions in this disease.[58] At the clinical stage of OA, there are changes in the synovial membrane and an inflammatory reaction is often seen (synovitis, which is usually associated with effusion of variable amounts, pain, and muscle spasm). A hypothesis explaining the pathological development of OA may be summarized as follows: the cartilage matrix breakdown produced by proteolytic enzymes releases increased amounts of matrix fragments into synovial fluid. Synovial cells ingest the cartilage breakdown products through phagocytosis, including an inflammatory process that leads to the production of proteases and soluble inflammatory mediators. These factors, through diffusion into the cartilage, increase the catabolic process and create a vicious cycle with more cartilage being degraded and subsequently provoking more inflammation inside the joint evoking pain of variable intensity.[28]

Several soluble mediators have been identified in articular tissues from OA disease. Of the proinflammatory cytokines, interleukin-1beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) appear to be the principal inflammatory mediators causing joint destruction.[59] Yet it is claimed, and substantiated by studies on animal models,[60-62]that IL-1β is of pivotal importance in cartilage destruction and considered to be the principal mover of the enzyme system. IL-1 promotes connective tissue degradation through the induction of proteases and the inhibition of extracellular matrix synthesis.

These proinflammatory cytokines are able to increase other inflammatory factors such as some prostanoides, nitric oxide (NO),and the synthesis of enzymes, to inhibit the synthesis of the major physiological inhibitors of these enzymes, and also, to inhibit the synthesis of matrix constituents such as collagen and proteoglycans. Thus, the actions of IL-1β and TNF-α on other inflammatory mediators and on the enzyme process, combined with the suppression of matrix synthesis, result in the severe degradation of cartilage and the appearance of conditions that we know to be characteristic of OA including cartilage erosion as well as a variable degree of synovial inflammation.[56]

Interleukin-1 is released in an autocrine fashion in joints affected with arthritis. IL-1 inhibits proteoglycan synthesis that could be reversed by Type II soluble IL-1 receptor. Endogenous or exogenous Type II IL-1 receptor can attenuate the effects of IL-1 with respect to induction of inflammatory mediators, matrix metalloprotease activity and proteoglycan synthesis in human chondrocytes and cartilage.[63]

IL-1β is primarily produced as a precursor (pro- IL-1β) that must be cleaved to generate its mature and active cytokine. The intracellular enzyme responsible for this cleavage is caspase-1, also called IL-1β-coverting enzyme (ICE).[22]

The biological effects of IL-1β can be inhibited by its natural inhibitors: IL-1 receptor antagonist (IL-1Ra) and type II IL-1 decoy receptor (IL-1RII), which can bind IL-1β without transmission of a signal. [64] Once bound to its type 1 receptor (IL-1RI), IL-1β can initiate several signal transduction pathways, leading to an increase in intracellular Ca^{2+}, activation of Protein kinase C (PKC), p38, Extracellular signal-regulated kinases

(ERK1/2), and Jun N-terminal kinase (JNK), and nuclear translocation of nuclear factor-kB (NF-kB), activating transcription factor (ATF), and activator protein 1 (AP1).[22]

Several lines of evidence-based studies of human and animal joints suggest a central role of IL-1β in OA.

IL-1β is localized to chondrocytes in the superficial zone of human OA cartilage, where degenerative changes have been identified on histological examination. Saha, et al found upregulation of IL-1β in human OA cartilage preferentially located at the superficial and upper intermediate layers of articular cartilage, and also found a corresponding upregulation of IL-1β converting enzyme in OA tissue where IL-1β was elevated.[65] In another study, Smith, et al reported the presence of IL-1β in synovial membranes from all patients with OA, with a significant decrease in the ratio of IL-1Ra/ IL-1β with increasing grades of OA.[66] Kubota, et al showed that IL-1β levels in synovial fluid of temporomandibular joints have a positive correlation with OA changes.[67] Loeser, et al reported a positive association between the presence of nitrotyrosine, an indicator of oxidative damage, and the presence of IL-1β on human OA chondrocytes.[68]Additionally, although articular resident cells from normal tissue produce a limited amount of IL-1β, this cytokine is markedly increased in chondrocytes as well as synovial cells from patients with OA.[69] Marks, et al compared patients with anterior cruciate ligament (ACL) deficiency (risk factor for OA) to normal individuals and reported a marked increase in expression of IL-1β in the synovial fluid of the former group, with a positive correlation between expression ofIL-1β and the severity of chondral damage.[70]

