synovial fluid level of UA and OA (RC.Murray et al. 2009). Similarly the plasma level of UA was observed to be insignificant in OA (G.M.Rao et al. 2005; Y.Sun et al. 2000). Shi et al., (2003) identified uric acid as one of the principal endogenous danger signals released from injured cells and mediating the immune response to antigens associated with injured cells. Uric acid may be a factor promoting the pathological process of OA through activation of the inflammasome (Anna et al. 2010). However, studies establishing an independent association between uric acid crystal disease and OA are sparse (Nowatzky et al. 2010).

There is a potential role for these biomarkers in drug development in OA. Thus, there is a need for such biomarkers to offer a potential non radiographical alternative to detect early, non symptomatic osteoarthritis. Despite, a remarkable growth of knowledge regarding the discovery of a number of useful biomarkers, the real breakthrough in this area is still not achieved.

### 3.1 Materials

<table>
<thead>
<tr>
<th>Name of the chemicals</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>SRL, India</td>
</tr>
<tr>
<td>Tween-20</td>
<td>&quot;</td>
</tr>
<tr>
<td>Ammonium persulphate</td>
<td>&quot;</td>
</tr>
<tr>
<td>N,N’-Methylene-bis-acrylamide</td>
<td>&quot;</td>
</tr>
<tr>
<td>TEMED</td>
<td>&quot;</td>
</tr>
<tr>
<td>SDS</td>
<td>Merck, India</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>&quot;</td>
</tr>
<tr>
<td>Tris base(hydroxymethylaminomethane)</td>
<td>&quot;</td>
</tr>
<tr>
<td>β-2-Mercaptoethanol</td>
<td>&quot;</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>&quot;</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>&quot;</td>
</tr>
<tr>
<td>Freunds complete adjuvant</td>
<td>Bangalore Genie Ltd, India</td>
</tr>
</tbody>
</table>
Anti-human IgG

TMB

Anti-Human IgG conjugate

MWM(3kD-205kD)

Pristane Himedia, India

DAB

BMP-7 antibody(sc-9305) Santa Cruz, Biotechnology Inc, USA.
Apparatus

- Dialyzing membrane
  Spectrum laboratories
- SDS-PAGE
  Inc, Netherlands
  Western Blotting apparatus
  Bangalore Genie Ltd, India
- NCP
  Bangalore Genie Ltd, India
- Microtiter plate
  Tarson, India

Common chemicals were all of analytical grade from reputed companies
3.2 Plan of the Study

3.2.1 Clinical Evaluation

This part of the study was carried out in osteoarthritic patients and healthy volunteers. Detailed history was taken and following investigations were done with synovial fluid and blood samples for:

1. Cartilage metabolic markers.
2. Oxidative stress markers.

3.2.2 Experimental Study

This part of the study was carried out in mice to produce the polyclonal antibody (anti-OP-1) against OP-1 in mouse ascitic fluid.

After 56th days of experimental schedule the following parameters were analyzed:

1. Anti-OP-1-IgG level
2. Osteogenic Protein -1 level by using anti-OP-1.
3.3 **Study design:** Experimental study with case-control design.

The study was conducted in the department of Biochemistry, Sikkim Manipal Institute of Medical Sciences, Gangtok. Subjects were selected from the patients attending the department of Orthopedics, Central Referral Hospital (CRH) and Sir Thutop Namgyal Memorial Hospital (STNM), Gangtok.

**Heparinized venous blood samples (5ml) were collected from:**

1. Clinically diagnosed patients with primary knee Osteoarthritis (n=75)
2. Normal control age and sex matched (n=75).

**Synovial fluid (about 3ml) was collected from:**

Clinically diagnosed patients with primary knee Osteoarthritis (n=75) who were undergoing arthrocentesis as part of their evaluation or therapy. Synovial fluid was aspirated and centrifuged to remove the debris and was divided into aliquots and stored at -80°C. For experiment, female white albino mice (n=4) weighing 40-45 grams were taken for immunization.
Sample size calculation:

The mean ±SD values were taken from a previous study done by Chubinskaya et al. (2006), Rush University Medical Center, USA, by using the following formula (Sathian, et al. 2010):

\[
n_1 = \frac{(r + 1) \sigma^2 (Z_{power} + Z_{\alpha/2})^2}{r \text{ difference}^2}
\]

(1.1)

where the variable Hyaluronic acid, potential marker in OA was summarized by means.

Standard deviation (\(\sigma\)) = 2.15

Ratio (\(r\)) = 3

Size of the difference \((p_1 - p_2)^2\) = 1.78

Power (\(Z\)) = typically 0.84 for 80% power

Level of statistical significance \((Z_{\alpha/2}) = 1.96\)

n=35 for case and control
3.4 Classification of Cases:

3.4.1 Inclusion criteria for test group: Patients were selected as per the criteria described by the American College of Rheumatology (ACR) (Altman et al. 1986) based on clinical and radiographic findings, which are as follows:-

1) Knee pain
2) Age: > 50 yrs
3) Morning stiffness lasting for 30 minutes or < 30 minutes.
4) Crepitus on motion
5) Radiological examination: presence of osteophytes.

3.4.2 Exclusion criteria for the control and test group

Participants with history of smoking and alcoholism was excluded from the study. Patients with diagnosed hypertension, diabetics, cardiovascular disorder, post traumatic knee OA and other systemic or neurological disorders were also excluded from the study. Detailed information of the study was given to all participants and written informed consent was taken prior to the study.

The study was approved by the Institutional Research Ethics Committee, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim.