CHAPTER I
INTRODUCTION

1.0. Introduction

Osteoarthritis (OA) also known as degenerative arthritis or degenerative joint disease or osteoarthrosis, is a group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone (Di Cesare et al. 2009). The word ‘Osteoarthritis’ originated from the Greek word “Osteo”, meaning “of the bone”, “arthro”, meaning “joint”, and “itis”, meaning inflammation (Arya and Vijay Jain 2013). Osteoarthritis also often called ‘degenerative joint disease,’ is the most common form of arthritis (Kelsey and Hochberg 1988). It is a leading cause of chronic disability between fourth and fifth decade of life (Lutzner et al. 2009). Osteoarthritis traditionally was considered as a disease of articular cartilage. It is one of the most frequently occurring health problems for middle-aged and older people (Woolf and Pfleger 2003, Buckwalter et al. 2004). Historically, Osteoarthritis was seen as a degenerative disease caused solely by the ‘wear and tear’ process of ageing cartilage. Now it is recognized as a more dynamic, complex disease involving numerous factors affecting the whole joint (Clouet et al. 2009). Osteoarthritis can occur in every synovial joint but is most common in hip, knee, hand, foot and spine (Buckwalter et al. 2003).

Osteoarthritis has been variously described as a part of an age-related change or disease. The incidence of Osteoarthritis is twice as high in women than in men and increases with age, especially after 60 (Felson 2000). Worldwide estimates are that 9.6% of men and
18.0% of women aged 60 years and over have symptomatic Osteoarthritis. For radiological Knee Osteoarthritis these estimates are somewhat higher, even at a younger age (45 years and over) 14.1% for men and 22.8% for women. Knee Osteoarthritis is twice as prevalent as hip osteoarthritis (Woolf and Pfleger 2003). The Kellgren and Lawrence (K & L) classification criteria are the most widely used radiographic classification criteria to identify and grade Osteoarthritis. The World Health Organization (WHO) adopted these criteria as the standard for epidemiological studies of Osteoarthritis (Kellgren et al. 1963). Osteoarthritis is mainly of two types: Primary Osteoarthritis (Idiopathic) and Secondary Osteoarthritis.

Primary Osteoarthritis (POA) is most cases of Osteoarthritis have no known cause and are referred to as primary osteoarthritis, is mostly related to aging and it can present as localized, generalized or as erosive Osteoarthritis. Secondary Osteoarthritis (SOA) is caused by another disease or condition. Osteoarthritis commonly affects the knee joint (Felson et al. 2000a; Lonner 2003) resulting in joint space narrowing and the development of osteophytes and sclerosis of the underlying bone (Felson et al. 2000a; Iorio and Healy 2003). Ligaments may also be compromised leading to instability in the knee (Iorio and Healy 2003).

The clinician must first differentiate Osteoarthritis from other types of arthritis. It is also important to determine whether a patient has primary Osteoarthritis or a secondary form of Osteoarthritis that is associated with other diseases (Ju Hee Ryu et al. 2012). Osteoarthritis can affect any or all three compartments (medial, lateral, patellofemoral) of
the knee joint (Ashraf et al. 2003) and about one third of patients have Osteoarthritis predominantly in only one compartment of the knee–unicompartamental Osteoarthritis of the knee (Ledingham et al. 1993). There are three compartments of the knee that can be affected by Osteoarthritis: the medial tibiofemoral compartment is more commonly affected than the lateral tibiofemoral compartments; but there is a lack of data addressing prevalence of patellofemoral osteoarthritis (chondromalacia patellae) and its correlation to tibiofemoral disease (McAlindon et al. 1992).

In many cases, the cause of Osteoarthritis cannot be identified (Lonner 2003), however, it is thought that a combination of systemic and local factors may be responsible (Felson et al. 2000a). Systemic factors such as age, sex, ethnicity and genetics may combine with local factors such as obesity, injury to the joint, deformity in the joint, sporting activities, occupational factors and muscle weakness to cause loss of articular cartilage (Felson et al. 2000a; Felson et al. 2000b; Felson et al. 2000c; Ashraf et al. 2003). Knee Osteoarthritis is the most common joint disorder affecting the elderly throughout the world. It is a leading cause of disability and has a formidable societal and public health impact. The Knee Osteoarthritis is mainly affecting knee joint and further articular cartilage degradation.

**The Knee joint:** The knee is a weight-bearing joint which functions to allow movement of the leg and is critical to normal walking. According to (Grey 1918) described that, it can be divided into three compartments; the femur (thighbone) and the tibia (shinbone) joins together to form the lateral and medial compartments of the tibio femoral (TF) joint, the patella (knee cap), which joins with the femur to form the patella femoral (PF) joint
In the normal knee it is mainly the medial TF compartment which is load bearing (Evans 2007). The joint is stabilized statically by collateral and cruciate ligaments and dynamically by muscles and tendons crossing the knee. The joint is enclosed by the joint capsule which inner surface is lined with the synovial membrane, or synovium, which is permeable to water, and small molecules and proteins (Simkin 2003). The cavity formed inside the capsule is filled with synovial fluid (SF) which is a viscous fluid rich in hyaluronan and lubricin (Swann et al. 1981). Synovial fluid (SF) serves both as a lubricant, minimizing friction between the articular surfaces (Swann et al. 1984) and a medium for transport of nutrition and waste products between the microvasculature and lymphatic vessels of the synovium and the avascular cartilage (Simkin 1991).

