2.1 Structure and function of the knee joint

The knee joint is essentially composed of four main bones; the femur, tibia, fibula and patella. The knee joint comprises two different articulations between the femur and tibia, and between the femur and the patella forming the femoro tibial and patella femoral joints respectively. Each joint is held in the correct plane by a series of ligaments and muscles with associated tendons. The knee is complemented with a selection of ligaments including the anterior and posterior cruciate, and the medial and lateral collateral ligaments. These serve to strengthen the knee structure as well as place restraints on the range of movements through which it can travel. Due to its location within the human skeleton, and the fact humans are bipeds, the knee joint is constantly exposed to varying forces which it must cushion and absorb to prevent the formation of pathological stresses. To cushion joint load, the articular surfaces are covered in cartilage, and the knee is equipped with the medial and lateral menisci which sit between the two articular surfaces of the femur and tibia (Bendjaballah et al. 1995).
2.2 Normal cartilage structure and function

Primarily the articular cartilage is composed of extracellular matrix with sparse population of cells, lacking the blood vessels, lymphatic vessels and nerves (Buckwalter et al. 2005; Bullough 2007). Articular cartilage cannot heal itself easily once it is damaged because it is avascular (Yuehuei et al. 2003; Katta et al. 2008). The metabolic activity is low and there is little or no cell division or cell death in normal articular cartilage, though the articular chondrocytes are capable of cell division (Aigner et al. 2004; Buckwalter et al. 2005; Loeser, 2009; 2010; Shane and Loeser, 2010). The chondrocytes which makes up only about 1% volume of adult human articular cartilage (Buckwalter et al. 2005) is the one type of cell present in the articular cartilage and they are responsible for the synthesis and degradation of the cartilaginous matrix (Buckwalter et al. 2005; Goldring et al. 2007; Loeser RF, 2009). Chondrocytes gets their nutrients from the synovial fluid by diffusion, compression and relaxation of tissue leading to fluid exudation and uptake (Yuehuei et al. 2003). The mechanism between the synthesis and degradation of the articular cartilage is not clearly understood but cytokines having both the anabolic and catabolic effects seems to have an important roles (Buckwalter et al. 2005; Goldring et al. 2007). To exert a normal function for the articular cartilage within the joint, it requires to be elastic and have tensile strength. The unique mechanical properties of articular cartilage depends upon the extracellular matrix (Buckwalter et al. 2005). The extracellular matrix consist of two components, the tissue fluid and the structural macromolecule consisting of collagen fibers type II, proteoglycans and non-collagenous proteins and glycoproteins, produced in the appropriate amounts and
assembled and organized into a highly ordered molecular framework by the chondrocytes (Buckwalter et al. 2005; Bullough, 2007). The collagen matrix gives the cartilage its tensile strength and form. Proteoglycan and non-collagenous proteins binds to the collagenous network and thus helps to stabilize the matrix macromolecular framework and helps the chondrocytes to bind the macromolecule of the network (Buckwalter et al. 2005). Aggrecan, the most abundant PG found in articular cartilage, is composed of a protein backbone bound by negatively-charged chondroitin sulfate and keratin sulfate groups. It binds with HA to form complexes within the ECM. Due to negatively charged sulfated groups, these complexes electrostatically interact with cations, ultimately forming ion-dipole interactions with water, allowing cartilage to function as a hydrated tissue that resists compression.

2.3 Epidemiology

Prevalence of osteoarthritis is difficult to determine because the clinical symptoms of OA (joint pain, swelling and stiffness) does not always corresponds with the structural abnormalities of the OA when seen radiographically (NICE, 2008). Limited literatures are available on the incidence and prevalence of OA because of the problems of defining and determining its onset (Wolf and Pfleger, 1990). Prevalence of pain associated with joint degeneration varies among joints and among individuals (Buckwalter et al. 2008). OA, being one of the most prevalent diseases in our aging population. The incidence of OA is predicted to increase as the senior population grows, placing a significant financial burden on healthcare providers and governments. In Asia, the percentage of people aged 65 years and above will be more than double rising from 6.8% in 2008 to 16.2% in 2040. India has shown an increase
by 274% (Kinsella and Wan, 2009). The prevalence of osteoarthritis increases with age and is higher in women than in men, especially among the elderly (Van et al.1988). A worldwide estimate indicates that 9.6% of men and 18% of women ≥ 60 years have symptomatic OA (Wolf AD et al.1990). OA was more in women (65%) compared to men (34.3%) (Pushpa et al.2012), study done by Iqbal et al. (2011) also observed that OA was more in women (74%) compared to men (26%) and a similar observation was also made by a study done (Sharma et al. 2007) which was 70.1% vs 41.6%. Prevalence of OA increases in females during premenopausal age and remains though out menopause indicating a loss of estrogen at the time of menopause increases a women’s risk of getting OA (Spector and Campion, 1989). As per a report published by Times of India (2010), over 40% of the Indian populations in the age group of 70 years or above suffer from OA (Dinesh et al.2013) with 5.3% of males and 4.8% of females are aged more than 65 years(Gupta,2001) Nearly 2% of these undergo severe knee pain and disability. According to Piramal Healthcare Limited in a nationwide campaign against chronic diseases, India is expected to be the chronic disease capital, with 60 million people with arthritis, by 2025. A study conducted from year 1997-2003 showed a prevalence of 22-39% of OA in India (Chopra et al.1997, Chopra et al.2001,Mahajan et al. 2003) and by 2009 the prevalence increased by 24.9% (Aggarwal,2009). According to a community based survey data from rural and urban areas of India showed the prevalence to be 17 - 60.6% (Sharma et al, 2007). Significant higher prevalence of knee pain was found in the rural areas (13.7%) compared with the urban (6%) (Joshi and Chopra, 2009). A community-based cross-sectional study was carried out in an urban resettlement colony in South Delhi showed a prevalence of knee OA in women to be 47.3% in aged group ≥40 years(Salve et al.2010). The number of arthritic persons and the
ensuing social impact are projected to increase by 40% in the next 25 years (Hootman et al. 2006).

Most of the prevalence surveys on the frequency of osteoarthritis have focused on radiological disease and on osteoarthritis per joint group. Incidence rates for osteoarthritis at multiple sites are not known. When the diagnosis is based on clinical signs and symptoms alone, the prevalence among adults is found to be lower, at 10%. The radiological demonstration of typical signs of osteoarthritis of the knee is not correlated with symptoms: Only about 15% of patients with radiologically demonstrated knee osteoarthritis complain of knee pain (Hannan et al. 2000). The incidence rate of symptomatic osteoarthritis at different joint groups according to age and sex, based on the American College of Rheumatology (ACR) criteria has reported that 100/100,000 person-years in the hands, 88/100,000 person-years in the hips and 240/100,000 in the knees (Oliveria et al. 1995). A report by the Clearwater Osteoarthritis Study indicated that patients with radiographic knee OA with elevated body mass index (BMI) had a greater possibility of knee pain when compared with those with normal BMI (Sharma et al. 2001). A more specific and well as sensitive method for knee assessment like MRI are required to further elucidate the prevalence and risk factor of the disease (Yusuf et al. 2011).

Future changes in the incidence and prevalence of OA are difficult to predict. As incidence and prevalence rise with increasing age and extending life expectancy will result in greater numbers with OA. The burden will be the greatest in developing countries where improvements in life expectancy are expected but access to arthroplasty and joint replacement is not readily available. Due to the paucity of data
on time trends in incidence rates for osteoarthritis, these rates have been assumed to be stable over time. Prevalence studies over the last 30 years were used to assess regional prevalence rates.