Chondrocytes, the major cellular targets for IL-1β, not only express the receptor for this cytokine, but also express higher type 1 IL-1 receptor (IL-1R1) in patients with OA. Shlopov, et al reported that chondrocytes located in cartilage proximal to macroscopic OA sites bound more IL-1β compared with chondrocytes isolated from morphologically normal cartilage from the same joint, and that this increase was due to upregulation of IL-1RI.[71] Thus, upregulation of IL-1RI in the chondrocytes renders these cells highly sensitive to the effects of this cytokine, leading to alteration of chondrocyte gene expression. Aigner, et al reported significant alteration in the expression of 79 genes in human chondrocytes following exposure of these cells to IL-1β, including upregulation of IL-1β itself.[72] However, from microarray studies of 78 normal and OA samples, Aigner, et al surprisingly reported a downregulation of IL-1β in the OA samples.[73] Yet, in the same study the IL-1β antagonist (IL-1RII) was also reported to be downregulated, and the authors noted the very low level of expression as a concern regarding reliability of data. In a followup study using immunohistochemistry, the same investigators described that while IL-1β expression and signaling mechanisms, phosphorylated JNK, and p38 were detectable in the upper zones of normal cartilage, they were more pronounced in the upper portions of OA cartilage.[74] The authors also described an autocrine-positive loop following stimulation of chondrocytes by IL-1β, in that these cells responded not only by producing the inflammatory cytokine IL-6 but also IL-1β.[74] A remarkable change in catabolic gene expression and signal transduction pathways also reported by Saas, et al using microarray studies in 2 donors.[75] In addition to chondrocytes, IL1RI has been reported to be expressed in human synovial fibroblasts and to be significantly upregulated in OA synovial cells.[76] Further, in OA synovium, a relative deficit in production of natural antagonists of IL-1 receptor has been reported.[77] Thus the downregulation ofIL-1 antagonists, along with

upregulation of IL-1β and its receptors (IL-1RI), may be a molecular explanation for the enhanced catabolic effects of IL-1β in OA joint tissues.

Genomic studies have also provided some support for involvement of IL-1β in OA. Genome-wide scans for genetic loci predisposing to OA revealed potential linkage with an IL-1 gene cluster on chromosome 2. When Loughlin, et al performed an association analysis in a case-control cohort of 557 cases, they observed that the IL-1 ligand cluster encodes susceptibility to knee OA.[78]

Experimental animal models point to a key role for IL-1β in the development of OA as well. Pelletier, et al reported that dog knee joints subjected to anterior cruciate ligament (ACL) resection had a marked increase in IL-1β-expressing cells, which promptly decreased following treatment.[79] Wheaton, et al reported that biochemical changes similar to those seen in OA were induced with an intraarticular injection of recombinant porcine IL-1β into the pig knee joint.[80] The same authors demonstrated that 100 ng of IL-1β injected into the intraarticular regions of porcine joints resulted in a significant decrease in proteoglycan (PG) content, especially in the middle zone of cartilage, along with a marked increase in cell infiltration in synovial fluid. Lai, et al reported that IL-1β activation as a transgene in the joint led to a number of structural changes characteristic of OA, such as cartilage surface fibrillation and erosion.[81]

Molecular outcomes of activation of IL-1β pathway in OA:

Destructive effects of IL-1β in OA include both elevation of cartilage catabolism and suppression of cartilage anabolism.

Elevation of cartilage catabolism: Induction of proteolytic molecules involved in cartilage degradation.

IL-1β has been considered the central mediator of cartilage loss in OA, and articular extracellular matrix (ECM) has been reported to be a target of catabolic activity of this cytokine. IL-1β upregulates the major extracellular proteolytic enzymes in cartilage degradation, such as MMP and A Disintegrin-like and Metalloproteinases with Thrombospondin Motifs (ADAMTS).

