Osteoarthritis is a degenerative joint disease involving the cartilage and its surrounding tissues. In addition to damage and loss of articular cartilage, in osteoarthritis remodeling of subarticular bone, osteophyte formation, ligamentous laxity, weakening of periarticular muscles and synovial inflammation occur (Hutton, 1989). Worldwide estimates are that 9.6% of men and 18.0% of women aged ≥60 years have symptomatic osteoarthritis whereas in India it ranges from 14-47% (Woolf et al., 2003; Salve et al., 2010; Ganvir et al., 2013). Epidemiological observations showed that after the age of 50, osteoarthritis, particularly of the knee, is more common in women than in men and suggesting that estrogen deficiency may play an important role in the onset or progression of osteoarthritis (Nevitt et al., 1996; Hart et al., 1999; Sangha et al., 2000). Clinically, the condition is characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and a variable degree of local inflammation.

**Structure of the knee joint:**

The knee is one of the largest and complex types of synovial joints in the body. It has three compartments namely- medial, lateral and patellofemoral. The femur (thigh bone) and tibia (shin bone) together to form a tibiofemoral joint which has two compartments medial (inner) and lateral (outer) and the patella (kneecap) and femur form a third joint, called the patellofemoral joint. The end of each bone is covered with cartilage which has a smooth, slippery surface that allows the ends of the bones to move against each other almost without friction. Cartilage is made up of four components: water, chondrocytes (basic cartilage cells), proteoglycan and collagen. The joint is surrounded by a membrane the synovium that produces a small amount of synovial fluid, which helps to nourish the cartilage and lubricate the joint. The synovium has a
tough outer layer called the capsule, which helps to hold the knee in place. Four major ligaments occur within the knee (anterior and posterior cruciate ligaments) and on the inner and outer sides of the knee (medial and lateral collateral ligaments) which stabilize the joint along with the capsule. The anterior and posterior cruciate ligaments provide front and back (anterior and posterior) and rotational stability to the knee. The medial and lateral collateral ligament located along the inner (medial) and outer (lateral) sides of the knee provide medial and lateral stability to the knee. Fibrocartilaginous structures called menisci line the top of the tibia and lie between the tibial and the femoral condyles. Menisci provide space, stability, and cushion for the knee joint (Arya et al., 2013). (Figure no-1)

**Figure no 1(a): Structure of a normal knee joint** (Source-Internet)

**Figure no 1(b): Anterior and lateral view of the normal knee** (Source-Internet)
Structural changes in the knee joint during osteoarthritis

Osteoarthritis is considered as an organ disease that involves the whole joint structure. The radiographic and structural changes occur in the knee joint during osteoarthritis include narrowing of the joint space, subchondral bone sclerosis, the formation of osteophytes at the joint margins, flattening and deformation of the subchondral cortical plate referred to as bone attrition and synovitis, which is often present and cannot be detected with standard radiographs. The narrowing of joint space occurs due to the loss of articular cartilage, which can be asymmetrical in the knee joint, and affecting principally the medial or lateral side. The thickening of the subchondral cortical plate accounts for the appearance of bone sclerosis. (Figure no-2)

Figure no 2: Radiographic and structural changes in osteoarthritis: Radiograph image courtesy of Douglas M. Mintz, Hospital for Special Surgery, Department of Radiology, New York, New York, USA. (Source: Goldring et al., Nat Rev Rheumatol 2016)
Classification of Osteoarthritis

Osteoarthritis is of two types:

**Primary Osteoarthritis:** Osteoarthritis has no known cause are referred to as primary osteoarthritis and is mainly related to aging and it can present as localized, generalized or erosive osteoarthritis.

**Secondary Osteoarthritis:** Osteoarthritis occurs due to another disease or conditions are referred to as secondary osteoarthritis. The causes of secondary osteoarthritis are:

- Congenital
- Mechanical: limb length discrepancy, malalignment, hyperlaxity, Ehlers-Danlos syndrome, Marfan’s syndrome
- Inflammatory: rheumatologic diseases, i.e., rheumatoid arthritis, SLE, all chronic forms of arthritis
- Traumatic: injury to joints or ligaments, postsurgical
- Infective: septic arthritis, Lyme disease
- Metabolic: hemochromatosis and Wilson’s disease, gout, calcium crystal deposition, alkaptonuria
- Endocrine: diabetes, acromegaly, hypothyroidism, obesity
- Neuropathic arthropathy
- Miscellaneous: hemophilia, osteonecrosis

Another classification of osteoarthritis is:

**Radiological classification of OA**

In 1957, Kellgren and Lawrence developed a classification system that sets out a series of radiological features that are considered evidence of osteoarthritis, and divides the disease into five grades:

Grade 0 (Normal) = no radiographic features of osteoarthritis
Grade 1 (Doubtful) = doubtful joint space narrowing and possible osteophytic lipping
Grade 2 (Mild) = definite osteophytes and possible joint space narrowing
Grade 3 (Moderate) = multiple osteophytes, definite joint space narrowing, some sclerosis, possible bone end deformity
Grade 4 (Severe) = large osteophytes, marked joint space narrowing, severe sclerosis and definite bone end deformity.

**Risk Factor for Osteoarthritis**

Osteoarthritis has a multifactorial etiology, can be considered the product of the interaction between systemic and local factors.

**Systemic Risk Factors for OA:**

**Age:**

Age is one of the strongest risk factors for osteoarthritis (Felson et al., 1998; Felson et al., 2000; Lawrence et al., 2008). The increase in the prevalence and incidence of OA with age probably is a consequence of cumulative exposure to various risk factors and biologic changes; these include cartilage thinning, weak muscle strength, poor proprioception, and oxidative damage.