2.4 Risk factors

Multiple studies have been done on the risk factors for OA. Classically they are divided into two categories a) systemic factors, which are associated with the development of OA and b) local factors, which affect biomechanical loading of the joint (Garstang and Stitik, 2006).

2.4.1 Systemic risk factor

Systemic risk factors are thought to increase the overall susceptibility to degenerative joint changes and to decrease the effectiveness of the reparative response resulting in a predisposition to OA.

Age: Aging is one of the strongest risk factor for development of OA in all the joints (Creamer et al. 1997; Felson et al. 1998). Although age related changes in the articular cartilage plays an important role, the mechanism by which age increases the susceptibility to joint degeneration are largely not known. The development of OA starts with the failure of cartilage collagen network (Verzijl et al. 2002). With increasing age, the stiffness of the collagen network in articular cartilage increases resulting in an increase susceptibility to mechanical induced damage (Grushko et al. 1989; Basser et al. 1998). The tensile properties of the articular cartilage showed significant changes with increasing age (Kempson, 1982; 1991) indicating the resistance of the collagen network to fatigue decreases with increasing age (Freeman,
1975; Weightman, 1976). Decrease response of chondrocyte to repair stimuli which could be due to an imbalance between chondrocyte anabolic and degradative activity leading to progressive thinning of articular cartilage (Buckwalter et al. 2000). The size of the proteoglycan (molecules that consist of central protein cores with multiple covalently bound chondroitin and keratan sulfate chains and that give articular cartilage its stiffness to compression, resilience and durability) aggregates decreases significantly with age (Buckwalter et al. 1985). Other mechanism which are less cartilage oriented such as decrease proprioception and muscle strength or unstable articulation with aging could also be implicated (Sharma et al. 1997; 2003). Hence, it appears that the increased prevalence and incidence of OA with aging is multifactorial resulting in an increased susceptibility to other risk factor of OA (Zhang and Jorden; Loeser, 2010).

**Gender and Estrogen**

Women are more susceptible to OA than men and they also have higher disease severity (Srikanth et al. 2005) during the menopausal age. Significant increase of OA in women around the time of menopause has led to multiple investigations that hormonal factors may play a role in the development of OA. Some of the studies have shown that loss of estrogen at the time of menopause increases the women’s risk of getting OA (Spector and Campion, 1989). The result from endogenous or exogenous effect of estrogen on OA from observational studies have been conflicting (Wluka et al. 2000; Hannan et al. 1990; Nevitt et al. 1996). However, there are increasing evidences that activity of the joint tissues are influenced by estrogen acting at multiple levels through complex molecular pathways (Roman-Blas et al. 2009). Study by Framingham concluded that the intake of estrogen in women had a modest but
insignificant effect on both radiographic OA and severe radiographic OA (Hannan et al. 1990). On the other hand, data from the Women’s Health Initiative reported that women on estrogen replacement therapy were less likely to require total knee or hip replacement (Cirillo et al. 2006; Nevitt et al. 1996). With long term use of estrogen the benefit seemed to increase however those who had taken for ten years or longer showed a decrease in the risk for hip OA but there was no significant decrease in disease symptoms (Nevitt et al. 1996; 2001). Moreover data on endogenous hormones, age at menarche/menopause etc in the hand, hip and knee OA showed no relationship between OA and female hormonal aspects. Thus it seems that further research is required to elucidate the complex role of estrogen in the pathophysiology of OA.

**Race/ethnicity**

Many studies have supported the role of ethnicity in the development of OA based on the racial and ethnic groups (Zhang et al. 2010). Both hip and hand OA was found to be infrequent among the Chinese in the Beijing Osteoarthritis Study when compared to the whites in the NHANES I study and Framingham study but Chinese women showed a higher prevalence of both radiographic and symptomatic knee OA by about 45% compared to the Framingham study (Nevitt et al. 2002; Zhang et al.; Zhang et al. 2001). Increased prevalence of Knee OA in Chinese women could be due to increase manual labour and crouching (Zhang et al. 2004). Report estimated by the Johnston County Osteoarthritis Project have shown that the prevalence of hip OA in African women (23%) was similar to that in white women (22%) and the 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 OA is inherited and genetics have a strong influence on the incidence of OA (Stecher, 1941). Osteoarthritis is multifactorial and polygenic disease in which environmental factors are key modulators of gene expression (Loughlin, 2001). Studies from the early 1960s have reported that first degree relatives are twice as likely to have radiographic generalized OA (Kellgren et al., 1963). Twin and family studies have estimated large genetic influences between 50-65% for hand, hip and knee OA (Spector et al., 1996; Palotie et al., 1989; Felson and Zhang, 1998). In a genome wide association study it was found that the C allele of rs3815148 on chromosome 7q22 was associated with a 1.14 fold increased prevalence of knee or hand OA and also with a 30% increased risk of knee OA progression (Kerkhof et al., 2010). The presence of osteophytes was the most sensitive biomarker of the hand OA heritability in a population with the clinical and radiographic hand OA and their siblings (Ishimori et al., 2010). A popular method to investigate the genetic etiology of OA is genetic association studies (Valdes and Spector, 2008). A comprehensive and systematic assessment of the current association studies (GAS) for OA was analyzed and catalogued in CUMAGAS-OSTEO, a web based information system reported that 19 gene variants significantly increases the risk for OA by 30% or GDF5 and LRCH1 being the most important variants (Zintzaras et al., 2010). Thus genetic markers can be used to identify individuals at high risk OA and also for the risk of total joint arthroplasty which may facilitate the application of preventive and disease management strategies (Valdes and Spector, 2010).
Nutritional factors

Nutritional factors have been the subject of considerable interest in OA though the results are conflicting. Vitamin D is one of the most important factor for development and remodeling of the bone. It has been suspected that since Vitamin D influences bone quality, so its status could have an effect on tests in the risk of the development/progression of OA (Bergink et al. 2009). The Framingham study subjects in the highest and the middle tertile of 25-hydroxy vitamin D showed 3 fold decrease risk for radiographic knee OA compared to those with lower intake (Mc et al. 1996). The Rotterdam study also observed that low dietary intake of vitamin D increases the risk of progression of knee OA especially in patients with low baseline bone mineral density (Bergink et al. 2009). Another study also found out that serum 25-hydroxy vitamin D levels and exposure to sunlight are associated with decrease loss of cartilage of the knee when assessed by MRI (Ding et al. 2009). Moreover a study concluded that low levels of serum 25 hydroxy vitamin D associated with increase incidence of hip OA (Lane et al. 1999). However, a study on bone turnover, vitamin D and calcium regulation in Caucasian twin with knee OA found no difference in these markers after adjusting age and BMI (Hunter et al. 2003). Results from two cohort studies concluded that Vitamin D status is not related with the risk of joint space and cartilage loss in knee of OA (Felson et al. 2007). Mc et al. (1996) were the first to document that high intake of β carotene and vitamin C decreases the risk of progression of radiographic knee OA Similarly, one of the study showed that high serum vitamin K levels were associated with a low prevalence of hand OA (Neogi et al. 2006). In a randomized placebo controlled study, vitamin E did not show any benefit for the management of symptomatic knee OA (Brand et al. 2001). Many
nutritional factors such as ascorbic acid, minerals, fatty acids, flavanoids and ginger have been studied, but also with variable results. Even though some studies suggest a correlation between these factors and OA or symptom improvement, the role of nutrition in slowing down the disease progression remains to be thoroughly assessed.