Fan, et al reported upregulation of MMP-1, -3, and -13, but not MMP-2, in both normal human and OA chondrocytes followingIL-1βtreatment.[82] Elliott, et al showed induction of MMP-1 and -13 from human primary chondrocytes and human chondrosarcoma cell lines following a 24-hour incubation of these cells with 10 ng/ml of recombinant IL-1β.[83] Inoue, et al described upregulation of MMP-3 release by both chondrocytes and synoviocytes from OA patients following IL-1β treatment, which was suppressed by IL-1Ra.[84] Kobayashi, et al observed upregulation of gene expression of MMP-1, -3, and -13 in articular cartilage from patients with OA, along with an increase in collagen and PG degradation, with all these effects being significantly suppressed by IL-1Ra treatment.[85] Mix, et al reported induction of mRNA for MMP-1, -3, and -13 following treatment of human chondrosarcoma cells by 1 ng/ml ofIL-1β,[86] and Tetlow, et al presented Western blots demonstrating the production and release ofMMP-1, -3, and -13 proteins by IL-1β-stimulated human articular chondrocytes.[87] Apparently the effect of IL-1β in induction ofMMP-1, -3, and -13 is well conserved between different species, as such induction is reported for rabbit, bovine, and equine chondrocytes.

ADAMTS, also called aggrecanases, are enzymes involved in degradation of aggrecans. Fan, et al reported upregulation of ADAMTS-4 in normal human and OA chonrocytes by IL-1β,and Bondeson, et al observed both ADAMTS-4 and -5 were upregulated by IL-1β in human OA synovial fibroblasts.[88] Cortial, et al observed that when bovine chondrocytes were cultured with IL-1β, ADAMTS-4 and -5 were substantially increased.[89] Using human OA chondrocytes, Dai, et al reported upregulation of IL-18 by IL-1βand upregulation of ADAMTS-5 by IL-18.[90] Thus, it is conceivable that some IL-1β catabolic effects are relayed by other factors, such as IL-18 in this system.

MMP and ADAMTS are not the only proteolytic mediators induced by IL-1β. For example, Mahraban, et al reported upregulation of cathepsin B expression in osteochondrocytes by IL-1β treatment,[91] and Milner, et al described IL-1β induction of fibroblast activation protein-∞ (FAP∞), a type II integral membrane serine proteinase, in the chondrocytes.[92] Additionally, Schwab, et al reported that urokinase-type plasminogen activator receptor (uPAR), which is involved in cartilage degradation by serine proteinases and is upregulated in OA, is stimulated on chondrocytes in a dose-dependent manner by IL-1β.[93]Shikhman, et al reported that stimulation of human chonrocytes with IL-1β resulted in an increase in extracellular lysosomal glycosidases, a distinct subset of cartilage matrix-degrading enzymes.[94]

Induction of inflammatory mediators and cell infiltration:

There is evidence of cell infiltration in OA that could be associated with and account for some of the histopathology characteristics such as thickening of the synovial membrane lining layer, increased vascularity, synovial fluid cellularity, and secretion of degradative enzymes. For example, Nakamura, et al observed an increase in the T cell population in all of the OA synovial tissues, especially in the perivascular area,[95] and Da, et al showed that synovial infiltration by B lymphocytes was present in almost half of cases of knee OA.[96] Walsh, et al reported an increase in synovial macrophages in patients with OA compared to a control group.[97]

In addition, IL-1β has the capacity to induce several proinflammatory mediators including cytokines, chemokines, angiogenic factors, and proteolytic enzymes involved in the increase of local hematopoietic cells during OA. For example, IL-1β-induced MMP have been shown to be involved in cell infiltration. Additionally, investigators have reported that IL-1β stimulation of articular cells such as chondrocytes leads to expression of TNF-alpha, IL-8, complement factors, and prostaglandin E_2, each having the capacity to induce hematopoietic cell infiltration and propagate local inflammation and tissue damage. Further, IL-1β induces angiogenic factors such as vascular endothelial growth factor, in addition to several chemokines such as regulated upon activation normal T cell expressed and presumably secreted (RANTES), and their receptors such as chemokine(C-C motif) receptor 4 (CCR4), leading to inflammatory cell infiltration into the synovia. Several investigators have reported a local increase in mononuclear cells/macrophages during OA progression, with these cells being an important source of proteolytic enzymes and free-radical molecules. Blom, et al reported that in experimental models of OA, macrophage depletion led to marked inhibition of osteophytes.[98] Bondeson, et al described a decrease in production of MMP-1 and -3 following depletion of synovial macrophages.[99] Hence, once generated, IL-1β can induce several molecules that act as cell chemoattractants. Thus, an increase in articular hematopoietic cells can induce thickening of the synovial