**Gender and Hormone:**

The incidence of the knee, hip, and hand OA is higher in women than men and in women, it increases dramatically around the time of menopause (Srikanth et al., 2005). The increase in incidence of osteoarthritis in women just after menopause has hypothesized that estrogen deficiency plays a role in onset and progression of disease (Spector et al., 1997; Felson et al., 2000; Cirillo et al., 2006; de Klerk et al., 2009). Epidemiologic studies of women who take estrogen replacement therapy have reported that those women who take hormone replacement therapy have a lower prevalence of osteoarthritis than women not taking estrogen and suggested a possible
therapeutic role for estrogen in osteoarthritis (Spector et al., 1997; Felson et al., 1998; Cirillo et al., 2006).

**Ethnicity and race:**

The prevalence of osteoarthritis and patterns of affected joints vary among racial and ethnic groups. Both radiographic hip and hand OA were much less frequent among Chinese in the Beijing Osteoarthritis Study than in whites in the Framingham Study (Zhang et al., 2001; Nevitt et al., 2002), but interestingly Chinese women had significantly higher prevalence of both radiographic and symptomatic knee OA than white women (Zhang et al., 2001). Results from the Johnston County Osteoarthritis Project have shown that the prevalence of hip OA in African American women was similar to that in white women, and prevalence was slightly higher in African American men (21%) than that in white men (17%) (Nelson et al., 2010).

**Genetics:**

Several studies have shown that osteoarthritis is inherited and may vary by joint site. Twin and family studies have estimated the heritable component of OA to be between 50 and 65% with larger genetic influences for hand and hip OA than for knee OA (Palotie et al., 1989; Spector et al., 1996; Felson et al., 1998). In a genome-wide association study, Kerkhof et al., 2010 reported that the C allele of rs3815148 on chromosome 7q22 was associated with a 1.14-fold increased prevalence of knee and/or hand osteoarthritis and also with a 30% increased risk of knee osteoarthritis progression.

**Nutrition:**

Dietary factors are the subject of considerable interest in osteoarthritis; however, results of studies are conflicting. One of the most promising nutritional factors for OA is vitamin D. Without sufficient vitamin D, bones become thin and brittle. Low
dietary intake of Vitamin D (McAlindon et al., 1996) and vitamin C has been associated with increased risk of progression of knee osteoarthritis (McAlindon et al., 1996).

**Congenital/ Developmental deformities:**
A few congenital or developmental abnormalities (i.e., congenital subluxation, Legg-Calve-Perthes disease, and slipped capital femoral epiphysis) have been associated with the occurrence of OA in later life (Murray et al., 1965; Stulberg et al., 1981; Harris et al., 1986).

**Local Risk Factors of OA:**

**Obesity:**
People with an elevated body mass index (BMI) as a measure of relative weight for obesity, has a positive association with knee OA. Obesity results in substantial overloading and damage to the knee joint (Toivanen et al., 2010).

**Occupation:**
The incidence of both knee and hip osteoarthritis is higher in the jobs requiring kneeling or squatting along with heavy lifting (Felson et al., 1991; Felson et al., 2000). Farmers, jackhammer operators, and mill workers have high rates of osteoarthritis.

**Joint Injury and trauma:**
Severe injury to the structures of a joint, particularly a trans-articular fracture, meniscal tear requiring meniscectomy, or anterior cruciate ligament injury, can result in an increased risk of OA development and musculoskeletal symptomatology (Roos et al., 2001; Lohmander et al., 2004)
Physical Activity/Sports:

Certain competitive sports increase the risk for osteoarthritis such as football or soccer. In China women practicing gymnastic or kung fu (traditional Chinese martial arts) regularly was at the risk of knee injury (Järvholm et al., 2005).

Muscle weakness:

Weak quadriceps muscles can lead to osteoarthritis of the knee (Slemenda et al., 1997).

Sign and Symptoms of Osteoarthritis:

Pain:

Pain is the first and predominant symptom of knee osteoarthritis, causing loss of ability and often stiffness. “Pain” is generally described as a sharp ache or a burning sensation in the associated muscles and tendons. The pain is intermittent and is worse with use and better with rest but in advanced disease, it may occur with rest also.

Stiffness:

It generally occurs in the morning and improves after 30 minutes of activity.

Crepitus:

Osteoarthritis of the knee can cause a crackling noise called “crepitus”, with the movement of joint or when the affected joint is touched, and patients may experience muscle spasm and contractions in the tendons.

Bony enlargement:

Heberden and Bouchard nodes, squaring of the first CMC, typically along the affected joint line in the knee.

Joint effusion:

Occasionally, the patient presents with swelling or joint effusion sometimes called water in the knee in lay terms due to the fluid within the joint.
Cyst:
Popliteal cyst also known as Baker’s cyst can also be seen with knee osteoarthritis.

Deformity:
In advanced cases, patients may present with lateral instability symptoms due to degenerated tears in cruciate ligaments and menisci or genu valgum (knock knee) or varum (bowleg). Varus deformity is more common than valgus deformity because the medial compartment of the knee is more commonly involved (Altman et al., 1986; Arya et al., 2013).