2.4.2 Local risk factors

The systemic factors are the key modulator of OA susceptibility and they are often associated with local biomechanical and biochemical factors. Mechanical factors are more closely linked with determining which anatomical sites will be affected.

Biomechanical factors

The progression of knee OA is found to be frequently driven by biomechanical forces. Tetsworth and Paley reported that 5° or less of genu varum leads to high compressive force on the medial knee compartment (Tetsworth and Paley, 1994). In a prospective cohort study, it was observed that, abnormal anatomic alignment in existing knee OA was found to be strongly associated with increase in structural deterioration in the compartment under greatest compressive stress (Sharma et al. 2001). Similarly another study demonstrated that varus alignment at baseline had 4 fold increased risk of progression of medial knee OA and those with valgus alignment at baseline with a 5 fold increase risk of progression of lateral progression. They also demonstrated the severity of the malalignment leads to the decline in physical function at 18 months (Cerejo et al. 2002). The association between malalignment and risk of knee OA in not so clear and it may not be a primary risk
factor for the occurrence of radiographic knee OA but can a marker of progression of the OA (Hunter et al. 2007).

**Muscle Strength**

The correlation between muscle strength and OA is not entirely clear and may vary by joint site. Contrast to malalignment, muscle strength is a more modified risk factor for OA. However, data on the association between the muscle strength of lower quadriceps and the incidence/progression of the knee OA are variable. Few studies have shown that 18% lower knee extensor strength in women are associated with higher incidence of OA (Slemenda et al. 1998). While some reported that high or moderate quadriceps strength in women reduced the risk of hip or knee OA, significantly by about 60% (Hootman et al. 2004). Moreover, one of the largest study in the field of research have reported that quadriceps weakness predicted risk for joint space narrowing of knee but not in men thus protecting them against reaching a threshold under which strength becomes a risk factor for OA (Segal et al. 2010). Muscle weakness and degeneration are commonly related with knee OA and are thought to be product of disuse resulting from pain-avoidance. Women with asymptomatic radiographic knee OA had no muscle atrophy showed muscle weakness indicating that this might be a risk factor for the development of symptomatic knee OA (Slemenda et al. 1997). One of the study demonstrated that subjects with both asymptomatic patellofemoral and tibiofemoral radiographic knee OA had weaker quadriceps strength than those who did not have OA (Baker et al. 2004). In a follow up study, quadriceps muscle weakness not only resulted from painful knee OA but also increases the risk of structural damage of the knee (Slemenda et al. 1998; Brandt et al. 1999). However, Sharma et al reported that in context of malalignment and laxity, greater muscle strength may be associated with a increased risk of progression of knee
In this circumstances, men with hand OA with greater grip strength in the Framingham cohort were at increased risk for developing carpometacarpal, metacarpophalangeal and proximal interphalangeal OA. The discrepancies between these studies require further assessment as they can have a major impact on management of OA in future (Fisher et al. 1993).

Exercise

Exercise is one of the most discussed and controversial nonpharmacologic management strategies for osteoarthritis (OA) of the knee. From various well recognized benefits, exercise is beneficial to the tissues of the joint (Scerpella et al. 2010). In assessing the risk of development of OA from sports participation, it is necessary to categorize recreational and professional sports participants as the latter are at higher risk for injury associated with the development of OA, especially those in sports involving repetitive movement (Buckwalter and Lane, 1997). Few population based studies have observed an increase in knee and hip OA with higher exposure to sport activities. Vingard and his colleagues showed a risk of 4.5 for men and 2.3 for women in higher activity group for developing hip OA (Vingard et al. 1993; 1998). Another study demonstrated that male runners who ran more than 20 miles per week below the age of 50 were at higher risk for developing OA (OR 2.4; 95% CI: 1.5-3.9) and increases more when combined with heavy load from occupation (Cheng et al. 2000). Some studies have also demonstrated that increase in prevalence of OA in the former elite middle and long distance runners but this has been conflicting as others have no increased prevalence of OA in such group (Buckwalter et al. 1997; Spector et al. 1996). As the joint are considered as a complex organ, the total effect of physical activity on the joint is multifactorial and
every part including bone density, muscle strength and risk of injury must be assessed to forecast the net effect.

Joint injury

Multiple studies have demonstrated that knee injury is one of the strongest risk factors for OA. Severe joint injury especially a trans-articular fracture, meniscal tear requiring meniscectomy or anterior cruciate ligament injury can lead to an increase risk to the development of OA (Lohmander et al. 2004; Roos et al. 2001). The Framingham study showed that the prevalence of meniscal damage was much higher with patients with radiographic knee OA when compared with those without OA. The prevalence of meniscal damage increased among those with moderate (K/L=3) and severe (K/L=4) radiographic knee OA (Englund et al. 2008). Since sports and recreational physical activity alone do not appear to be risk factor for development of OA, it has been studied that injuries can counteract the beneficial aspects of sports and lead to secondary OA. A number of joint injuries are associated to the incidence of OA, especially in the knee but can almost affect all the joint. A study reported that almost 15% of sports related injuries in the children and teenagers were associated with growth disturbance (Maffulli et al. 2011).

Knee injury is the primary risk factor for OA in men and the second in women. The prevalence of knee OA is much higher (26%) in soccer players than those in runners (14%) which can be explained with a high risk of injury in the former group (Kujala et al. 1995). The most commonly reported injuries are anterior cruciate ligament (ACL) injuries and meniscal tears. The incidences of OA after ACL reconstruction is high as 50% at 6 years (Keays et al. 2010). Till date ACL
reconstruction and meniscectomy cannot completely repair the joint and prevent the development of OA.

**Obesity**

Obesity and overweight have long been accepted as a modifiable risk factor for OA, commonly the OA of the knee due to high mechanical stresses imposed on the joint. In the Framingham study it was observed that reduction of 5kg in women showed 50% reduction for the risk of developing symptomatic knee OA (Felson *et al.*1992;2000). From the same study it was observed that weight loss was strongly related with a low risk of development of radiographic knee OA (Messier *et al.*2004;Christensen *et al.*2007). The Arthritis, Diet and Activity Promotion Trial reported that weight loss by exercise alone were effective in decreasing pain and improving function in OA among symptomatic obese elders (Messier *et al.*2004). Study from meta-analysis of randomized controlled trials reported that subjects whose weight reduced by about 5% within a period of 20 week experienced relief of knee OA (Christensen *et al.*2007). The relation between overweight and hip OA is inconsistent and is weaker than that with knee OA (Tepper and Hochberg, 1993; Van Saase *et al.*1988). However there are evidences that shows obesity increases the risk of bilateral radiographics and symptomatic OA of hip (Heliovaara *et al.*1993). Since obesity is also a risk factor for non weight bearing joints like hands (Carman *et al.* 1994), it cannot be explained by mechanical factors. One of the study, found a significant dose-effect relationship for overweight (BMI >30) as a risk factor for knee OA, but not for hip OA effect relationship for overweight (BMI >30) as a risk factor for knee OA (Grotle *et al.*2008). As numerous studies are focusing on assessing the presence of possible obesity-related systemic factors in the pathogenesis
A recent study observed that adipokines, molecules implicated in the systemic factors of OA (Hu et al. 2011). Till date the major adipokines implicated in OA are leptin, adiponectin, resistina and visfatin. Although their roles still needs to be clarified, it seems that adipokines may soon be essential to the diagnosis and prognosis of, and pharmacological approaches to, rheumatoid arthritis and OA (Wu et al. 2011; Zhang et al. 1994). Increase load on the joint is possibly the main mechanism by which obesity can cause knee or hip OA. Overloading the knee and hip joints could result to the breakdown of synovial joint and failure of ligamentous and other structural support (Zhang and Jordan, 2010).

