CHAPTER IV

MATERIALS AND METHODS

4.0. Overview of the Chapter

This chapter discusses with the materials and methods. It brings out the various criteria, number of subjects were selected, details of ELISA (Enzyme-Linked Immunosorbent Assay) kits and other materials, equipments were used for the study. Further, discussed about the cross-sectional demographic data especially study area, sampling design, sample size, study method, inclusion and exclusion criteria. Followed by calculation of Body Mass Index (BMI), WOMAC (Western Ontario and McMaster Universities) scores, Bio-chemical measurement of Erythrocyte Sedimentation rate (ESR), detection of serum enzymes of Matrix Metalloproteinases of MMP-3 and MMP-13 by ELISA in Knee Osteoarthritis patients and control groups and finally, various statistical applications were used for analyzing the research data.

4.1. Materials

The first phase of the research study was recruiting 150 Knee Osteoarthritis patients were diagnosed by Orthopaedic doctors. The clinically and radiologically evaluated based on the American College of Rheumatology Criteria for OA (Altman et al.1986)xxi. The Erythrocyte Sedimentation rate was measured from subjects belonging to 150 Knee Osteoarthritis and 15 control groups. The overview of the 150 Knee Osteoarthritis patients and 15 control groups was given below (Table 4.1):
Table 4.1: Subjects were selected in the study

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>150</strong></td>
<td>Knee Osteoarthritis patients were recruited based on Orthopaedic doctors diagnosed</td>
</tr>
<tr>
<td><strong>15</strong></td>
<td>Control groups were recruited for the study</td>
</tr>
<tr>
<td><strong>150</strong></td>
<td>Body Mass Index (BMI) of Knee Osteoarthritis patients was calculated by using an Anthropometric rod to measure the stature; body weight was measured by using weighing balance.</td>
</tr>
<tr>
<td><strong>150+15</strong></td>
<td>Blood collection for biochemical experiments of Erythrocyte Sedimentation Rate (ESR) by the method of Westergren.</td>
</tr>
<tr>
<td><strong>150</strong></td>
<td>Knee Osteoarthritis patients were interviewed by using structured questionnaire. The questionnaire comprising of demographic profile, clinical background, self-administered WOMAC scores, dietary patterns and food habits and daily activities.</td>
</tr>
</tbody>
</table>

The second phase of the research study was selected 72 Knee Osteoarthritis patients out of 150 based on an exclusive and inclusive criteria and 8 were selected from 15 control groups for detecting of serum enzymes for Matrix metalloproteinases-3 and Matrix metalloproteinases-13 by using one step sandwich ELISA (Enzyme-Linked Immunosorbent Assay). The overview of the 72 Knee Osteoarthritis patients and 8 control groups was given below (Table 4.2).
Table 4.2: Subjects were selected based on the ESR level

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>A total of 72 Knee Osteoarthritis patients were selected based on the ESR of $1 \geq 20 \text{mm/hr}$ and more elevated ESR in different age groups among the study subjects.</td>
</tr>
<tr>
<td>8</td>
<td>Eight controls were selected having normal and low rate of ESR levels among the control group.</td>
</tr>
</tbody>
</table>

4.1.1. ELISA kits

1. Abcam (United States of America) ab100607 MMP-3 Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human MMP-3 pro and active forms in serum enzymes in Knee Osteoarthritis patients and controls.

2. Abcam (United States of America) ab100605 MMP 13 Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme linked immunosorbent assay for the quantitative measurement of Human MMP-13 in serum enzymes in Knee Osteoarthritis patients and controls.

**Ethics statement/approval:**

Informed consent was obtained from all study participants. All procedures, study protocol and informed consent were approved by Institutional Human Ethical Committee, University of Mysore, Mysuru. (IHEC-UOM No. 82 Ph.D./2013-14) and Dean Director, Krishna Rajendra Hospital, Mysuru (order no. 2558025 dated: 08/05/2012).
4.1.2. Materials: different instruments or materials were used for biochemical experiments.

Centrifuge machine: It was used to make the separation of clear supernant or serum from the whole blood. Speed between 1000 to 5000 rpm (revolution per minute). Centrifuge tubes are made with the glass and in order to maintain proper balance were kept in opposite directions that is diagonally to each other.

Micropipette: While doing test it was used for dispensing the liquids.