Pathophysiology of Osteoarthritis:
Osteoarthritis is a complex joint disease, affecting all joint tissues that are articular cartilage, subchondral bone, and synovium. Articular cartilage is composed of an extracellular matrix that contains a single type of cell: the chondrocyte - which is responsible for the synthesis of extracellular matrix. This matrix is mainly composed of water, proteoglycans and type II collagen. One of the roles of cartilage is to absorb the mechanical stress between two mobile bone surfaces. Under normal conditions, articular chondrocytes maintain a dynamic equilibrium between synthesis and degradation of extracellular matrix (ECM) components, including type II collagen and aggrecan, the most abundant proteoglycan in articular cartilage (Sandell et al., 2001; Nakata et al., 1993). In osteoarthritic states, however, a disruption of matrix equilibrium leads to progressive loss of cartilage tissue, clonal expansion of chondrocytes in the depleted regions, induction of oxidative states, and eventually, apoptosis of cells (Lane et al., 2011; Bauer et al., 2006). In osteoarthritis, a biological or mechanical stress is followed by an increase of pro-inflammatory mediator production by chondrocytes (i.e., chondrocyte activation) but also by synoviocytes and osteoblasts, in each respective tissue. Among those proinflammatory mediators,
cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-17 (IL-17), tumor necrosis factor-α (TNF-α), radical oxygen species (ROS), advanced glycation end products (AGE) and bio-active lipids (prostaglandin E2 (PGE2) are the most occurred. This local inflammation induces an increased production of the proteolytic enzymes (metalloproteinase (MMP) and aggrecanases) by all 3 cells (i.e., chondrocytes, synoviocytes and osteoblasts) that will digest the cartilage matrix. In addition, chondrocytes dedifferentiate and die by apoptosis, leading to a synthesis of an altered matrix (type 10 instead of type 2 collagen) and to a loss of matrix synthesis respectively. While physiologically cartilage is non-vascularized, neoangiogenesis is promoted in the deep layer of cartilage facilitating the passage of soluble mediators between subchondral bone and cartilage by specific pro-angiogenic mediators and leads to the development of vascular channel. In addition to cartilage disruption, subchondral bone undergoes remodeling with condensation and overgrowth (osteophytes) under the influence of growth factors such as insulin-growth factor and transforming growth factor (Courties et al., 2016). Finally, synovium inflammation occurs in the adjacent area of cartilage degradation due to cartilage debris. Infiltration of inflammatory cells such as lymphocytes and macrophages are responsible for overproduction of cytokines, oxidative stress and proteolytic enzymes which in turn aggravate cartilage degradation and creating a vicious circle (Sellam et al., 2010) (Figure no -3).
**Figure no 3: A Molecular and cellular mechanism that perpetuate osteoarthritis:**

BMP, bone morphogenetic protein; MMP, matrix metalloproteinase; NO, nitric oxide; PA, plasminogen activator; PG, prostaglandin; TGF, transforming growth factor; TIMP, tissue inhibitor of MMP; TNF, tumor necrosis factor. (Source: Abramson et al., Arthritis Res Ther 2009)
Type 2 Diabetes and Osteoarthritis:

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia due to relative insulin deficiency, or insulin resistance or both and often accompanied by glycosuria, polydipsia, and polyuria (American Diabetes Association, 2005). Depending on the etiology, diabetes mellitus can be divided into two major forms, Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM). T1DM occurs when the insulin-producing \( \beta \)-cells in the pancreas are destroyed, typically by an autoimmune, T cell-mediated mechanism, resulting in the production of insufficient amounts of insulin (Rother, 2007). Type 2 DM is characterized differently and is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion which in some cases becomes absolute. Despite the different pathogenic mechanisms of T1DM and T2DM, they share common symptoms including glucose intolerance, hyperglycemia, hyperlipidemia and similar complications. The effect of diabetes mellitus includes long-term damage, dysfunction, and failure of various organs. Mortality and morbidity in type 2 DM are due to the occurrence of microvascular complications such as diabetic nephropathy, neuropathy, retinopathy and musculoskeletal disorders (MSDs) along with macrovascular complications such as accelerated atherosclerosis causing ischemic heart and cerebrovascular disease (Morrish et al., 2001). In contrast to various vascular complications of diabetes mellitus which are life-threatening, musculoskeletal manifestations lead to considerable morbidity (Sarkar et al., 2003).

Type 2 Diabetes is a frequently co-occurring disease in people with osteoarthritis (Reeuwijk et al., 2010; Louati et al., 2015). In diabetes, hyperglycemia is the main trigger for joint degradation (Berenbaum, 2011). Hyperglycemia induces oxidative stress, overproduction of proinflammatory cytokines and advanced glycation end
products which leads to matrix stiffness, subchondral bone destruction and chondrocytes dysfunction (Zhuo et al., 2012). Hyperglycemia also induces a low-grade systemic inflammation that contributes to a toxic internal environment and exacerbates osteoarthritis (Berenbaum, 2011; Zhuo et al., 2012) (Figure no-4).

**Figure no 4:** A general paradigm for a diabetes-induced osteoarthritis phenotype. (Source: Berenbaum, Ann Rheum Dis. 2011)

**Common Risk factors for Osteoarthritis and Type 2 Diabetes mellitus:**

**Ageing**

Age is one of the recognized links between Osteoarthritis and Type 2 Diabetes Mellitus since they share several common risk factors. The increased risk for OA and T2DM with aging is multifactorial. One factor is the decline in cell function with aging. For example, aging is associated with T2DM because pancreatic beta-cell function declines with aging (Cnop et al., 2001). Aging also causes osteoarthritis. In
Metabolism

OA, senescent chondrocytes are more likely than young chondrocytes to secrete inflammatory mediators involved in cartilage degradation (Berenbaum, 2011). Ageing also promotes cumulative joint loads and consequent cartilage wear and OA. Finally, aging has been attributed to a decline in mitochondrial health and decreased mitochondrial health has been theorized to contribute to both diabetes and cartilage degradation (Harman, 1956; Harman, 1972; Trounce et al., 1989; Goldring, 2000; Kahn et al., 2006).

Obesity

Obesity is increasingly prevalent among individuals with T2DM and participates in the pathogenesis of T2DM. It is also a risk factor for OA. Obesity contributes to the development of OA via biomechanical and systemic pathways. The biomechanical pathway is based on the direct effects of increased body weight. For example, increased body weight imposes greater loads on the weight-bearing joints, which has shown to affect cartilage wear (Felson et al., 1997; Reijman et al., 2007). Excess body weight has also been associated with misalignment of weight-bearing joints (particularly the knee joint), which increases joint stress and promotes cartilage degradation that leads to OA (Sharma et al., 2000). Moreover, obesity has been linked to decreased strength in muscles necessary for joint stabilization and therefore decreased the ability to sustain mechanical joint stress (Slemenda et al., 1998; Syed et al., 2000). Obesity also causes chronic low-grade inflammation in adipose tissue and enhanced expression and secretion of proinflammatory cytokines (e.g., IL-6, IL-1, and TNF-α) as well as adipokines (Goldring, 2000; Fernandes et al., 2002). Adipokines and proinflammatory cytokines such as IL-1 and TNF-α have been shown to mediate the OA pathophysiology, possibly by modulating chondrocyte expression of proteases involved in matrix breakdown.
Hypertension and Dyslipidemia