**Occupation**

For about 60 years, studies have clearly demonstrated the relationship of occupation with the incidence of OA. Cross-sectional studies have shown that the risk of knee osteoarthritis is 1.9 to 13.0 times higher among underground coal miners than in a control population (Kellgren and Lawrence, 1958; Kellgren and Moore, 1952; Greinemann, 1988). Most probably, the main risk factor in this occupational group is frequent work in the kneeling or squatting position. Construction workers, too, particularly floorers, have a significantly elevated prevalence of knee osteoarthritis (Jensen et al. 2000). Construction workers have been identified at risk for osteoarthritis (Vingard et al. 2001). Study from the Johnston County Project reported an association between physically demanding occupational tasks (lifting over 10 pounds, crawling, heavy work while standing) and both symptomatic knee and hip OA but failed to demonstrate the radiographic changes (Allen et al. 2010). The agriculture sector has been the first to be identified in association with OA of the hip and the knee (Walker and Palmer, 2002). The intense exposure to heavy labor of varied nature and repeated...
local stresses especially at young age could favour a systemic mechanism to the development of OA (Felson et al. 2000). The early onset and severity of OA in certain occupations requires an urgent need for occupation specific studies for the development and evaluation of preventive strategies in this leading cause of disability (Rossignol et al. 2005).

2.5 Pathogenesis

The biochemical, structural and metabolic changes in the joint cartilage has been well documented, though the etiology of OA is not clearly understood. Cytokines, mechanical trauma and altered genetics are now recognized as the factor involved in the pathogenesis leading to degeneration or alteration of articular cartilage in OA. Now it has become evident that OA is a disease process affecting the entire joint structure including the cartilage, synovial membrane, subchondrol bone, ligaments and periarticular muscles resulting in inflammation, pain and structural damage leading to loss of function (A Mahajan et al. 2005).

OA results from the failure of the chondrocytes to maintain the homeostasis between the synthesis and breakdown of these extracellular matrix components (Aigner et al. 2004; Buckwalter et al. 2005; Buckwalter et al. 2006; Goldring et al. 2007; LoeserRF, 2010). The disruption of homeostasis results in increased water content and decrease proteoglycan content of the extracellular matrix; decrease synthesis of type II collagen leading to weakening of the collagen network and increased breakdown of pre-existing collagen (Buckwalter et al. 2005). Moreover there is increase apoptosis of chondrocytes. Initially the compensatory mechanism like an increase synthesis of matrix molecules and proliferation of chondrocytes in the
deeper layers of the cartilage are able to maintain the integrity of the articular cartilage but eventually loss of chondrocytes and changes in extracellular matrix predominates and osteoarthritic changes develop (Buckwalter et al. 2005; Bullough 2007).

But with progression of OA, the surface of the joint becomes thinner, the cartilage gets soften, the integrity of the surface gets disrupted and vertical clefts develops (fibrillation). Deep cartilage ulcers, extending to bone may appear. One of the major features of OA is remodeling and hypertrophy. Despite the loss of bone and cartilage in some parts of joint, net effect of new cartilage and bone formation is an increase in joint size and remodeling of shape. Bone growth occurs at the subchondrol region, resulting to a bony sclerosis when observed radiographically. Growth of the cartilage and the bone at the joint margins leads to osteophytes which modify the shape of the joint and may restrict the movement. Chronic synovitis and thickening of the joint capsule can further restrict the movement. Periarticular muscle wasting is common and plays a major role in symptoms and in disability (Brandt, 2000). Separated fragments of the cartilage and bone may form a loose body which gets incorporated into the synovium and proliferate locally. Synovium becomes thick and hypertrophied and capsule contracts with infiltration of lymphoid follicles, lymphocytes and macrophages. Calcification may occur as calcium crystal deposit in the cartilage with presumed secondary uptake in synovium (Doherty et al. 2004). The inflammatory mediators involved with osteoarthritic bone are not clear than those produced by cartilage and synovium. Nitric oxide could have implication for OA by resulting in subchondrol bone changes. The endothelial isoform, endothelial cell nitric oxide synthase (ecNOS) is constitutively expressed in bone which likely regulates the
osteoblast activity and bone formation thus mediation the effect of mechanical loading on the skeleton. ecNOS acts along with prostaglandins to promote bone formation and suppress bone resorption (van’t Hof RJ et al. 2001). In contrast, IL-1 and TNF induce iNOS in bone cells, and nitric oxide (NO) derived from this pathway potentiates bone loss (Pelletier et al. 2001). Local production of anabolic growth factors like insulin-like growth factor-1 (IGF-1) and TGF-β, which is highly expressed in osteophytes of the femoral head in OA patients, contribute to osteophyte formation and subchondral bone remodeling (Bettica et al. 2002).

The synovial cells and the chondrocytes of osteoarthritic patients produce large number of inflammatory cytokines such as interleukin-1b (IL-1b) and tumor necrosis factor-a (TNF-a), which in turn will decrease the synthesis of anabolic collagen synthesis and increase the catabolic and inflammatory mediators like matrix metalloproteinases (MMP), IL-8, IL-6, prostaglandin E2 and nitric oxide (NO) (Krasnokutsky et al. 2007; Pelletier et al. 2001). NO, in turn promotes cartilage degradation, including inhibition of collagen and proteoglycan synthesis, MMP activation, and increased susceptibility to other oxidant injury. Premature senescence and apoptosis also result from nitric oxide and other oxidative injury thus indicating that OA is a disease of premature aging of the joint (Kuhn et al. 2004). Studies have demonstrated that oxidative stress causes telomere shortening and reduction in number and function of mitochondria in the chondrocytes of osteoarthritic patients (van’t Hof et al. 2000). The findings of premature senescence and apoptotic acceleration in OA confirms that OA is age dependent, mechanically driven, and chemically mediated (Svetlana et al. 2007). The classification of OA as a noninflammatory arthritis is due to the leukocyte count which is typically less than
2000 cells/mm². But the clinical presentation like swelling, effusions and stiffness in the osteoarthritic joints obviously reflects synovial inflammation, as a small role in the pathogenesis of the disease. Arthroscopic studies suggest that in the synovium of OA patients up to 50% of localized proliferation and inflammatory changes takes place indicating synovitis in even in early OA (Ayral et al. 1996). A synovial hypertrophy or effusion and subchondral change is associated with pain, now considered as inflammatory (Roemer et al. 2009). The histological changes of the synovium are synovial hypertrophy and hyperplasia, with increase number of cells lining sometimes accompanied by infiltration of the sublining tissue, with scattered foci of lymphocytes. The inflammation of the synovium in OA is mostly restricted to areas adjoining to damaged bone and cartilage. This activated synovium can release proteinase and cytokines that may accelerate destruction of nearby cartilage. Even though the cartilage itself produces most of these destructive molecules in a vicious autocrine and paracrine manner, the synovium also produces some of the chemokine and metalloproteinase that breaks down the cartilage. As a result of cartilage breakdown produced form mechanical or enzymatic destruction can provoke the release of collagenase and other hydrolytic enzymes from synovial cells leading to vascular hyperplasia in the synovial membranes. This further induces the release of other inflammatory substances like IL-1β and TNF-α (Pelletier et al. 2001) and this process are likely to occur in earlier stages of the disease, as demonstrated by a recent study with early OA that synovial tissue from early OA had elevated levels of IL-1β and TNF-α and increased number of mononuclear cell infiltration compared to late OA (Benito et al. 2005).