Test tube stand: It was used to place the test tubes in an upright position.

Sterile swabs: It was wetted with the spirit or 70 per cent alcohol made to use for cleaning of the skin before and after taking the blood.

Disposable syringe with the needle: It was used to draw the blood from subjects.

Eppendorf's tubes: It was used to preserve the serum at -20ºC before used for the assay of MMPs.

4.2. Methods

4.2.1. For demographic data

Study area: The present study was conducted in Sri. Krishna Rajendra Hospital, Mysuru from June 2013 to May 2014.

Study sampling design: A sample is defined as a representative part of a larger group. The purposive sampling method was used to select the patients from Out Patient department in the section of Orthopaedic, K.R. Hospital.

Sample size: Orthopaedic doctors or surgeons diagnosed clinically as well as radiologically confirmed total one hundred and fifty Knee Osteoarthritis patients were
selected and collected the data for the present study among the patients those who are having OPD cards and visited hospital from June 2013 to May 2014. The study included 95 females and 55 males were recruited from the department of Orthopaedic, main, single-centered Sri Krishna Rajendra Hospital, Mysuru city. Fifteen subjects were enrolled as control groups among people without personal and family history of Knee Osteoarthritis and participated voluntarily.

For ELISA (Enzyme-Linked Immunosorbent Assay) of serum enzymes of MMP-3 and MMP-13, a total of 72 Knee Osteoarthritis patients were selected based on the ESR of 1≥20mm/hr and more elevated ESR in different age groups among the study subjects and eight controls were selected having normal and low rate of ESR levels among the control groups.

**Study method:** Structured interview scheduled were used to elicit the socio-economic status, physical activity, dietary pattern and medical history etc. The questionnaire were consists of a series closed-ended and opened-ended questions. An Anthropometry was used to measure the stature; body weight was measured using weighing machine. Body Mass Index (BMI) was calculated by these measurements. Blood collection for biochemical experiments of Erythrocyte Sedimentation Rate (ESR) by the method of Westergren, serum enzymes of Matrix Metalloproteinase-3 and Matrix Metalloproteinase-13 by ELISA (Enzyme-Linked Immunosorbent Assay).
**Inclusion criteria:**

- Age between 40-65 both male and female.
- Patients diagnosed with primary Osteoarthritis.
- Orthopaedic doctors diagnosed Knee Osteoarthritis patients (radiologically confirmed).

**Exclusion criteria:**

- Patients with any systemic illness, secondary diseases such as diabetic, blood pressure and obesity etc.
- Patients who have undergone total knee replacement in both the knees, patients with Osteoarthritis secondary diseases like rheumatoid arthritis and gout.
- Patients with restricted mobility.

4.2.2. For BMI, WOMAC scores, ESR and ELISA

In the present study Anthropometric equipments were used for the measurement of physical characteristics.

**Body Mass Index (BMI):** Height (cm) and weight (kg) were used to calculate Body mass index.

**Height:** It was measured by using an anthropometric rod/anthropometry. It consists of four segments which when joined together form a rigid rod of 200cm. One side of this rod is graded in an ascending order from the bottom to the top provided with a fixed socket through which can adjustable cross bar can be fixed. There is another socket having provision for an adjustable cross bar and this socket can be slided up and down on the rod. It is in centimeters (cm). The Knee Osteoarthritis patients and controls were
asked to stand in erect with both the heels touching each other and head was in front-horizontal plane. Anthropometric rod was held vertically behind the patients. The horizontal movable arm of the anthropometric rod was brought to the vertex and height was recorded without footwear.

**Weight:** Body weight of the Knee Osteoarthritis patients and controls was measured by weighing balance. Before measuring the weight zero error of the scale was checked. Patients were asked to stand in erect position with light clothing, without footwear on the machine and weight was recorded in kilograms (kg).

**Clinical features:** Pain involved in the knee, duration of the disease, diagnosis of knee signs, symptoms either by Orthopaedic doctors or surgeon, general health history or medical history used to screen for exclusion criteria or documented history of other forms of arthritis, joint injury or surgery. How much pain patients were getting while performing their daily activities.

**Activities:** Regular hard work like carrying heavy loads, power walk/jogging, bicycling, exercise, house hold activities like sweeping, mopping and cleaning etc.