membrane, increases in oxidative burst activity and decreases in O_2 concentration, and generation of additional inflammatory and proteolytic enzymes that can lead to progression of OA.

Suppression of cartilage anabolism: Several studies have shown that apart from increased degradation of ECM, IL-1β can decrease ECM synthesis by decreasing the anabolic activities of chondrocytes and/or the cell densities of articular cartilage.

Downregulation of proteoglycan(PG) and collagen biosynthesis:

Pfander, et al reported a decrease of more than 40% in aggrecan mRNA following treatment of human OA chondrocytes with IL-1β.[100] Stove, et al demonstrated thatIL-1β downregulated aggrecan transcripts in human OA chondrocytes by 2-3 fold.[101] Venkatesan, et al revealed a time-dependent decrease in PG synthesis of rat femoral explants by IL-1β, along with a decrease in PG accumulation.[102] Eger, et al showed that both types of chondrocytes from normal human knees and ankles responded to IL-1β with decreased PG synthesis.[103] Attur, et al reported that 5 ng/ml of IL-1β could significantly suppress PG synthesis from both human and bovine chondrocytes.[104] Stabellini, et al described release of matrix sulfated PG into culture media and inhibition of sulfated PG synthesis following IL-1β treatment of bovine cartilage explants.[105] The deleterious effects of IL-1β on the anabolism of PG could involve suppression of galactose- β-1, 3-glucuronosyltransferase I (G1cAT-I), the key enzyme in the biosynthesis of glycosaminoglycan that is linked to PG core proteins. Gouze, et al reported suppression of G1cAT-I mRNA (38%) following treatment of rat articular chondrocytes with IL-1β, which correlated with 32% inhibition of PG synthesis.[106]

Collagen, the major articular joint protein, is another target whose synthesis is suppressed by IL-1β. For example, Shakibaei, et al reported the downregulation of type II collagen in human chondrocytes treated with IL-1β.[107] Using Western blotting, Yudoh, et al reported significant reduction in production of type II collagen by rabbit chondrocytes incubated in presence of 10 ng/ml of IL-1β.[108] Golring, et al reported downregulation of type II collagen mRNA by Northern blots following treatment of human chondrocyte cell lines.[109] At least some part of the inhibitory effect is due to downregulation of collagen transcription, since IL-1β suppressed the collagen promoter in a reporter assay. The downregulation was very specific, as several enzymes and transcriptional factors were upregulated by IL-1β. In a study using human fibroblasts, Nawrat, et al reported a 50% reduction in collagen biosynthesis following a 24-hour treatment with 10 ng/ml of IL-1β.[110]

Induction of chondrocyte apoptosis:

IL-1β may also induce apoptosis of chondrocytes. Several investigators have reported reduced numbers of chondrocytes due to an increase in apoptotic chondrocytes in patients with OA, with a potential link to IL-1β as the possible culprit in these processes. For example, Lopez-Armada, et al reported a depolarization of mitochondria and upregulation of proapoptotic Bcl-2 family proteins (characteristics of apoptotic cells) following treatment of human articular chondrocytes with IL-1β.[111] Heraud, et al described that in human OA cartilage 18-21% of chondrocytes showed apoptotic features and that IL-1β could increase the percentage of apoptotic cells in both normal and OA

cartilage in a dose-dependent manner.[112] Also, apoptosis could be significantly induced in rabbit chondrocytes cultured in presence ofIL-1β.