2.6 Clinical features
The main symptoms of knee OA is pain, stiffness and altered function. Initially the pain gets worse with weight bearing and movements. As the disease progresses the pain may become persistent with the loss of cartilage ultimately leading to bone to bone contact. Nocturnal pain interrupting with sleep is observed mainly in advanced OA of the hip. Joint stiffness in the morning or after a period of inactivity may be prominent and usually last less than 20 min. Since articular cartilage is aneural, pain in the joint may arise from the other structures. In some cases it may be due to stretching of nerve ending in the periosteum covering the osteophytes while in others it may be due to microfractures in subchondral bone or from medullary hypertension caused by distortion of blood flow by thickened subchondral trabeculae. Joint instability, resulting to stretching of the joint capsule, and muscle spasm may be sources of pain.

Some of the OA patient’s joint pain may due to synovitis which may be due to phagocytosis of debris of cartilage and bone from the abraded joint surface. Palpation may reveal some warmth over the joint. Atrophy of the periarticular muscle may be due to disuse or to reflex the inhibition of muscle contraction. In advanced stages there may be deformity, bony hypertrophy, subluxation, loss of joint motion (Mankin, 1993).

Less commonly, patients may present with a valgus or knock-knee deformity, indicative of more advanced disease in the lateral compartment of the knee. On occasion, and much less commonly, patients may present with isolated OA in the patellofemoral joint, which can of itself be very symptomatic. Loss of cartilage of the knee can lead to malalignment of the leg with a varus deformity or bow-legged
positioning of the leg being evident. This angulation of the knee applies to medial compartment OA of the knees (Manal and Rhonda, 2010).

2.7 Investigations

The diagnosis of OA is based on clinical and radiographic features.

**Clinical features:** In early phase of OA, the radiograph may appear normal, but due to the loss of the articular cartilage joint space narrowing becomes evident. Other radiographic characteristics finding include subchondral bone sclerosis, subchondral cyst and osteophytosis. Due to remodeling and subluxation, a change in contour of the joint may be seen. Patients having early OA who do not show radiographic evidence of bony changes, joint space narrowing alone does not accurately indicate the status of cartilage thinning though tibiofemoral joint space narrowing has been considered to be radiographic surrogate for articular cartilage thinning. Knee OA mainly involves the medial or lateral femoraltibial compartment or the patellocompartment. Palpation may reveal bony hypertrophy (osteophytes) and tenderness.

**Laboratory Investigations:** There is no laboratory studies are diagnostic for OA but specific test may be helpful in identifying the cause of secondary OA. As primary OA is not systemic, so the parameters like erythrocyte sedimentation rate, serum chemistry determinations, blood counts, and urinanlysis are normal. Synovial fluid analysis reveals mild leukocytosis (<2000 white blood cells per microliter) with a predominance of mononuclear cells. Before the appearance of radiographic changes, to diagnose OA clinically without an invasive procedure such as arthroscopy is limited. MRI(magnetic resonance imaging) and ultrasonography have not been
sufficiently validated to justify their routine clinical use for the diagnosis of OA /or for monitoring the progression of disease (Brandt, 2001). Although routine histological diagnosis is rarely performed for the diagnosis of human OA, the microscopic features of OA include loss of chondrocytes and proteoglycan from the cartilage surface, cartilaginous ulceration and erosion, subchondral degeneration and osteophytosis Histological diagnosis is useful for the assessment of OA in laboratory animals and other smaller species (Bendele, 2001).

2.8 Diagnostic Criteria

In clinical practice, OA is routinely graded using standard radiographic films. The radiographic features associated with OA has been already mentioned and are graded by using a system first described by Kellgren and Lawrence, 1957 (Table 1.1). This system assigns one of five grades to osteoarthritic lesions at various sites in comparison to a radiographic atlas. According to the ACR - for the purposes of classification - it should be specified whether OA of the knee is of unknown origin (idiopathic, primary) or is related to a known medical condition or event (secondary).
TABLE 1.1 Kellgren and Lawrence radiographic scoring system grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>OA Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Doubtful narrowing of join space and possible osteophytes.</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Definite osteophytes and possible narrowing of the joint spaces</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Moderate multiple osteophytes, definite narrowing of joint spaces, some sclerosis, and possible deformity of bone ends.</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Multiple large osteophytes, marked narrowing of joint spaces, severe sclerosis and definite of bone contour.</td>
</tr>
</tbody>
</table>

- Morning stiffness > 30 minutes
- Crepitus on motion
- Age >40 years
- Osteophytes on radiological examination

TABLE 1.2 Criteria for American College of Rheumatology
The most widely used criteria for the diagnosis of OA were developed by the American College of Rheumatology (Altman et al. 1991). The criteria combines clinical features with the radiographic grading system of Kellgren and Lawrence (Table 1.2). Knee OA is confirmed if knee pain is experienced for most days of the prior month in addition to osteophytes, synovial fluid changes, morning stiffness and crepitus.

### 2.9 Treatment

Recommendations for the treatment of OA underwent a NICE review in 2008, to include a more holistic approach to disease management. Aerobic exercise and local muscle strengthening are indicated in addition to weight loss in over-weight patients. Pain management recommendations suggest that the administration of paracetamol and topical non-steroidal anti-inflammatory drugs (NSAIDS) in the first instance. Oral NSAIDS (including COX-II inhibitors) are only indicated if other analgesics are

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR &lt;40mm/hr</td>
</tr>
<tr>
<td>RF&lt;1:40</td>
</tr>
</tbody>
</table>
insufficient, and should be prescribed with caution due to potential gastrointestinal and cardiovascular side-effects. Surgery is only indicated in those patients were the severity of disease is having a substantial impact on their quality of life (NICE, 2008).

3.1 Cartilage Metabolic Markers

Biomarkers like Osteogenic protein-1 (OP-1), Keratan sulfate (KS) and Hyaluronic acids (HA) present in the synovial fluid have been studied as cartilage metabolic markers (Chubinskaya et al., 2006). The biomarkers have many advantages over conventional methods like the changes in the biomarker concentration are seen earlier than changes in radiographs. They are sensitive to minor changes in articular cartilage as compared to radiography so they can be used as a surrogate for radiography. They are appropriate to detect degenerative diseases at their initial stages. Biomarkers provide relevant information regarding assessment, progression and treatment efficacy in OA so combined use of markers along with radiography is necessary to predict their use in clinical practice (Pravin et al., 2009).

3.1.1 Osteogenic Protein-1

Osteogenic protein-1 (OP-1), also known as Bone morphogenetic protein-7 (BMP-7), is one of the 30 currently known BMPs, belonging to the transforming growth factor (TGF-β) superfamily. BMPs have been successfully tested in clinical trials and subsequently approved for treating skeletal defects (Vukicevic and Sampath, 2004). OP-1 are highly conserved across animal species including mature human and mouse sharing 98% identical amino acid sequence (Ozkaynak et al., 1990). BMP-7 was originally identified as a regulator of cartilage and bone induction activity (Urist,
They are found in species ranging from worms and insects to mammals. BMP-7 exist in two forms, mature form generated by the intracellular proteolytic cleavage and an inactive precursor form (Jones etal. 1994). In the cartilage of the osteoarthritis, mature BMP-7 was detected in the superficial layer whereas the proform in the deep layer suggesting the possibility that proteinase could regulate the anabolic activities through the conversion of pro-BMPs to mature BMPs, which can stimulate the matrix synthesis (Linda and Thomas, 2001).