**WOMAC scores:** Well-experienced Orthopaedic doctors or surgeons diagnosed the Osteoarthritis patients of knee complained of joint pain in the knee from clinical symptoms, thorough examinations and radiological findings and diagnosis mainly focused on the inclusive criteria to differentiate the Osteoarthritis from other types of arthritis and patients were having only primary Osteoarthritis. Patients were recruited those who were willing to participate in this study, willing to give blood. The patients who underwent Orthopaedic examinations had completed the Western Ontario and
McMaster Universities Osteoarthritis Index (WOMAC). It was based on self-administered questionnaire to evaluate the functional disability and pain score. The WOMAC index produces scores for three subscales: pain, stiffness and physical function.

At the time of examination, age, sex ratio, and general living area were similar between the control groups and the patient groups. At the time of selection of the patients and enrolment of control groups in to the study, signed an informed consent form was obtained from all study participants, who were verbally explained about the purpose and procedures of this research study.

**Functional disability and pain score evaluation:** Functional disability was assessed. Questionnaire-based assessment of pain, stiffness and physical function were done using the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index. The WOMAC index produces scores for three subscales: pain, stiffness and physical function.

**Food habits and dietary pattern:** How many times patients consuming per day starch content food, fruits, vegetables, non-vegetarian food like meat, poultry, egg, fish and drinking beverages etc.

**For Erythrocyte Sedimentation rate (ESR)**

**Blood collection:** From one hundred and fifty Knee Osteoarthritis patients and 15 control subjects 5ml of venous blood samples were collected into blood collection tubes and the time range from 10am to 4pm. In that, 2ml of blood has taken to another sterilized tube for measuring ESR levels of the Knee Osteoarthritis patients and control groups. The erythrocyte sedimentation rate levels were measured by the method of Westergren and it is expressed in mm/hr. Informed consent was obtained from each patient and controls
including permission for the use of serum to be collected throughout the study for the assay of MMP-3 and MMP-13.

**Precautions:**

- Before drawing the blood, investigator has to wear gloves;
- Subjects skin were cleaned with spirit moist sterile swabs before collection of the blood;
- Exact vein were located where the investigator has to draw the blood;
- Clean, dry and sterile blood collection tubes, Westergren tubes, pipettes’ tips were used throughout the collection as well as analysis of the samples;
- Care was taken to during the collection of the blood, obtained serum into well labeled tubes;
- Disposable syringe with needles were used and
- Serum and prepared reagents were loaded into the proper Elisa 96-wells.

**Estimation of Erythrocyte Sedimentation Rate (ESR):** Estimation of erythrocyte sedimentation rate was done by Westergren method.

Disposable ESR pipette with vacuum plug offers a method is simple, risk free, safe, accurate, reliable avoiding contamination of infectious blood of the patient. The concept of this pipette is completely different from the conventional method. The risk of accidental contact of infected blood with mouth during sucking has led to various diseases. To avoid sucking disposable ESR pipettes with vacuum plug were used.

(i) **Blood collection tubes:** Tubes were used for the collection of blood from study participants.
(ii) **Disposable ESR pipette with vacuum plug:** This is 240mm length with both sides open. The bore of the pipette is of 2.25 mm diameter. The pipette is calibrated in mm from 0 to 200 from above to downwards. The blood column of the pipette is 200mm and made from optically clear polystyrene powder.

(iii) **Tube stand:** It is a stand with the 6 tubes accommodative capacity at a time. It was used to place the blood collected tubes (12/13*75mm) with the pipette in vertical position.

(iv) Sterile solution of 3.8 per cent sodium citrate.

(v) Disposable syringe and needle.

(vi) Sterile swab moist with alcohol.

**Procedure:**

From Knee Osteoarthritis patients and controls 1.6 ml of the blood was taken by vein puncture in the blood collection tubes containing 0.4 ml of 3.8 per cent sodium citrate. Blood was mixed gently. So the final volume is 2.0ml. Care was taken while doing this. Lower end of the pipette bearing the vacuum plug was inserted into the tube pushed it down slowly making sure that no air bubble was trapped within the blood column. The citrated blood will automatically rise into the pipette and will reach the zero mark to create a blood column of 200mm. The stopper at the zero mark of the pipette will not allow the blood to move beyond the zero mark of the top end of the ESR pipette. Assembled tube and pipette was placed and it was fixed absolutely vertical on suitable tube stand. Further, allowed the blood cells to sediment. The time was noted and the tube stand was allowed to stand vertically undisturbed for 1 hour. Exactly after one hour the
reading of the level of the blood column was taken and the results were recorded in mm/hr.