How IL-1β induces the death of chondrocytes is not entirely understood, but NO has been strongly suggested as a culpable mediator. For example, Pelletier, et al reported that although NO production by normal human cartilage was undetectable, OA cartilage spontaneously produced NO, and this release was upregulated by IL-1β.[113] In the study by Tenor et al, IL-1β induced human OA chondrocytes to produce a large amount of NO in a time- and concentration-dependent fashion.[114] Additionally, Clancy, et al demonstrated that in bovine chondrocytes, NO mediated IL-1β-dependent apoptosis under conditions of oxidant stress, where induction of NO led to subsequent accumulation of intracellular oxidants including peroxynitrite and superoxide anion.[115] Thus, IL-1βmay induce chondrocyte apoptosis by increasing NO concentration via upregulation of the enzyme inducible NO synthetase, the inducible enzyme responsible for NO production. This can result in formation not only of NO, but also its derivatives such as peroxynitrite (ONOO-), which can eventually lead to chondrocyte apoptosis.

All the studies demonstrating the role of inflammation in OA refer to either arthroscopy, magnetic resonance imaging (MRI), or bone scintigraphy findings.[116] In daily practice, such investigations are rarely performed in the monitoring of OA patients but it has been suggested that the presence of certain symptoms might be suggestive of an inflammatory episode of the disease. Such symptoms include:

- Sudden increase in pain,
- Night pain,
- Morning stiffness of more than 20 minutes
- Presence of hydarthrodial effusion, and
- Physical signs of inflammation (redness, etc.).

Arthroscopic classification of cartilage breakdown:

In humans, the arthroscopic classification of severity of chondropathy differentiates grade I from the others (grades II to IV).[117-120] The arthroscopic definition of this chondropathy is the presence of swelling and/or softening of the cartilage. Histologically, these lesions are defined by the presence of edema together with cellular infiltrates. Grade I chondropathy (also called closed chondropathy or chondromalacia) can be considered as a “chondritis” usually observed after direct trauma on the cartilage.[121] These lesions can resolve spontaneously or can progress to an open chondropathy of grades II to IV.[122] Grade II is characterized by superficial cartilage fibrillation; grade III by deep and large cartilage fibrillation; and grade IV is defined by visualization of the subchondral bone. In patients with a more advanced OA disease, the demonstration of “chondritis” is less well established but some in vitro studies clearly show hypercellular “activity” within the cartilage.

Diagnosis

Clinical criteria for the classification of idiopathic OA of the knee were developed through a multicenter study group.[123] Comparison diagnoses included rheumatoid arthritis and other painful conditions of the knee, exclusive of referred or para-articular pain. Variables from the medical history, physical examination, laboratory tests, and radiographs were used to develop sets of criteria that serve different investigative purposes.

❖ **Clinical and laboratory:**[123]

Knee pain plus at least five of nine:

1. Age >50 years
2. Stiffness<30 minutes
3. Crepitus
4. Bony tenderness
5. Bony enlargement
6. No palpable warmth
7. Erythrocyte sedimentation rate <40 mm/h
8. Rheumatoid factor <1: 40
9. Synovial fluid signs of OA

❖ **Clinical and radiographic:**[123]

Knee pain plus at least one of three:

1. Age>50 years
2. Stiffness <30 minutes
3. Crepitus

+ Osteophytes

❖ **Clinical:**[123]

Knee pain plus at least three of six:

1. Age>50 years
2. Stiffness<30 minutes
3. Crepitus
4. Bony tenderness
5. Bony enlargement
6. No palpable warmth

Clinical features for diagnosis of knee OA:

The diagnosis of knee OA in the most cases can be made by the clinical findings and physical examination. The important three features for the clinical diagnosis of knee OA by the EULAR include persistent knee **pain**, **limited morning stiffness** and **reduced function.**[124] Stiffness is the earliest symptom of joint degeneration, due to lack of lubrication and articular attrition. In addition **crepitus**, **restriction of joint movement** and **bony enlargement**.

Pain is the most common symptom in knee OA, a leading cause of chronic disability and a major source of the disability attributable to OA. Pain in knee OA typically exacerbates by activity and relieves by rest. In the presence of the above **6 symptoms and signs** the probability of having radiographic knee OA increases to 99%.[125]

In advanced cases synovitis may appear and leads to pain at rest or night.

Short duration of stiffness less than 30 minutes may be seen in OA patients in the morning or following periods of inactivity.