This protein undergoes proteolytic cleavage at Arg-XX-Arg sites to yield carboxy-terminal mature active dimmers of disulfide linked monomers (dimerization). Mature OP-1 is a dimer of two 139 amino acid chains connected by a disulfide bond with an approximate molecular weight of 36 kDa. Each monomer contains C-terminal seven cysteine domain and three N-linked glycosylation sites with the major site of glycosylation at Asn 80. There are three intrachain disulfides and one interchain disulfide bond (Wozney, 1988; Israel etal. 1992; Kingsley, 1994). BMP-7 has been known to elicit wide range of biological activities such as the development, homeostasis, and repair of different tissues. OP-1 gene and protein expression have been detected in all of the connective tissues of the joint including cartilage, meniscus, synovium, ligament, and tendon (Rueger and Chubinskaya, 2004). BMPs are believed to promote bone formation by inhibiting myogenesis, they activate the expression of inhibitor of differentiation–inhibitor of DNA binding (Id) genes. Id proteins then repress the transcription by basic helix–loop–helix heterodimers containing myoD/myogenin, which results in the inhibition of myogenesis and formation of osteoblast. Apart from the local application of BMPs for the regeneration of bone, BMPs have also been systemically used to increase the volume of the
skeleton (Simic et al., 2006), and to regenerate the kidney following acute and chronic failure in rats (Vukicevic et al., 1998; Simic & Vukicevic, 2005).

BMP-7 has been implicated in the stimulation of cartilage repair and maintenance of articular cartilage integrity (Soder et al. 2005). Invitro studies have demonstrated that OP-1 up regulates metabolism of the chondrocytes and synthesis of proteins without causing uncontrolled cell proliferation and formation of osteophytes (Chubinskaya et al. 2002; Fan et al. 2004; Flechtenmacher et al. 2006; Loeser et al. 2003; Nishida et al. 2000). OP-1 in the chondrocytes stimulates only cartilage specific extracellular proteins like collagen type II and VI, aggrecan, decorin, fibronectin, H) etc (Chubinskaya and Rueger, 2007; Loeser et al. 2005; Flechtenmacher et al. 2006; Nishida et al. 2000). One of the most significant properties of OP-1 is its anti-catabolic activity. It effectively counteracts the chondrocytes catabolism (inhibition of prostaglandin and Hyaluronic acid synthesis(HA)) induced by various catabolic mediators such as proinflammatory (IL-1 and IL-6) and fragments of cartilage matrix proteins (fibronectin fragments or HA hexasaccharides) and metalloproteinase-1 and metalloproteinase-13(MMP) (Huch et al. 1997; Koepp et al. 1999; Im HJ et al. 2003). A study have shown that OP-1 when injected in sheep with acute cartilage trauma, could successfully reduce the number of the apoptotic cells which associated with the overall improvement in joint morphology and cartilage structural integrity (Hurtig et al. 2004). OP-1 is the only BMP studied thus far in cartilage that exhibits both the pro-anabolic and anti-catabolic activities as well as it is a better stimulator of PGs than BMP-2, 4, 6 and cartilage-derived morphogenetic proteins (CDMPs) 1 and 2 (Rueger et al. 2004). A recent antisense study have demonstrated a decrease in proteoglycan synthesis in a cultured articular chondrocytes due to the down regulation
of OP-1/BMP-7 mRNA suggesting this protein is very important in articular cartilage homeostasis. OP-1 has been detected in the synovial fluid (SF) of normal joints and also from OA and rheumatoid arthritis (RA) patients. Chubinskaya et al found the normal range of OP-1 in the synovial fluid to be 50ng/ml which were comparable with those extracted from normal articular cartilage (about 50g/g dry tissue) (Chubinskaya et al. 2002, 2006; Rueger et al. 2004). Recombinant OP-1 has been produced and extensively characterized both biochemically and biologically since the discovery of the OP-1 gene in the late 1980’s. To evaluate the therapeutic potential in the applications of bone repair, a variety of animal models have been experimented. These studies have led to the demonstration of bone repair in humans and have resulted in OP-1 receiving regulatory approval as the first commercial BMP (Chubinskaya et al. 2007).

3.1.2 Keratan sulfate

Keratan sulfate, also known as kerato sulfate is a sulfated glycosaminoglycan that is distributed in extracellular matrix of cornea, cartilage and bone. It is also synthesized in the Central nervous system (CNS) and glial scar (Zhang et al. 2006). It is a linear polysaccharide consisting of repeating disaccharide unit consisting of alternating galactose and N-acetylglucosamine residues linked to β-1, 4 and β-1, 3 glycosidic bond. The repeating sequence is sulphated to various degrees at the C-6 position of both sugar residues is a linear polymer of N-(Meyer et al. 1953). The term keratan was first discovered in the corneal tissue of the molecule but similar polysaccharide was also identified in cartilage. Keratan sulfate found in two of the tissues i.e. cornea and cartilage are designated as keratan sulfate I and II differing in the oligosaccharides linking the polymer to protein. Corneal keratan sulfate (KSI) is attached to Asn in
core protein via a complex-type N-linked branched oligosaccharide, whereas in cartilage, KSII is O-linked via GalNAc to Ser or Thr residues via a structure known as mucin core-2 (Krusius et al. 1986). Keratan sulfate are highly hydrated molecule and acts as cushion to absorb the mechanical shock. Serum keratan sulfate was proposed as a useful marker of OA when measured using an ELISA however it did not correlate with the X-ray grading (Campion et al. 1991; Thonar et al. 1985; Budsberg et al. 2006). Later Wakitani and colleagues estimated its concentration by High performance liquid chromatography (HPLC) were reported to be more sensitive and more accurate than ELISA and demonstrated a higher value of serum keratan sulphate in patients with early-stage damage of articular cartilage which was not detected by X-ray suggesting that KS may serve as a screening test to detect articular cartilage injury which may contribute greatly to the decision on a therapeutic strategy for the management of OA or cartilage injury (Wakitani et al. 2007). Tao Tang et al concluded that serum KS was associated with the progression of OA when estimated by HPLC (Tang et al. 2008). It has also been documented that serum concentration of KS is not only a marker of knee articular cartilage, but also of other joints and intervertebral disc because of its distribution (Wakitani et al. 2007). Although serum KS can reflect acute major loss of cartilage in experimental OA (Block et al. 1989) and is often regarded as a marker of cartilage turnover in assessing its prognosis (Sharif et al. 1995) still remains in question. However, high levels of KS in the SF of OA knees, and the lack of correlation with the serum levels (Campion et al. 1991) suggest that SF levels of KS may prove a more sensitive indicator of local joint processes (C. Belcher et al. 1997).