**For Matrix metalloproteinases (MMPs)**

**Serum collection:** Remaining 3ml of blood samples were allowed to coagulate for 1 hour at room temperature and then centrifuged at 2000rpm for 15 minutes. The serum samples were transferred to another sterilized eppendorf’s tubes and kept frozen at -20ºC until analyzed. Collected serum samples were thawed at room temperature and analyzed for enzymatic profiles in both control groups and Knee Osteoarthritis patients. Sample analysis was performed by abcam ab100607 MMP-3 and ab100605 MMP-13 Human ELISA Kit (USA), according to the manufacture’s instruction. The Human ELISA was done in the Institution of Excellence and Department of Bio-chemistry, University of Mysore, Manasagangotri, Mysuru.

Among the one hundred and fifty Knee Osteoarthritis patients who were fulfilling the inclusion criteria of both the sex, 72 patients were selected based on the ESR of 1≥20mm/hr and more elevated ESR in different age groups among the study subjects and 8 control serum samples were selected having normal and low rate of ESR levels among the control group and 8 standards of duplicates decided to do measurement of two serum enzyme levels of MMP-3 and MMP-13 enzyme-linked immnosorbent assay ELISA kit is a commercially available. Thus, a total of 96 were enrolled for the assay.

**Sensitivity:** The minimum detectable dose of MMP-3 is typically less than 0.3ng/ml and of MMP-13 is typically less than 6pg/ml.
Procedure:

Briefly, for MMP-3 100 µL of 1:2, for MMP-13 100 µL of 1:50 fold diluted serum samples were prepared initially using diluents given by the manufacturer and was loaded onto 96-well ELISA plates, pre-coated with anti-human MMP-3 and MMP 13. After incubation at room temperature for 150 min, the wells were washed with 300 µL of wash buffer (provided by the manufacturer) for 4 times. Next, 100 µL of biotin conjugated antibody (provided by the manufacturer) was added to each well and incubated for 60 min at room temperature. Further, the wells were washed with 300 µL of wash buffer for 4 times and then incubated with streptavidin solution (provided by the manufacturer) for 45 min at room temperature. After incubation the washing step was repeated and 100 µL of TMB substrate solution (provided by the manufacturer) was added and incubated for 30 min at room temperature. At last 50 µL of stop solution (provided by the manufacturer) was added and the development in colour was monitored at 450 nm immediately using an ELISA plate reader (Flow Chart 4.1).
Flow chart 4.1: ELISA – Procedure

For MMP 3 100 µL of 1:2, for MMP 13 100 µL of 1:50 diluted serum were prepared using diluent given by the manufacturer instructions.

Serum was loaded onto 96-well ELISA plates, pre-coated with anti-human MMP 3 and MMP 13.

After incubation at room temperature for 150 min, the wells were washed with 300 µL of wash buffer (provided by the manufacturer) for 4 times.

Next, 100 µL of biotin conjugated antibody (provided by the manufacturer) was added to each well and incubated for 60 min at room temperature.

Further, the wells were washed with 300 µL of wash buffer for 4 times and then incubated with streptavidin solution (provided by the manufacturer) for 45 min at room temperature.

After incubation the washing step was repeated and 100 µL of TMB substrate solution (provided by the manufacturer) was added and incubated for 30 min at room temperature.

At last 50 µL of stop solution (provided by the manufacturer) was added and the development in colour was monitored at 450 nm immediately using an ELISA plate reader.
4.3. Statistical Applications

Statistics is the study of how to collect, organizes, analyze and interpret numerical information from data. Descriptive statistics involves methods of organizing, picturing and summarizing information from data. Inferential statistics involves methods of using information from a sample to draw conclusions about the population. Bio-statistics is the study of distribution and determinants of health related states or events in specified population. The following statistic tools and applications are applied for the present study.

4.3.1. Frequency distribution

A frequency distribution is an organized tabulation of the number of individuals located in each category on the scale of measurement. The frequencies procedure provides statistics and graphical displays that are useful for describing many types of variables. The frequencies report can be suppressed when a variable has many distinct values. It can be labelled charts with frequencies or percentages.