Tenderness to palpation of the involved joint may be evident on physical examination.

Joint effusion may be present, which typically exhibit a mild pleocytosis, normal viscosity and modestly elevated protein.

Crepitus during joint motion or walking is common.

Limitation of range of motion is a common sign of knee OA especially in advanced cases. In advanced cases[125] also malalignment may be apparent and may be severe (genu varum or genu valgum)

Imaging:

Plain radiography and MR imaging:

A) Conventional plain radiograph is**:**

The first diagnostic procedure which is usually requested to demonstrate the structure-pain relationship in knee OA.

Radiographic examination has several limitations and it predominantly visualizes bone whereas MRI has the ability to directly visualize all the structures within the joint, including soft tissue, cartilage, subchondral bone marrow lesions[126]. Greater levels of structural changes at earlier stage can be revealed by MRI.

Identification of bone changes in early knee OA may not be possible due to **low** sensitivity of plain radiography. The major radiographic features of knee OA include: joint space narrowing, subchondral sclerosis, osteophytes and subchondral cysts[30]. Osteophyte formation is the radiographic feature that associates best with knee pain. It predicts pain more accurately than joint space narrowing. Radiographic assessment of both patellofemoral and tibiofemoral regions should be performed in all studies of knee OA[30] .

B) MR imaging:

MRI is **not** necessary for most patients with suggestive symptoms of OA and/or typical plain radiographic features. However, MRI of the knee has a diagnostic role in patients with joint pain and symptoms such as locking, popping or instability that suggest meniscal or ligamentous damage of the knee joint [30]. The presence of two MRI findings concomitantly correlates with painful knee OA. Several lesions may be diagnosed in knee OA by MRI. These include cartilage lesions, osteophytes, bone oedema, subchondral cysts, bone attrition, meniscal tears, ligament tears, synovial thickening, joint effusion, intra-articular loose bodies, infrapatellar pad of fat inflammation and periarticular cysts.[126-128]

Relationship between knee pain and radiographic changes[30]:

Available data suggest that many individuals with radiographic knee OA are asymptomatic and in contrary in many patients with knee pain suggestive of OA radiographic findings are absent. It has recently been shown that **synovial hypertrophy, synovial effusion**, **bone marrow oedema**, **effusion**, **synovitis**, **meniscal tears** and **subarticular bone attrition** being the origin of knee pain in knee OA. In another study of comparing radiographic and MRI findings of knee OA, there was a significant association between pain, radiographic severity of knee OA and **seven parameters identified by MR imaging**. Moreover, in middle-aged women, there were significant association between pain, radiographic severity of knee OA and seven MR imaging identified parameters.[129] Women report symptomatic OA more frequently than men with a similar degree of radiographic OA.

Laboratory findings[30]:

Knee OA is a degenerative joint disease which is a focal lesion with no systemic manifestations. Although mild synovits may be seen in patients with knee OA but markers of inflammation such as erythrocyte sedimentation rate (ESR) and C- reactive protein (CRP) levels are normal. Synovial fluid in knee OA is of non-inflammatory type. Serum and synovial fluid levels of CRP in OA are markedly lower than inflammatory arthritis.

Determination of IL-1 in synovial fluid in patients with knee OA:

Recent reports have suggested that IL-1 promotes the early degenerative changes in OA. It induces cartilage degeneration through induction of proteases and the inhibition of extra-cellular matrix synthesis.[130] and causes deterioration of the functional properties of articular cartilage.[131] IL-1 induces the production of collagenase and prostaglandin and results in decreased synthesis of cartilage specific collagens and proteoglycans.

Elevated levels of IL-1 occur in the synovial fluid and cartilage tissue of patients with OA compared to healthy individuals,[21] implying a role in disease pathogenesis. Thus, IL-1 may be a fairly attractive therapeutic target in OA. Clinical trials have also generated optimism for anti-IL-1 therapy as a novel approach in OA treatment.[22] Inhibition of IL-1 was achieved by the use of diacerein for patients with OA and recommendations for combination therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported by many investigators.