Hypertension and dyslipidemia, both widely recognized risk factors for T2DM (Fukui et al., 2011). Hypertension and dyslipidemia both have been proposed to contribute to the development of OA (Hart et al., 1995; Velasquez et al., 2010; Zhuo et al., 2012). Hypertension might affect OA via narrowing of blood vessels and subchondral ischemia, which would initiate cartilage degradation (Findlay, 2007; Zhuo et al., 2012). Evidence for the contribution of dyslipidemia in OA is also not conclusive. Increased lipid deposits in the chondrocytes and deregulation of cellular lipid metabolism might initiate osteoarthritis development (Hart et al., 1995). There is also evidence that fatty acids are elevated in OA bone and that excessive intake of polyunsaturated fatty acids is associated with increased risk of bone marrow lesions (Lipiello et al., 1991; Wang et al., 2008). Lipid alterations affect chondrogenesis, osteogenesis, and adipogenesis and may induce abnormalities in mesenchymatous cell differentiation (Aspden et al., 2001) (Figure no-5)

**Figure no 5: Common Risk Factors for Osteoarthritis and Type 2 Diabetes Mellitus** (Source: Piva et al., Clin Geriatr Med 2015)
Metabolic changes link Type 2 Diabetes and Osteoarthritis:

Cartilage health is dependent upon a number of metabolic processes that regulate cartilage growth and nutrition, which is altered can lead to its degradation (Mobasher et al., 2002). Altered glucose metabolism could be a direct link between osteoarthritis and T2DM (Rosa et al., 2009; Velasquez et al., 2010; Berenbaum, 2011; Sellam et al., 2013; Yan et al., 2013). Chondrocytes are glycolytic cells able to sense the concentration of glucose present in the cartilage matrix, the synovial fluid and to a less extent the subchondral bone (Maroudas et al., 1968). By sensing glucose, chondrocytes will then respond appropriately by adjusting their cellular metabolism. Chondrocytes express multiple isoforms of the glucose transporter especially GLUT-1, GLUT-3 and GLUT-9 which represent the first rate-limiting step in glucose utilization (Mobasher et al., 2008). Among them, GLUT-1 is especially important as it is regulated by anabolic and catabolic stimuli (Shikhman et al., 2001). Under glucose deprivation, normal chondrocytes increased GLUT-1 expression and membrane incorporation whereas they decreased under high glucose condition. During OA this capacity of normal chondrocytes to adapt themselves to the local glucose level is lost, results in accumulating more glucose and producing more ROS (Rosa et al., 2009). ROS are harmful to chondrocytes as they favor production of cytokines, such as IL-1β, and transcription factors, such as NF-κB, which give rise to catabolic processes implicated in cell degradation and cell apoptosis (Goldring, 2006). High glucose concentration also decreases dehydroascorbate transport into chondrocytes, which can shift the synthesis pattern of chondrocytes from type II collagen to reactive oxygen species, potentially mediating cartilage destruction (Henrotin et al., 2003; McNulty et al., 2005).
In diabetes, the intracellular glucose concentration is found to be high which promotes the formation of AGEs, the products of non-enzymatic glycation and oxidation of protein and lipid. Advanced glycation end products (AGEs) production and their accumulation in articular cartilage contribute to a toxic environment that might facilitate OA pathogenesis. AGE compounds interact with membrane receptors called RAGE and toll-like receptor give rise to a cascade of events that promote the release of pro-inflammatory and procatabolic factors (Brownlee, 2001). The activation of these receptors in chondrocytes induced a decrease of PPAR-gamma and an activation of Nf-kappa B and MAP kinase pathways (Rasheed et al., 2011; Rasheed et al., 2012; Chen et al., 2013; Wang et al., 2016) which cause inflammation and oxidative stress intracellularly and might promote cartilage degradation (Brownlee, 2001). AGEs accumulation has also been occurring with aging, and in vitro studies demonstrated that it contributes to cartilage stiffness and degradation (Verzijl et al., 2000; Verzijl et al., 2002; Steenvoorden et al., 2006) (Figure No 6 & 7).

AGEs may also contribute to the progression of OA through diabetic peripheral neuropathy; excess accumulation of AGEs may compromise proprioceptive and nociceptive receptors in joint structures (Leaverton et al., 2012). Impaired joint proprioception has been occurring in OA due to dysfunctional articular mechanoreceptors and reduced muscle spindle sensitivity in weak and atrophied muscles around the joints (Knoop et al., 2011). Thus, impaired sensation in OA and diabetic neuropathy may conceal the perception of pain and further perpetuate joint damage by allowing constant harmful mechanical workloads.

Hyperglycaemia can also trigger a low-grade systemic inflammation and have an impact on the progression of osteoarthritis (Berenbaum, 2011) that play role in the progression of osteoarthritis (Yerneni et al., 1999; Esposito et al., 2002; Ling et al.,
Low-grade systemic inflammation is directly linked to cartilage loss and also exacerbates the oxidative stress that leads to local toxicity (Stannus et al., 2010).

