INTRODUCTION

Osteoarthritis (OA), the most common form of arthritis, is progressive disease involving cartilage degradation, subchondral bone thickening, and new bone formation (Peat et al. 2001). It is characterized by joint pain and limited function of the joint. OA occurs in all the synovial joints of the knee, hip, hand, spine, wrists and ankles.

Knee OA represents one of the most prevalent forms of OA among them (Arden and Nevitt, 2006). It is the second most common disorder with a prevalence of 22% to 39% in India (Chopra A et al. 1997; 2001; Mahajan et al. 2003). According to World Health Organization (WHO), over 40% of the Indian populations in the age group of 70 years or above suffers from OA. Women have higher rates of cartilage loss and progression of knee cartilage defects than men (D.T. et al. 1987; Hanna et al. 2009). A report estimated by WHO indicates that knee OA is likely to become the fourth most common cause of disability in women and the eighth most common cause in men (Lopez et al. 1997) hence leading to a significant economic burden on patients and health care resources (March et al. 1997).

The treatment of OA is symptom driven (mainly pain and anti-inflammatory medication in combination with exercise treatment and lifestyle changes) but unfortunately, such treatment cannot prevent or cure OA. The symptomatic treatment often fails to provide satisfactory relief (Felson et al. 2003; Buckwalter et al. 2006). Joint replacement may be possible in developed countries for patients with
severe OA and significant disability (Buckwalter etal.2006). Research efforts during the past decades have focused on the search for disease modifying treatments (Mc Alindon, 1999). Most of these disease modifying treatments were directed towards regeneration of the cartilage and were tested in patients with evident OA. However, so far, these efforts have not been very successful and have not had a significant influence on the symptoms of OA.

Despite a substantial amount of clinical and scientific research, the mechanism of cartilage degradation is not clearly understood in knee OA, it is thought that Reactive Oxygen Species (ROS) formed in both physiological and pathological conditions (TAS et al. 2005) may be one of the causes. Alteration of the redox status is known to occur in rheumatic diseases (Mezes etal. 1983; Ostalwska etal. 2006). To prevent the ROS toxicity, our bodies possess a well co-ordinated antioxidant system like superoxide dismutase (SOD), glutathione peroxidase (GPx) and non enzymic antioxidant like uric acid (UA) among others.

Cartilage might be tissue in which the pathophysiological process of OA starts, but biochemical and imaging studies have shown that synovium and bone can also be starting points. However, it remains unclear which of these three types of tissue, or some combination thereof, might serve as the key tissue for OA. Regeneration and repair of the cartilage have become one of the major obstacles in current orthopaedics. Radiography being the gold standard for diagnosing OA, has shown a poor association with the clinical features (Lawrence etal. 1996; Sowers, 2001). This technique does not allow early detection of pathological changes in joint tissues and
damage (Creamer et al. 1999). Thus the accuracy of radiology in clinical and population-based studies has been questioned (Kallman et al. 1989; Lane et al. 1993). On the other hand arthroscopy, ultrasound, MRI, CT scan etc. are used specially for experimental studies and not recommended for routine clinical use (Mahajan, 2005).

Biological markers in the blood might provide significant information much earlier than imaging techniques and may contribute to our understanding the mechanisms that causes the clinical efficiency of treatments for OA (Rousseau and Delmas, 2007). There is no diagnostic tests, which are able to find the underlying disease process, so OA represents a great and unmet clinical need (Flannery 2010). Hence there is an urgent need for an improved method which can evaluate the disease process and treatment response.

The body fluid level for biochemical markers of structural or metabolic changes in joint tissues has begun to provide clinically useful information (Thonar et al. 2001). Keratan sulphate (KS) estimation has been proposed as a potential marker of cartilage destruction in arthritis (Budsberg et al. 2006). Serum HA has been suggested as a useful indicator in assessing knee osteoarthritic activity (Turan et al. 2007; Alan et al. 2005). Osteogenic Protein-1 (OP-1) has shown a great potential of having a beneficial effect in cartilage as anabolic and anti catabolic factor (Rueger et al. 2007). The cost of this protein is one of the major disadvantages (Desmyter et al. 2008) for the use in management of OA. Knowing the beneficial effect of OP-1 in cartilage regeneration and repair, it has become important to estimate the concentration of OP-1 in osteoarthritic patients in a simple and feasible manner.
Immunodiagnosis could be helpful in the early diagnosis of OA for its effective cure and control of the disease. Polyclonal antibody developed against OP-1 has been successfully produced in western countries but is way beyond the reach of lower economics and emerging nations. Hence, there is a need to search for an alternative sources for producing polyclonal antibody against OP-1 in a cheap and feasible way. Although studies regarding the plasma and synovial fluid level of OP-1 have been examined in knee OA, there have been no detailed studies on synovial fluid levels of OP-1 in various clinical stages of primary OA along with its correlation with the biochemical parameters (Chubinskaya et al. 2006; 2007; Kaneko et al. 2006; Parke et al. 2008, Pilichou et al. 2008). Our study could establish the correlation of the oxidative stress markers, OP-1 with the disease progress and age of the patients.
AIMS

To produce the polyclonal antibody (anti-OP-1) against osteogenic protein-1 in the mouse peritoneal cavity and to find any correlational relationship between the oxidative stress markers (SOD, GPx, UA) and cartilage metabolic markers (HA, KS, OP-1) in osteoarthritic patients.

OBJECTIVES

1. To isolate and characterize the osteogenic protein -1(OP-1) from synovial fluid of OA patients for production of polyclonal antibody (anti-OP-1) in mouse.

2. To detect the presence of OP-1 in the synovial fluid of OA patients by using anti-OP-1.

3. To grade the severity of disease by Kellgren-Lawrence scoring system and to correlate with the OP-1.

4. To determine the immunodiagnostic parameters (KS and HA) and oxidative stress makers (SOD, GPx and UA).

5. To establish diagnostic index for any correlational relationship between the immunodiagnostic findings and oxidative stress markers in the treatment and monitoring of OA patients.
**Hypothesis:**

Polyclonal antibodies produced against OP-1 can detect the presence of OP-1 in the synovial fluid of OA patients and can be used as a biochemical parameter for diagnosing OA based on its severity.

**Motivation of the study:**

Osteoarthritis of the knee is one of the most common forms of OA affecting thousands of people in India. There is a lack of reliable methodology which has delayed the research for decades leading to only small advancement in the pathogenesis and treatment of the disease.

**Limitations of the study.**

1) Synovial fluid could not be taken from healthy volunteers due to ethical reason.

2) Comparison of OP-1 in the synovial fluid of normal joint could not be done and comparison with CT/MRI could not be done due to the cost

3) This study did not include changes of OP-1 with exercise due to the cross-sectional study design.
Scope for further work:

1) Osteogenic protein-1 can be estimated in blood (serum/plasma) and can be compared with control.

2) Blood and synovial fluid level of OP-1 can be correlated.

3) OP-1 level can be compared in OA patients and normal subjects before and after exercise.

4) The severity of the disease can be assessed by MRI/CT scan.