Treatment:

Historically, only symptom relieving therapy has been available for OA.[132] Because pain is the most common complaint, the perennial focus of therapy has been on pain control. Nonpharmacologic interventions, such as those to achieve weight loss, temperature modalities and physical therapy are recommended. Simple analgesics, topical agents, NSAIDs, and intra-articular glucocorticoids can subsequently be prescribed. More recently, intra-articular hyaluronic acid (HA) derivatives and nutriceuticals have been adopted as symptom relieving agents.

Although progress continues to be made in the field of symptomatic therapy, advances in our understanding of the pathogenesis of OA have yielded the possibility of new therapeutic options. Forestalling the deterioration of articular tissues and thus modifying the disease course has become the goal of many researchers.

Symptom modification:

Symptom modification interventions can be subdivided into nonpharmacologic and pharmacologic.

- **Nonpharmacologic measures:**

Education, exercise, and weight loss are mainstays in the management of OA and the promotion of general health.[132]

Exercise therapy is considered to be an important nonpharmacologic treatment approach.[133] The goal of exercise therapy in OA patients is to reduce pain and disability. In order to achieve this, exercise therapy aims at the improvement of muscle strength, stability of joints, range of motion, and aerobic fitness. These functions are frequently impaired in patients with OA, presumably contributing to pain and disability.[134] Improving these functions is assumed to result in a reduction of pain and disability.

Weight loss has been shown to produce symptomatic improvement in OA of the knee.[135] Recent studies [136,137] have confirmed this finding and have also shown that the benefit of pain reduction may persist up to 1 year if weight loss is maintained. The combination of exercise and weight loss may be superior to either intervention alone.[138]

- **Pharmacologic measures:**

Pharmacologic measures are widely used and include analgesics, NSAIDs, intra-articular agents, and nutritional supplements.

Non-narcotic analgesics:

Acetaminophen is the first-line pharmacologic agent recommended for the treatment of OA by the American College of Rheumatology and the European League Against Rheumatism. [132] Acetaminophen is effective in the relief of pain associated with OA. Because of its comparatively favorable side-effect profile, it should remain the initial pharmacologic agent of choice.

Nonsteroidal anti-inflammatory drugs:

Nonsteroidal anti-inflammatory drugs are effective agents in the treatment of OA but they remain burdened by their side-effect profile. Gastrointestinal and renal toxicity are well described. Selective cyclo-oxygenase-2 inhibitors have reduced gastrointestinal toxicity but concerns about cardiovascular risks have led to voluntary withdrawal of rofecoxib from the market in the USA. There have also been concerns about celecoxib at a dose of 200 mg twice daily because of the increased relative risk for myocardial infarction identified in an adenomatous polyp prevention trial. [139] Currently, there is no evidence of increased cardiovascular risk associated with the use of celecoxib at dose of 200 mg/day in the management of OA.[132]

Potential Structural Effect:

There is in vitro and in vivo evidence pointing to a possible beneficial structural effect of the use of NSAIDs in OA.[140,141] Despite these expectations, there are presently no data suggesting a beneficial structural effect for any available NSAID. In fact, there are data suggesting a deleterious structural effect of long-term, daily indomethacin

intake,[142,143] although there are also data suggesting a lack of such effects for long-term daily use of other NSAIDs such as naproxen [144] and contradictory results concerning long-term daily intake of diclofenac.[145,146]

Two mechanisms have been suggested to explain the potential deleterious structural effect from the use of NSAIDs. The first is based on the analgesic properties of NSAIDs—one could argue that the disappearance of pain permits greater physical activity, the latter being responsible for the deleterious structural effect. The second mechanism is based on the vascular properties of NSAIDs and, in particular, the vasoconstriction induced by such therapy. Perfusion of the osteoarthritic femoral head is greater than normal.[147] It has been suggested that the osteoarthritic joint requires adequate perfusion to maintain the repair of joint structures and that if this is interfered with by inhibition of prostaglandin synthesis, the already compromised joint will deteriorate more rapidly.