Figure no 6: Interaction of AGE with RAGE leading to oxidative stress and initiation of inflammation cascade which ultimately leads to diabetic complication. (Source: Singh et al., J Physiol Pharmacol 2014)

Figure no 7: Effect of hyperglycemia on chondrocytes. (Source: Courties et al., Diabetes Res Clin Pract 2016)
Osteoarthritis and Menopause

The prevalence of osteoarthritis in both men and women is similar up to the age of 50 years, but after the age of 50, the disease becomes more prevalent, severe and generalized in women (Lawrence et al., 1966; Tsai et al., 1992). For the first time Cecil et al., 1925 described women develop “menopausal arthritis” which consists of the rapid development of hand and knee osteoarthritis coinciding with cessation of menses and suggested estrogens may play a role in osteoarthritis. However, studies of the prevalence and incidence of osteoarthritis in postmenopausal women with and without hormone replacement therapy (HRT) have also provided strong support for a beneficial effect of estrogens in osteoarthritis. Subsequently, the identification of the two estrogen receptors alpha and beta (ERα and Erβ) in chondrocytes provided further evidence that the cartilage is sensitive to estrogens (Ushiyama et al., 1999). Several in vitro studies and in vivo animal experiments confirmed that chondrocytes respond to estrogens and also provided insight into the mechanisms by which estrogens may influence chondrocyte metabolism. Estrogen may affect tissue directly by acting on estrogen receptor found on human articular chondrocytes or indirectly using secondary messenger (Tsai et al., 1992). Estrogen has been shown to affect cytokine levels both in vitro and in vivo and affect cartilage metabolism. The production of IL-6 by human chondrocytes is affected by estradiol, suggesting a possible mechanism whereby it may affect cartilage metabolism (Guerne et al., 1990). Furthermore, polymorphisms in the ER-alpha gene have been suggested to be associated with radiographic osteoarthritis of the knee (Bergink et al., 2003; Jin et al., 2004). Estrogen also influences the risk of osteoarthritis by protection against vascular defects in subchondral bone, greater neuromuscular protection against excessive joint loading,
and through its antioxidant potential. The effect of estrogen and its periarticular structure is shown in Table No-1.

**Table No 1: Effect of estrogen on joint and periarticular tissue**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Main effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartilage</td>
<td>Increases PG/glycosaminoglycan synthesis</td>
</tr>
<tr>
<td></td>
<td>Decreases NF-kB, iNOS, COX-2, ROS</td>
</tr>
<tr>
<td></td>
<td>Regulates intracellular calcium concentration</td>
</tr>
<tr>
<td></td>
<td>Decreases cartilage damage</td>
</tr>
<tr>
<td></td>
<td>Decreases cyclooxygenase-2 mRNA expression</td>
</tr>
<tr>
<td></td>
<td>High doses increases PG degradation and metalloproteinase production</td>
</tr>
<tr>
<td>Subchondral bone (SB)</td>
<td>Regulates bone growth and SB remodeling</td>
</tr>
<tr>
<td></td>
<td>Controls OB development and function</td>
</tr>
<tr>
<td></td>
<td>Regulates matrix production and mineralization</td>
</tr>
<tr>
<td></td>
<td>Reduces bone formation and prevalence of marginal osteophytes in OVX monkeys</td>
</tr>
<tr>
<td></td>
<td>Decreases MRI likelihood of bone attrition in women</td>
</tr>
<tr>
<td>Synovium</td>
<td>Increases synovial levels of components of the IGF pathway in animals</td>
</tr>
<tr>
<td></td>
<td>Reverses autoimmune arthritis development in mice</td>
</tr>
<tr>
<td></td>
<td>Decreases RF, anti-ds-DNA and anti-type II collagen serum levels in mice</td>
</tr>
<tr>
<td>Muscles</td>
<td>Promotes myoblasts proliferation and differentiation</td>
</tr>
<tr>
<td></td>
<td>Decreases muscle cell apoptosis</td>
</tr>
<tr>
<td></td>
<td>Reverses muscle contractile dysfunction in rats</td>
</tr>
<tr>
<td></td>
<td>Decreases muscle atrophy and calpain levels in rats</td>
</tr>
<tr>
<td></td>
<td>Enhances muscle performance and structure in women</td>
</tr>
<tr>
<td>Ligaments</td>
<td>Contrasting effects on ACL mechanical properties in animal models</td>
</tr>
<tr>
<td></td>
<td>High estrogen levels during menstrual cycle have been associated with ACL ruptures in young women</td>
</tr>
</tbody>
</table>

ACL: anterior cruciate ligament; COX-2: cyclooxygenase-2; IGF: insulin-like growth factor; iNOS: inducible nitric oxide synthase; MRI: magnetic resonance imaging; OB: osteoblasts; OVX: ovariectomized; PG: proteoglycan; RF: rheumatoid factor; anti-ds-DNA: anti-double-stranded DNA. (Source: Martin-Millan et al., Joint Bone Spine 2012)
Adhesion molecules

Cell adhesion molecules are membrane-associated cell surface glycoproteins that are involved in either cell-cell or cell-matrix interactions. They can be subdivided into four different groups, which are categorized on the basis of structures and functional characteristics: the cadherins, integrins, selectins, and immunoglobulin (Ig)-like proteins (Takeichi, 1990; Reichardt et al., 1991; Lasky, 1995; Brümmendorf et al., 1996). Leukocyte/endothelial cell adhesion molecules play essential roles in many immune and inflammatory responses, including leukocyte migration and activation, lymphocyte homing and recirculation, platelet adhesion, and phagocytosis (Springer 1994; Langer et al., 2009). These molecules also participate in the pathogenesis of inflammatory diseases including atherosclerosis, rheumatoid arthritis, systemic lupus erythematosus (SLE), psoriasis, diabetes, multiple sclerosis, inflammatory bowel diseases, osteoarthritis as well as other disorders (Bevilacqua et al., 1994; McMurray, 1996; Vainer, 1997; Kevil et al., 1999). The other form of adhesion molecule is soluble cell adhesion molecules. These molecules can be derived from cell surface adhesion molecules following cell stimulation or activation. Alternatively, soluble adhesion molecules can result from the alternate splicing of the adhesion molecule gene and are then directly released from the cell without first being in or on the cell membrane (Volin, 2005). Soluble forms of adhesion molecules are distinguished as sICAM-1, sVCAM-1, sE-selectin and sP-selectin. The cellular expression of CAMs is difficult to assess clinically, but these soluble forms are present in the circulation and may serve as markers for CAMs (Abe et al., 1998).
### Table No 2: Leukocyte/endothelial cell adhesion molecules (Source: Bullard, Immunol Res 2002)