3.1.3 Hyaluronic acid
Hyaluronic acid (HA) is a linear polysaccharide formed from the disaccharide unit, composed of D-glucuronic acid and D-N-acetyl glucosamine linked via alternating β-1, 4 and β-1, 3 glycosidic bonds. It is found in most connective tissues and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, umbilical cords and chicken combs. In articular cartilage, hyaluronan and aggrecan form large aggregates, binds huge amount of water and are responsible for the resilience of the cartilage. The human knee contains 2 ml of synovial fluid, with a concentration of HA of 2.5 to 4.0 mg/ml, studies have reported that hyaluronan has beneficial effects on cartilage during the development of OA (Moreland and Willoughby, 2003). It endows the synovial fluid with its viscoelastic properties which are important for the lubrication of the diarthrodial joints. D. R. Blake and his colleagues postulated that when synovial fluid gets exposed to free radicals like superoxide and hydrogen peroxide, HA gets depolymerized and the fluid loses its lubricating properties (D. R. Blake et al., 1981). According to National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) HA may be a useful biomarker to detect the presence and severity of OA. Studies have reported that serum HA level was strongly associated with the presence and severity of radiographic knee OA in their large-scale study (Elliott et al. 2005; Inoue et al. 2011). Similarly HA has also been demonstrated as a potential biomarker for the establishment of a proper management system in knee OA (Poole, 2000; Ishijima et al. 2011; Sharif et al. 1995; Elliott et al. 2005). The concentration of HA correlated with the degree of joint inflammation and synovial proliferation as well as with the radiographic signs of OA (Horslev-Petersen et al. 1988; Larsson et al. 2002; Mazieres et al.; Jung et al. 2006). Though a negative correlation was found between the concentration of HA and severity of OA (Plickert et al. 2013). Hyaluronic acid (HA) plays an important role in the protection of
articulart cartilage and soft tissue surfaces of the joint (Vanden Bekerom etal.2006). In vitro studies shows that HA acts as an antioxidant by altering the profile of inflammatory mediators like NO, cytokines, proteases, such that the balance between cell matrix synthesis and degradation is shifted away from degradation(Comer etal.1996).

Exogenous HA may facilitate the production of newly synthesized HA. Intra-articular treatment with HA has recently become more widely accepted in the therapies for OA pain (ACRS, 2000). HA may have direct or indirect effects on substance P which may be involved in pain (Moore Willoughby etal.1995) either due to its effect on nerve impulses and nerve sensitivity as inflammation of knee joint influences the excitability of nociceptors of articular nerves(Pozo etal.1997). The efficacy and tolerability of intra-articular HA for the treatment of pain in with knee OA have been demonstrated in several clinical trials (Adams etal.1995; Lussier etal.1996; Wobig etal.1998). HA treatment has also been shown to have protective effects on cartilage in experimental models of OA (Shimizu etal. 1998; Yoshioka etal 1997; Schiavinato etal.1989). In vitro studies also show that HA has beneficial effects on the extracellular matrix, immune cells, and inflammatory mediators(Smith and Ghosh,1987;Frean etal 1999;Takahashi etal 1999;Nonaka etal 2000;Balazs etal 1973;1981). The mechanism of action of HA injections is unclear, but it seems to inhibit inflammatory mediators, decrease cartilage degradation, and promote cartilage matrix synthesis (Hochberg etal;Wen,2000). It also insulates synovial pain fibres, thus decreasing perception of pain(Simon,1999) Effects of HA have been found to last longer than the actual compound does, suggesting that intra-articular HA stimulates synthesis of natural HA(Guyatt,1993).
3.2 The Oxidative Stress

In the last two decades, there has been a considerable amount of interest in the role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in clinical medicine. A free radical is defined as any chemical species that contains unpaired electrons. This unpaired electron usually produces a highly reactive free radical. In biological systems, the most common source of free radicals is oxygen. The harmful effects and biological damage caused by ROS and RNS is termed oxidative stress and nitrosative stress (Valko \textit{et al.} 2007). Oxidative stress may also be defined as the disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells. This definition of oxidative stress implies that cells have intact pro-oxidant/anti-oxidant systems that continuously generate and detoxify oxidants during normal aerobic metabolism. Unfavorable side effects occur when there is an imbalance between overproduction of ROS/RNS and decrease of antioxidant molecules in body. Generally, ROS and RNS play dual roles in body: deleterious and beneficial effects (Valko \textit{et al.} 2006). Usually the beneficial effects of ROS involves defense against microbial pathogens. This role occurs by low concentration of these molecules. However, overproduction of ROS or RNS can damage and inhibit the normal functions of lipids, proteins and DNA. This effect is due to intracellular reduction of O2 into ROS or free radicals, which is toxic to cells and tissues (Wickens, 2003). ROS can be produced from both endogenous and exogenous cellular substances. Potential endogenous sources include mitochondria, cytochrome P450, peroxisomes, and inflammatory cells activation (Inoue \textit{et al.} 2001). Mitochondria generate significant quantities of hydrogen peroxide and use ~90% of cellular O2. During the mitochondrial process of reducing oxygen for production of water, several short-lived intermediates are produced, including superoxide (O2), hydrogen peroxide (H2O2)
and the hydroxyl radical [OH]. Superoxide and hydroxyl radicals are toxic to cells. Cell destruction also causes further free radical generation (Wickens, 2003).

Reactive Oxygen Species (ROS) are deleterious agents involved in cartilage degradation and chondrocyte survival (Henrotin et al. 2003). ROS serves as intracellular signalling molecules that amplify the inflammatory responses (Sutipornpalangkul et al. 2009). They cleave the collagen and proteoglycan, activate MMPs and molecular signaling pathways as well as alter the cellular synthetic activity and chondrocyte apoptosis (Monboisse and Borel, 1992; McCord JM, 1974; Klamfeldt and Marklund, 1987; Burkhardt et al., 1986; Lander et al., 1986; Burdon, 1995). Oxidative stress occurs in an organism when there is a serious imbalance between production of ROS and antioxidant defenses. Oxidative stress elicited by reactive oxygen species (ROS) disturbs cartilage homeostasis and promotes catabolism via induction of cell death, breakdown of matrix components, up regulation of latent matrix-degrading enzyme production, inhibition of ECM synthesis, and oxidation of intracellular and extracellular molecules (Sandell and Aigner, 2001). The theory about the role of oxidative stress in joint pain involves the movement of the joints and blood flow. Articular cartilage is non vascular, it functions at low oxygen pressure as compared to other tissues and depends directly on movement for its nourishment. Oxygen and other nutrients must diffuse through the synovial fluid and cartilage extracellular matrix, creating a non uniform oxygen environment throughout the thickness of the tissue. At rest, the synovial joint is a relatively hypoxic environment compared with a mobilized joint, and the joint becomes even more hypoxic during stress due to inflammation or mechanical loading. Movement of the ligament and tendons surrounding a joint, facilitates nutrient to the cartilage by allowing the nearby blood
vessels to dilate. Joint nourishment is enhanced, as long as pressure in exercising blood vessels exceeds the pressure in the synovial cavity. But when the movement is absent and the synovial pressure becomes greater than the pressure in nearby blood vessels, the vessels can collapse and a process known as hypoxic reperfusion injury may begin (Mappa and Grootveld, 1995). This process involves the greater ironies of oxygen metabolism and ROS production and risk of cell damage. ROS production and risk of cell damage is greatest when oxygen concentrations are lowest. It seems logical that as more and more oxygen is delivered to a cell, the cell is more likely to be damaged by oxygen-related metabolism (Henrotin et al. 1992). However, it appears the situation is exactly the opposite. When cells are deprived of oxygen, they seem at greater risk of oxygen related damage than when supplied with ample of oxygen. Ramasamy and his colleagues showed that in rheumatoid arthritis synovial cavity damage correlates with fluctuating oxygen pressure in the joint, overproduction of ROS and of oxygen processing enzymes and free radical scavenging molecules (Ramasamy et al. 1997). Another theory involves the nature of connective tissue itself. Connective tissue is non cellular predominantly and is primarily composed of an extracellular matrix (ECM). The three basic components found in ECM are fibers (especially collagen fiber), ground substance (made up of glycosaminoglycan and glycoprotein) and fluid. The collagen fibers are the most abundant proteins in the body constituting about 30% of all body protein by weight. The stability of the collagen is highly sensitive to inflammatory messenger molecule such as interleukin-1 or tumour necrosis factor alpha in the synovial fluid. When the concentrations of these inflammatory messengers are high, the damage to collagen and arthritis risk is greatly increased (Pinnals and Bartley, 1992; Joosten et al. 1994).
Hence, minimizing oxidative stress may prevent cellular death, decrease inflammation, and prevent some morbidity and mortality (Christman et al. 2000)

### 3.3 Antioxidants

Protective enzymatic and non enzymatic antioxidant defense mechanism reduces oxidative stress by degrading the ROS. Antioxidants are the compounds which dispose, scavenge and suppress the formation of ROS or oppose their actions. They are other enzymes, vitamins and other substances which protect the cells against oxidation (Alvarez et al., 1987).