4.3.2. Descriptive statistics

Descriptive statistics defined as summarizing and exploring data. Descriptive statistics are procedures specifically used to organize, make sense of a set of scores or summarize or describe numeric observations referred to as data. This statistics includes the construction of graphs, charts, tables and the calculation of various descriptive measures such as mean, median, Standard deviation and percentiles etc. The various graphs and charts were constructed. Mean and Mean deviation were extensively used in this research study. The mean defined as a given set of observations and divided by their sum. Usually denoted by $\bar{X}$. Computing the Arithmetic Mean is sum of all the observations (numbers)
and divides by number of observations (numbers). The formulas for calculating
Arithmetic Mean and Standard Deviation are as follows:

Formula for calculating Arithmetic mean an individual series  \( \overline{X} = \frac{\sum x}{N} \)

Where,

\( \overline{X} \) = is the symbol for the mean.

\( \sum \) = is the symbol for summation.

\( X \) = is the set of observations

\( N \) = is the number of observations in given series.

Formula for calculating Arithmetic Mean in Continuous series=

\[ \overline{X} = a + \frac{\sum fd'x}{\sum x} i \]

Where,

\( \overline{X} \) = is the symbol for the mean.

\( a \) = Assumed Mean

\( \sum fd'x \) = Sum of the product of frequencies and the deviations from assumed mean

\( \sum x \) = Sum of the frequencies

\( i \) = Size of the class intervals
4.3.3. Standard Deviation

Standard deviation is also called as Root Mean Square (RMS) average of all the deviations from the mean. It is denoted by sigma (\(\sigma\)). The standard deviation is the most common measure of variability, measuring the spread of the data set and the relationship of the mean to the rest of the data. If the data points are close to the mean, indicating that the responses are fairly uniform, then the standard deviation will be small. Conversely, if many data points are far from the mean, indicating that there is a wide variance in the responses, then the standard deviation will be large. If all the data values are equal, then the standard deviation will be zero. The standard deviation is calculated using the following formula.

\[
\sigma = \sqrt{\frac{\sum fd^2}{N} + \left(\frac{\sum fd}{N}\right)^2 \times i}
\]

Where,

\(\sigma\) = Standard Deviation

\(\sum fd^2\) = Sum of the frequencies and deviation squares

\(\sum fd\) = Sum of the frequencies and deviations

\(N\) = Number of observations

4.3.4. Cross tabulation

A cross tabulation (or cross-tab for short) in that the rows represent the values of one variable and the columns represent the values of second variable. Crosstabs' statistics and measures of association are computed for two-way tables only. If specify a row, a column and a layer factor (control variable), the Crosstabs procedure forms one panel of
associated statistics and measures for each value of the layer factor (or a combination of values for two or more control variables).

4.3.5. Kruskal-Wallis H Test

It is a non-parametric method for testing whether samples originate from the same distribution. The Kruskal-Wallis test evaluates whether the population medians on a dependent variable are the same across all levels of a factor. The Kruskal-Wallis test was appropriate under the following circumstances: (a) compare three or more variables; (b) each variable is performed by a different group of participants; and (c) the data do not meet the requirements for a parametric test.

Using the Chi-Square table with Kruskal-Wallis H

The statistical test known as Chi-Square Goodness of Fit to determine whether there is a significant difference between the number of actual and expected genotypes. Chi-Square Goodness of Fit calculated using the formula: $\chi^2 = \sum [(\text{observed value} – \text{expected value})^2 / \text{expected value}]$. Using this test will get the probability (P) value. The P value tells that the lower the probability generated by the test, the greater the likelihood that the difference it can be seen between the observed and expected (Tishkoff and Kidd 2004).

The critical value for the Kruskal-Wallis H test comes from the Chi-square table with k-1 degrees of freedom, where k is the number of groups being tested. Since the critical value is taken from the Chi-square table, the computed value must be larger than the critical value in order to reject the null hypothesis. The critical value for this example is 5.99.
Since $H = 10.10$ is larger than the critical value of 5.99, reject the null hypothesis.