<table>
<thead>
<tr>
<th>Adhesion molecule</th>
<th>Primary expression</th>
<th>Major ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin</td>
<td>Endothelial cells, platelets</td>
<td>PSGL-1</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Endothelial cells</td>
<td>PSGL-1, ESL-1</td>
</tr>
<tr>
<td>L-selectin</td>
<td>Polymorphonuclear leukocytes, monocytes, majority of lymphocytes</td>
<td>CD34, GLYCAM-1</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Endothelial cells, lymphocytes, fibroblasts, other</td>
<td>LFA-1, Mac-1</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>Endothelial cells, lymphocytes</td>
<td>LFA-1</td>
</tr>
<tr>
<td>ICAM-3</td>
<td>Polymorphonuclear leukocytes, monocytes, lymphocytes</td>
<td>LFA-1, CD11d/CD18</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Endothelial cells</td>
<td>VLA-4</td>
</tr>
<tr>
<td>MAdCAM-1</td>
<td>Endothelial cells</td>
<td>o4β7</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>Endothelial cells, platelets, polymorphonuclear leukocytes, lymphocytes</td>
<td>PECAM-1</td>
</tr>
<tr>
<td>LFA-1</td>
<td>Polymorphonuclear leukocytes, monocytes, lymphocytes, other</td>
<td>ICAM-1, -2, -3</td>
</tr>
<tr>
<td>Mac-1</td>
<td>Polymorphonuclear leukocytes, monocytes, other</td>
<td>ICAM-1, Factor X, iC3b, fibrinogen</td>
</tr>
<tr>
<td>p150/95</td>
<td>Monocytes, polymorphonuclear leukocytes, dendritic cells</td>
<td>ICAM-1, iC3b, fibrinogen</td>
</tr>
<tr>
<td>CD11d</td>
<td>Polymorphonuclear leukocytes, lymphocytes, monocytes, specialized macrophages</td>
<td>ICAM-3, VCAM-1</td>
</tr>
<tr>
<td>VLA-4</td>
<td>Lymphocytes, monocytes</td>
<td>VCAM-1, fibronectin</td>
</tr>
<tr>
<td>VAP-1</td>
<td>Endothelial cells</td>
<td>Unknown</td>
</tr>
<tr>
<td>CD44</td>
<td>Lymphocytes, monocytes</td>
<td>Hyaluronan</td>
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Vascular Cell Adhesion Molecule 1

VCAM-1 was originally identified as an inducible molecule on human umbilical vein endothelial cells (HUVEC) that is capable of binding lymphoid and tumor cell lines (Osborn et al., 1989; Rice et al., 1989). DNA sequence analysis revealed that VCAM-1 belongs to the immunoglobulin superfamily of cell adhesion molecules and is expressed primarily on the endothelial cells. It is glycoprotein in nature. The molecular weight of VCAM-1 is 110 kD. It is not appreciably expressed on resting vascular endothelium but is rapidly induced in response to a number of inflammatory stimuli, such as TNF-α, IL-1, lipopolysaccharide and IL-4 (Osborn et al., 1989; Thornhill et al., 1991; Carlos et al., 1994). It is an important mediator of leukocyte recruitment to the sites of inflammation. VCAM-1 up-regulation has been shown in various inflammatory diseases including atherosclerosis, rheumatoid arthritis, and osteoarthritis. When endothelial cells are activated by inflammatory cytokines, soluble isoform of VCAM-1 has been generated. The generation of sVCAM-1 is consistent with proteolytic cleavage at a site close to the point of membrane insertion, although the mechanisms involved are unknown. Recently, TACE (ADAM 17) as the enzyme is identified responsible for phorbol myristate acetate (PMA)-induced cleaving of soluble VCAM-1 from the membrane of murine endothelial cells (Garton et al., 2003). Alternatively, in rodents, alternate splicing can result in a mRNA containing only the first three of seven immunoglobulin domains that compose VCAM-1. The product of this smaller VCAM-1 transcript is expressed on endothelial cells as a GPI-anchored molecule that mediates endothelial-leukocyte adhesion by binding VLA-4. A soluble form of this GPI-anchored VCAM-1 can be cleaved from the cell surface by phosphatidyl-inositol specific phospholipase C (Terry et al., 1993). It is not yet known if there is a human GPI-anchored VCAM-1. The soluble isoforms
Metabolism

of VCAM-1 (sVCAM-1) have been found in human serum, in the culture supernatant of cytokine-treated endothelial cells and in the synovial fluid of patients with rheumatoid arthritis or osteoarthritis. Soluble VCAM-1 has an approximate molecular weight of 85-90 kD. Soluble VCAM-1 can function as a chemoattractant for T lymphocytes (Kitani et al., 1998) and monocytes (Tokuhira et al., 2000) and may promote angiogenesis (Koch et al., 1995).

Structural characterization of VCAM-1:

Structurally, these proteins consist of an extracellular region comprising a varying number of Ig like homology domains that contain disulfide-linked loops, a single type I transmembrane domain, and a 19 amino acid carboxyl-terminus cytoplasmic domain (Osborn et al., 1989; Kumar et al., 1994) (Figure no-8). Human VCAM-1 comprises a large extracellular region consists of seven Ig-like domains containing a number of N-linked glycosylation sites. There is homology within these Ig-like domains, such as domains 1 and 4 have sequence homology, domains 2 and 5 have sequence homology, and domains 3 and 6 have sequence homology (Hession et al., 1991; Polte et al., 1991; Cybulsky et al., 2003). The cytoplasmic domain containing a number of consensus sites for potential serine/threonine phosphorylation (Kishimoto et al., 1985; Woodgett et al., 1986; Pinna et al., 1990) follows a single transmembrane region of hydrophobic residues; however, it is not known whether the cytoplasmic tail of VCAM-1 interacts with signaling pathways. The amino acid sequence of this cytoplasmic domain is 100% identical among several species including rat, mouse, human, and rabbit (Polte et al., 1991; Hession et al., 1992; Pepinsky et al., 1992; Banning et al., 2004; Gibbs et al., 2004). Human VCAM-1’s also has another splice variant that contains six Ig-like domains in the extracellular region. This human
VCAM-1 lacks domain 4 in its structure (Cybulsky et al., 1991; Cybulsky et al., 1991) (Figure no-9).