#### 3.3.1 Superoxide dismutase (SOD)

SOD is the first enzyme involved in the antioxidant defense. It is a metalloprotein found in both the prokaryotic and eukaryotic cells. SOD dismutates $O_2^{−}$ anion to oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$). The reaction is as follows:

$$2O_2^{−} + 2H^+ \rightarrow H_2O_2 + O_2$$

In human three forms of SOD exist: SOD1 located in the cytoplasm, SOD2 in the mitochondria and SOD3 in the extracellular (Ec-SOD). SOD1 and SOD3 contain copper and zinc whereas SOD2 contains the manganese. EC-SOD is a secreted tetrameric glycoprotein with a positively charged heparin-binding site (Fattman et al. 2003). The active center contains copper and zinc and is homologous with SOD1, but the EC-SOD molecule is immunologically distinct. It localizes to the ECM of tissues by binding to the negatively charged proteoglycan and collagen (Petersen et al. 2004; Oury et al. 1994). In this location, it can protect the vulnerable proteins and macromolecules of the ECM from oxidant injury. This important antioxidant has been studied in the vascular, pulmonary, and neurologic systems, but not in cartilage or...
joints (Fattman et al; Kinnula and Crapo et al, 2003; Juul et al. 2004). Later Regan et al demonstrated that EC-SOD is present in 10-fold higher concentrations in articular cartilage as compared with lung tissue and that it is markedly decreased in OA cartilage as compared with normal cartilage suggest that EC-SOD plays a key role in modulating ROS in cartilage in human and animal mode (Regan et al. 2005). It has been studied that a decrease in SOD2 is associated with the earliest stages of OA. A decrease in SOD2 was found to be associated with an increase in ROS (JL Scott et al. 2010; Gavriilidis et al. 2013). Extractive or recombinant SOD seems to be the most valid choice for such targeted therapeutic approach (A. Petkau, 1986). Intra-articular SOD (orgotein for injection) has been used for management of OA in Europe for many years and has demonstrated to be effective in reducing the symptoms of OA upto three months (Harris Mc et al, 1989).

### 3.3.2 Glutathione peroxidase (GPx)

GPx catalyses the reduction of hydroperoxides (H₂O₂) or organic hydroperoxides to water or corresponding alcohol using reduced glutathione (GSH). It is a selenoenzyme and about two third of which is present in the cytosol (liver) and one third in the mitochondria (Freeman B.A and Crapo JD, 1982).

The main reaction that glutathione peroxidase catalyzes is:

\[
2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS-SG} + 2\text{H}_2\text{O}
\]

Chondrocytes produces reactive oxygen species, including H₂O₂, in response to a number of stimuli (Tiku et al. 1990). Hydrogen peroxide at concentrations of millimolar range (≥ 4 mM) has been shown to provoke apoptosis in cartilage (Lo MY and Kim
EC-SOD, cytosolic copper, zinc SOD, and mitochondrially located manganese SOD (MnSOD) play a major role in the formation of hydrogen peroxide, whereas GPx, catalase, and peroxiredoxins all play a role in the enzymatic catabolism of this ROS. GPxs are a family of enzymes homologous to the selenocysteine (Sec)-containing mammalian GPx-1 that uses GSH as an obligate co-substrate in the reduction of hydrogen peroxide to water. Not all GPxs (defined by homology), however, use GSH, nor do they all contain Sec at the active site; rather, some of these. GPx-1 is one of the most abundant members of the GPx family of enzymes that include an epithelial-specific enzyme that is highly expressed in intestine (GPx-2); a secreted subtype (GPx-3); and GPx-4, which is widely expressed and differs in its substrate specificity compared to the other family members. GPx-1 is a crucial antioxidant enzyme involved in preventing the harmful accumulation of intracellular hydrogen peroxide. It is present in all cells; found in cytosolic, mitochondrial, and, in some cells, in peroxisomal compartments (Esworthy et al. 1997; Flohe and Schlegel, 1991; Singh et al. 1994; Li et al. 2000) and has been found to be more effective than catalase at removing intracellular peroxides under many physiological conditions (Cohen and Hochstein, 1963; Antunes et al. 2002).

Studies reported that diet supplemented with vitamins/selenium might be important in prevention or therapy of OA confirming the protective role of vitamin E supplementation against oxidative stress mediated biomolecular deterioration in OA (Kurz et al. 2002; Ijen et al. 2012).

### 3.3.3 Uric acid (UA)

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$. Uric acid is a product of the metabolic breakdown of purine
nucleotides. Uric acid is an endogenous aqueous antioxidant in human and contributes as much as 2/3rd of all free radical scavenging capacity in plasma (Squadrito et al. 2000).

Uric acid is one most potent radical scavenger which exists in the form of urate within the physiological pH range. Its ability to act as an antioxidant is associated with its ability to inactivate an oxidant via an electron transfer before the oxidant can react with the targeted biological molecule (Simic and Jovanovic, 1989). UA acid can inactivate strong oxidants, like nitrite, halogenated peroxy radical a hydroxyl generated radical (Simic and Jovanovic, 1989). It can be oxidized following the nonenzymatic degradation, and has been proven to be selective antioxidant, capable especially, of reacting with hydroxyl radicals (K.Banu and S.Kazim, 2008). UA may act either as an antioxidant (primarily in plasma) or pro-oxidant (primarily within the cell) (Yuri and Richard, 2008). It can scavenge superoxide, the hydroxyl radical, and singlet oxygen (Ames et al. 1981; Davies et al. 1986). UA may assist in the removal of superoxide by preventing against the degradation of superoxide dismutase, the enzyme that is responsible for clearing superoxide from the cell (Pacher et al. 2007). Several studies have reported an association of uric acid and OA. These include a study of hip replacement patients wherein elevated serum uric acid concentrations were associated with the presence of multijoint hand OA (Y.Sun et al. 2000). A second study noted the apparent co-localization of gout attacks and radiographic OA at a multiple joint sites like bi toe, midfoot, knee and concluded that OA may facilitate the localized deposition of gout (monosodium urate or MSU) crystals (Roddy et al. 2007). K.Banu and his colleague stated the relationship between the levels of IL-8, CP, GSH, and UA concentrations (K.Banu and S. Kazim, 2008). Synovial fluid level of UA, was found to be lower as compared to serum in inflammatory joint disease (Beutler et al. 1996) whereas few studies has found to be elevated in serum and correlated negatively with the disease duration in knee OA (Ostalowska et al. 2006). However, no association was found between the