4.3.6. The Mann-Whitney U Test

It is a non-parametric test is equivalent to the independent samples t-test is the Mann-Whitney U. The logic behind the Mann-Whitney test is to rank the data for each condition and then see how different the two rank totals are. If there is a systematic difference between the two conditions, then most of the high ranks will belong to one condition and most of the low ranks will belong to the other one. As a result, the rank totals will be quite different. On the other hand, if the two conditions are similar, then high and low ranks will be distributed fairly evenly between the two conditions and the rank totals will be fairly similar. The Mann-Whitney U computes two U values with the following formulas:

$$U_1 = n_1n_2 + \frac{n_1(n_1 + 1)}{2} - \Sigma R_1 \quad U_2 = n_1n_2 + \frac{n_2(n_2 + 1)}{2} - \Sigma R_2$$

Where,

$n_1 = \text{number of observations in group 1}, \quad n_2 = \text{number of observations in 2}, \quad R_1 = \text{sum of ranks assigned to group 1 and} \quad R_2 = \text{sum of ranks assigned to 2.}$

Computing the Mann-Whitney U

The smaller of $U_1$ and $U_2$ is the U test statistic and is compared to $U_{cv}$ to determine whether to reject the null hypothesis. The computed U statistic must be less than the critical value in order to reject the null hypothesis.
4.3.7. Bivariate Correlations

Bivariate correlation test is used to whether the relationship between two variables is linear or not. Bivariate correlations procedure computes Pearson's correlation coefficient, Spearman's rho, and Kendall's tau-b with their significance levels. Correlations measure how variables or rank orders are related. Before calculating a correlation coefficient, screen the data for outliers (which can cause misleading results) and evidence of a linear relationship. Pearson's correlation coefficient is a measure of linear association. Two variables can be perfectly related but if the relationship is not linear, Pearson's correlation coefficient is not an appropriate statistic for measuring their association.

Karl Pearsons correlations

This type of bivariate correlation test requires that the variables both have a scale level of measurement. Karl Pearsons coefficient of correlation is also known as the product moment correlation coefficient. The value of ‘r’ lies between +1 or –1. Positive values of ‘r’ indicate positive correlation between the two variables. (i.e., changes in both variables take place in the statement direction), whereas negative values of ‘r’ indicate negative correlation (i.e., changes in the two variables taking place in the opposite direction). A zero value of ‘r’ indicates that there is no association between the two variables.

When \( r = (+) 1 \), it indicates perfect positive correlation and when it is (-) 1, it indicates perfect negative correlation, meaning thereby that variations in independent variable (X) explain 100% of the variables in the dependent variable (Y). One can also say that for a unit change in independent variable, if there happens to be a constant change in the
dependent variable in the same direction, then correlation will be termed as perfect positive. But if such change occurs in the opposite direction, the correlation will be termed as perfect negative. The value of ‘r’ nearer to +1 or -1 indicates high degree of correlation between the two variables. The negative sign shows that inverse relationship between indicators and positive sign shows that direct relationship between indicators.

**Correlation Matrix**

Correlation Matrix is a statistical test for comparing two or more variables matrix. A correlation matrix is characterized as being a real, square symmetric matrix with ones on the diagonal and with non-negative given values.

**Scatter Plot or diagram**

Scatter diagram is a graph of observed plotted points where each points represents the values of X and Y as a coordinate. It portrays the relationship between these two variables graphically. A scatter plot matrix is table of scatter plots. Each plot is small so that many plots can be fit on a page. When need to look at several plots, such as at the beginning of a multiple regression analysis, a scatter plot matrix is a very useful tool.
4.4. Summary

This chapter summarizes the various materials and methods were used for the study. It emphasized on criteria for selection of Knee Osteoarthrosis patients, procedures, protocol, various materials, equipments were used for Biochemical measurements of ESR level. Detection of serum enzymes of Matrix Metalloproteinases in Knee Osteoarthrosis patients and controls by ELISA. WOMAC scores index were used to evaluate the functional disability and pain scores of Knee Osteoarthrosis patients. The various statistical applications were discussed are more appropriate and relevant to present study. For example, Kruskal -Wallis test and Mann Whitney U Test for finding the difference between two conditions and Chi-Square table were used to know the goodness of fit at the different level of significances. Finally, bivariate correlation was used to know the linear association of variables and correlation matrix were used to correlate among the MMP-3, MMP-13, ESR etc. Scatter plot was used plot MMP-3, MMP-13 and WOMAC scores of Pain, Stiffness and Physical functions to know the association among the variables.