**Figure no 8:** General structure of vascular cell adhesion molecule-1 (VCAM-1) and related immunoglobulin superfamily molecules. The cytoplasmic domain (C-terminus) is indicated as COOH (Source: Carter et al., Arthritis Rheum 2001)

**Figure no 9:** VCAM-1 splice variants- Human VCAM-1 has two splice variants that contain either six or seven immunoglobulin-like domains with disulfide linkages (Source: Barthel et al., J Leukoc Biol 2008)
Ligands and VCAM-1 binding regions:

The predominant receptor for VCAM-1 is the integrin α4β1 (very late activation antigen 4 ([VLA-4] [CD49d/CD29]), which is expressed by eosinophils, basophils, lymphocytes, mast cells, and monocytes (Elices et al., 1990). α4β1 integrin also binds to fibronectin, heparin, and junction adhesion molecule-B in endothelial junctions (Qasem et al., 2008; Ludwig et al., 2009; Schwinn et al., 2010). In addition to α4β1, VCAM-1 can bind to other integrins such as α4β7 integrin and αd β2 integrin (Ruegg et al., 1992; Chan et al., 1992; Grayson et al., 1998). X-ray crystallography and mutagenesis studies have revealed that integrin binding sites are located in domains 1 and 4 of VCAM-1 molecule. Amino acid substitution analyses defined a conserved 6–amino acid sequence critical for integrin binding in both domains 1 and 4 (Osborn et al., 1992; Osborn, 1994; Vonderheide et al., 1994). Similar motifs are involved in integrin binding to other IgSF adhesion molecules (Shimizu et al., 1999). Domain 2 provides structural stabilization and may facilitate ligand binding (Pepinsky et al., 1992; Osborn et al., 1992; Vonderheide et al., 1994). The binding to VCAM-1 is regulated by the activation state of the integrins (Chan et al., 2003; Kilger et al., 1995). Integrins at low-affinity roll on VCAM-1, whereas the high-affinity conformation of the integrins mediates firm adhesion to the endothelium that can withstand the force of the blood flow (Alon et al., 1995; Gerszten et al., 1996; Weber et al., 1996). In addition, integrin binding to domains 1 versus domain 4 of VCAM-1 is modulated by the degree of activation of the α4β1 integrin. The α4β1 integrin binding to domain 4 has a higher requirement for activation than for binding to domain 1. Moreover, α4β1 integrin versus α4β7 integrin differs in their activation requirements for binding to domains 1 and 4 of VCAM-1 ((Kilger et al., 1995). For half-maximal binding to domain 4 of VCAM-1, α4β1 integrin requires significantly
higher activating concentrations of divalent cations than α4β7 integrin (Kilger et al., 1995). The binding activity of α4β1 integrin to domain 1 of VCAM-1 is also regulated by CD24 expression (Kilger et al., 1995). Moreover, α4β7 integrin binding to VCAM-1 requires a higher activation state than for its binding to the mucosal addressin cell adhesion molecule-1, an endothelial cell adhesion molecule (Berlin et al., 1993). Thus, the α4-integrins bind to two domains of VCAM-1 and this binding to VCAM-1 domains is regulated by the activation state of the integrins. In addition to integrins, VCAM-1 can also bind galectin-3 (Figure no-10).

Figure No 10: Ligand binding to VCAM-1: Integrin binding to VCAM-1 is regulated by the integrin activation state. Solid arrow shows major ligand binding site. Dashed arrow, ligand binding requires higher integrin activation. Largely filled arrow, galectin3 binds to N-glycosylation sites. ADAM, a disintegrin and metalloprotease. (Source: Cook-Mills et al., Antioxid Redox Signal 2011)
Regulation of VCAM-1 expression:

VCAM-1 functions in combination with other adhesion molecules to regulate immune surveillance and inflammatory joint diseases. VCAM-1 is not appreciably expressed on resting vascular endothelium but is rapidly induced in response to a number of inflammatory stimuli, such as TNF-a, IL-1, IL-4, high levels of ROS, oxidized low density lipoprotein (oxLDL), 25-hydroxycholesterol, turbulent shear stress, high glucose, and microbial stimulation of endothelial cell TLRs (Osborn et al., 1989; Carlos et al., 1990; Thornhill et al., 1991; Willam et al., 1999; Yoshida et al., 2000; Naito et al., 2005) Upregulation of adhesion molecule expression appears to involve both transcriptional and posttranscriptional mechanisms. The promoters of VCAM-1 include binding motifs for the transcription factors nuclear factor-kB (NF-kB) and activator protein 1 (AP-1). Many of the agents such as proinflammatory cytokines upregulate VCAM-1 expression probably do so by activation of transcription factors. Similarly, many agents such as anti-inflammatory cytokines suppress VCAM-1 expression by inhibition of transcription factor activity (Carter et al., 2001). In human umbilical vein endothelial cells, regulation of VCAM-1 expression occurs primarily at the level of transcription. Using human endothelial VCAM-1 constructs in transfection assays, Neish et al., 1992 characterized a small region of the VCAM-1 promoter that was capable of directing cytokine-induced gene expression. Iademarco et al., 1993 found octamers within this cytokine-responsive region that bind the pit-1, oct-1, oct-2, and unc-86 (POU) family of transcription factors and function as transcriptional silencers, thereby preventing VCAM-1 induction in unstimulated human umbilical vein endothelial cells. Cytokine stimulation overcomes the negative influence of POU octamers by activating promoter activity through 2 adjacent NF-kB binding sites (Iademarco et al., 1992; Iademarco et al., 1993). NF-kB is critical for
endothelial VCAM-1 expression since deletion or mutation of the NF-kB sites abolishes cytokine-inducible VCAM-1 transcription (Shu et al., 1993). The NF-kB proteins are a family of ubiquitously expressed transcription factors that play an essential role in most immune and inflammatory responses. In mammals, the NF-kB family consists of five members: RelA (p65), RelB, c-Rel, NF-kB1 (p50 and its precursor p105), and NF-kB2 (p52 and its precursor p100). They form a variety of homodimers and heterodimers, each of which activates its own characteristic set of genes, and share a 300-amino acid domain that is designated the Rel homology domain which mediates their DNA binding, dimerization and nuclear translocation (Silverman et al., 2001; Li et al., 2002; Ghosh et al., 2002; Carlsen et al., 2004; Bouwmeester et al., 2004). Although the most prevalent activated form is the heterodimer RelA (p65) and p50, different dimers can bind to the same or distinct sites in NF-kB-dependent promoters regulating the transcription of response genes in a cell-type and stimulus-type manner (Udalova et al., 2002; Dejardin et al., 2002). Electrophoretic mobility shift assays indicate that p65 alone and p65 in combination with p50 bind to the human VCAM-1 promoter in TNFa-stimulated human umbilical vein endothelial cells (Shu et al., 1993) (Figure no 11).