Intra-articular agents:

Intra-articular corticosteroids have been widely utilized in treatment of OA for many years. Nevertheless, there are few long-term data on their efficacy to guide the practitioner.[132]

Nutriceuticals:

Preparations containing nutritional supplements ('nutriceuticals') such as glucosamine and chondroitin sulfate are often used by patients to self-manage OA. However, well designed, randomized controlled trials have not reached consistent conclusions regarding the efficacy of glucosamine.[132]

Disease modification:

4,5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid (diacerein), a symptomatic slow-acting disease modifying OA drug (DMOAD) with IL-1β inhibitory properties in vitro,[148] was rated as a therapeutic option in OA.

- **Mechanism of Action:**

It directly inhibits IL-1 synthesis and release in vitro and down modulate IL-1 induced activities and have been shown to posses disease modifying effect in experimental models of OA in human subjects with finger joint and knee OA.[25]

By inhibiting IL-1 diacerein retards all pathological prepossess initiated in OA. Diacerein also inhibits IL-1 induced expression of cartilage degrading enzymes. It also enhances expression of transforming growth factor (TGF)-beta1 and TGF-beta 2 thus favoring matrix synthesis and turnover in articular chondrocytes, thereby accounting for disease modifying property of diacerein. [149] It also inhibits superoxide production, chemotaxis and phagocytic activity of neutrophils in addition to effect on macrophage migration and phagocytosis.[150]

- **Pharmacology:**

Diacerein has efficacy on functional manifestations of OA and on structural component. It exerts its pharmacologic action through its active metabolite –rhein.[151] Diacerein is entirely converted to rhein before reaching systemic circulation and rhein later

gets eliminated by renal route (20%) or conjugated in liver to rhein glucronide (60%) and rhein sulphate (20%), these metabolites are mainly eliminated by renal route. The pharmokinetics after a single oral dose are linear in normal therapeutic doses with equal efficacy in normal young and elderly volunteers. The absorption in systemic circulation is delayed with standard meal but is associated with 25% increase in amount absorbed. In contrast to other NSAIDS the interactions are minimal as highly binding of rhein to plasma proteins is not saturable.[152] It does not alter renal or platelet COX activity and can be tolerated easily by patients with prostaglandin dependent renal function. Though dose modification is required in mild to severe renal insufficiency [50% dose reduction in severe renal failure],[153] no reduction in initial dose is proposed in liver cirrhosis.[154]

- **Structural effect:**

Various clinical trials have confirmed the efficacy of diacerein in patients with OA. The effect of the drug in acute exacerbations of OA has been documented in approximately 30 studies. It is much superior to that of placebo and over a common NSAID tenoxicam at the 60th treatment day.[155] Diacerein has been also shown to be effective in modification of symptoms and structure in patients of Knee OA.[156]

- **Safety profile:**

The optimal daily dose which relieves symptoms on OA knee calculated from effect on visual analogue scale assessment criteria of pain on movement was found to be 100mg/day.[156] Diacerein is well tolerated, the predominant adverse effect include transient change in bowel habits.[151] It seems neither responsible for gastrointestinal bleeding nor for renal, liver nor hematological toxicities. Non significant discoloration of urine occurs during treatment because of urinary elimination of metabolites of dicerein. No allergic cutaneous reaction were reported in knee OA trial.[156] No severe allergic reaction has been reported till date.

The main differences between 4,5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid (diacerein) and NSAIDs are as follows:

- A delayed onset of action. In an 8-week trial comparing placebo, tenoxicam, and diacerein, the treatment effect of tenoxicam (ie, the effect observed in the tenoxicam group over that observed in the placebo group) was stable at 17% to 19% during the 8-weeks of the study. By contrast, the diacerein was 0%, 5%, 11%, and 12%, at weeks 2, 4, 6, and 8, respectively. [157]
- A carry-over effect. This is the maintenance of the symptomatic effect of a drug after dosing has ceased. In the study evaluating diacerein and diclofenac, the relief of pain obtained with diclofenac was no longer observed 4 weeks after discontinuation of the drug. On the other hand, the relief of pain obtained at month 4 in the diacerein group (ie, the end of the treatment period) was maintained until the end of the follow-up (ie, at months 5 and 6). [158]
- A potential beneficial structural effect. Two recent published trials (one evaluating glucosamine sulfate in knee OA [159]) and the other diacerein in hip OA [160] suggested that demonstration of a beneficial structural effect is possible. It remains to be seen whether such a statistical difference has any clinical relevance.