The AP-1 site on the endothelial VCAM-1 promoter also appears necessary for transcriptional activation by several agents that do not affect NF-kB activity. For example, crosslinking of ICAM-1 on human umbilical vein endothelial cells and CD44 on synovial lining cells result in AP-1 activation and subsequent VCAM-1 induction, independent of NF-kB (Fujii et al., 1999; Lawson et al., 1999).

Fibroblast-like synovial lining cells constitutively express VCAM-1. The molecular basis of constitutive VCAM-1 expression is not well understood. Iademarco et al., 1993 used a series of mutated VCAM-1 promoter constructs in TNFa-stimulated
umbilical vein endothelial cells and a skeletal myoblast cell line that constitutively expresses high levels of VCAM-1. These studies revealed that an enhancer element present in the muscle VCAM-1 promoter (but not detected in the endothelial promoter constructs) overrides the activity of octamer and NF-kB sites, resulting in constitutive VCAM-1 expression. Mutation analyses and gel retardation studies suggest that the activity of the enhancer element is controlled by a distinct, but as yet uncharacterized, transcription factor (Iademarco et al., 1993).

Figure no 11: Regulation of VCAM-1 expression: (Source: Asgeirsdottir et al., Am J Physiol 2012)
Role of VCAM-1 in Osteoarthritis:

Vascular cell adhesion molecule-1 play an important role in the pathogenesis of inflammatory joint disease by adhesion of leukocyte to activated endothelium, retention of infiltrating leukocytes within extravascular compartments, angiogenesis, invasion of pannus, T and B lymphocyte activation, and survival within the synovial compartment (Carter et al., 2001). Osteoarthritis is firstly described as a non-inflammatory disease in order to distinguish it from ‘inflammatory arthritis’, such as rheumatoid arthritis or the seronegative spondyloarthropathies. But now it becomes evident that inflammation is increasingly recognized as contributing to the symptoms and progression of osteoarthritis (Spector et al., 1997; Conrozier et al., 1998). Inflammation may be both a primary event in osteoarthritis and secondary to other aspects of the disease, such as biochemical changes within the cartilage. Histological and serological evidence of osteoarthritis showed that synovitis is an early feature in osteoarthritis and not restricted to the patient with end-stage disease (Spector et al., 1997; Sowers et al., 2002; Haywood et al., 2003). Although the pathogenesis of the disease remains elusive, there is increasing evidence indicating that mononuclear cells migration plays an important role in the perpetuation of inflammation in synovium. VCAM-1 is involved in the process of adhesion and infiltration of synovium with mononuclear cells leading to the initiation and progression of the disease. In joints, VCAM-1 is locally expressed on endothelial cells, fibroblasts, and chondrocytes (Kienzle et al., 1998). In chondrocytes, pro-inflammatory cytokines such as interleukin 1, tumor necrosis factor alpha and hyaluronic acid released during articular cartilage damage induces VCAM-1 production (Osborn et al., 1989; Oertil et al., 1998). Then, chondrocytes interact with immune cells through VCAM-1 and thus VCAM-1 could thus by itself contributes to immune-mediated cartilage damage in
osteoarthritis (Kienzle et al., 1998). Up-regulation of VCAM-1 has also been shown in the synovial lining of osteoarthritis patients by immunohistochemical staining and in cultured human osteoarthritis synovial fibroblasts by Western blotting (Schett et al., 2009; Kalichman et al., 2011). Reduction in the levels of VCAM-1 in synovial fluid may suppress the inflammatory response in osteoarthritis (Karatay et al., 2004). The vascular recruitment of leukocytes into the inflamed synovium is a three-step process involving low affinity rolling of leukocytes on the endothelium followed by the arrest of the leukocyte on the endothelium through high-affinity adhesion, and the transmigration of the leukocyte through the endothelium (Figure no-12).

![Figure no 12: Leukocyte transendothelial migration](image)

**Figure no 12: Leukocyte transendothelial migration:** During inflammation, cytokines produced in the tissue induce endothelial cell adhesion molecule expression. In addition, chemoattractants released by both the tissue and endothelial cells increase leukocyte adhesion molecule affinity as well as provide direction for
leukocyte. (Source: Cook-Mills et al., Antioxid Redox Signal 2011)

Soluble VCAM-1 also induced chemotaxis of human endothelial cells in vitro and possesses angiogenic activity in rat cornea, which is an in vivo model of angiogenesis (Koch et al., 1995). Angiogenesis is the growth of new capillary blood vessels from pre-existing vasculature. Angiogenesis is a critical event in inflammatory processes and it appears to be strongly correlated with synovial hyperplasia, which is frequently observed in OA-affected joints (Mapp et al., 2012). This process occurs at the osteochondral junction as well as within the osteoarthritic synovium (Pufe et al., 2001). Angiogenesis with increased neo-formation of sensory fibers underlines the involvement of this process not only in joint changes but in addition, facilitate pain through structural reorganization of the joint (Mapp et al., 2012; Walsh et al., 2010).