**Articular cartilage:** Articular cartilage is a living material composed of a relatively small number of cells known as chondrocytes surrounded by a multicomponent matrix. Mechanically, articular cartilage is a composite of materials with widely differing properties. Articular cartilage is composed of 68-85 per cent water, 10-20 per cent collagen and 5-10 per cent by wet weight of proteoglycans (Mow and Ratcliffe 1991). Cartilage is classified in three types – elastic, fibrous and hyaline – based on its structure and composition. Articular cartilage is a smooth, hyaline cartilage that contains an extracellular matrix (ECM) in which cells are sparsely positioned. It is an avascular tissue and more than 60 per cent of its content is water (Mankin and Thrasher 1975), the remainder is to the largest part composed of collagens, aggrecan and other matrix molecules (Heinegard 2007) and only a few per cent of the tissue consists of cells. The joint cartilage is organized into four zones, ranging from the superficial zone at the
articular surface, via the transitional or intermediate zone to the deep zone which connects to the subchondral bone via the calcified cartilage zone (Martel-Pelletier et al. 2008). The extracellular matrix also has a distinct organization around the cell which is described as peri-cellular closest to the cell territorial and inter-territorial.

1.1. Risk factors for Osteoarthritis

The main risk factors for Osteoarthritis include increasing sex difference, age, obesity, and joint trauma. At present, overweight and obese people commonly suffer from Knee Osteoarthritis. The details are discussed given below:

1.1.1. Sex or Gender differences

Women have twice the risk that men have of developing bilateral Knee Osteoarthritis, while men are more likely to develop unilateral Osteoarthritis of the Knee. The definite increase in Osteoarthritis in women around the time of menopause has led investigations hypothesize that hormonal factors may play a role in the development of Osteoarthritis. However, results on effect of estrogen, either endogenous or exogenous on Osteoarthritis from observational studies have been conflicting (Wluka et al. 2000; Nevitt et al. 1996).

1.1.2. Age

Age is the most powerful risk-factor for Osteoarthritis (Cicuttini and Spector 1997; Brown and Forbes 1974). Various risk factors and biological changes were occurring with ageing such as cartilage thinning and degradation, weak muscle strength, poor proprioception and oxidative damage. Studies have shown that Knee Osteoarthritis greatly diminishes health status in the elderly (Dominick et al. 2004; Fryback et al. 1996).
As Osteoarthritis incidence increases with age, Osteoarthritis will become a major health issue and socio-economic problem in the coming decades (Sangha 2000).

1.1.3. Prevalence

The prevalence of Osteoarthritis is dependent on the precise definition used and on the site of interest. In the Asia-Pacific region, the prevalence of Knee Osteoarthritis was 7.50 per cent in China (Wigley et al. 1994), 5.78 per cent in rural India (Chopra et al. 1997), 22.00 per cent to 28.00 per cent in urban and 25.00 per cent in the rural population of North Pakistan (Farooqi and Gibson 1998) and 10.20 per cent in Bangladesh (Haq et al. 2005). Prevalence of Osteoarthritis in India is reported to be in the range of 17 to 60.6 per cent (Sharma et al. 2007). The reported prevalence of Osteoarthritis from a study in rural India is 5.78 per cent (Lone et al. 2011). In the Bhigwan population in India, six percentages of the respondents had chronic knee pain without clinical evidence of Osteoarthritis (Syed et al. 2011). Prevalence increases with age, so that about 11 per cent of all women over the age of 60 years have symptoms due to Knee Osteoarthritis. Most Knee Osteoarthritis is managed by primary care physicians rather than rheumatologists (Creamer et al. 2000). Osteoarthritis accounts for half of all chronic conditions in persons aged over 65 with about 25 per cent of people over the age of 60 have significant pain and disability from Osteoarthritis (Planning Commission 2011).
1.2. Clinical features

1.2.1. Symptom

Pain is the chief complaint. This is due to stimulation of capsular pain fibers, mechanoreceptors (increased intra-articular pressure due to synovial hypertrophy), periosteal nerve fibers and by perception of subchondral micro-fractures or painful entheses and bursae. Stiffness is other complaint described as gelling of joint after inactivity with difference in initiating movement. Some patients may complain of joint swelling, deformity, coarse crepitus and osteophytes at joint margins (X-ray) (Mahajan et al. 2005).

1.2.2. Signs

Coarse crepitus due to irregularity of articular surface, bony enlargement due to remodelling and osteophytes, deformity, instability, restricted ability and stress pain (Buckwalter et al. 2003). Pain when moving the knee, stiffness especially after rest, crepitus a grinding sensation when move the joint and hard swelling (Osteophytes) and soft swelling (extra fluid in the joint) (Arthritis Research UK 2011).

1.2.3. Diagnosis

The diagnosis of Osteoarthritis is established using a combination of clinical information derived from history, physical examination, radiologic and laboratory evaluation. An algorithm of diagnostic criteria for Osteoarthritis of the knee has been proposed by the American College of Rheumatology (ACR). The first classification criteria developed by the American College of Rheumatology (ACR) in 1986 are often used to standardize case definitions for research purposes (Li et al. 2007). At present, there is no standard
guideline primarily for the purpose of clinical diagnosis of Knee Osteoarthritis. Radiography is often used for diagnosis but it is not the only marker for Osteoarthritis (Zhang et al. 2009). Definition of Knee Osteoarthritis may change according to different levels of care and clinical requirements.

### 1.2.4. Causes

Osteoarthritis often affects multiple members of the same family, suggesting that there is hereditary susceptibility to this condition. A number of studies have shown that there is a greater prevalence of the disease between siblings and especially identical twins indicating a hereditary basis. Up to 60% of Osteoarthritis cases are thought to result from genetic factors. Researchers are also investigating the possibility of allergies, infections, or fungi as a cause. There is some evidence that allergies, whether fungal, infectious or systemically induced may be a significant contributing factor to the appearance of Osteoarthritis in a synovial sac (Broward Research Group, 2007).

### 1.3. Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate (ESR) is a simple laboratory test for assessing the inflammatory or acute response. The International Committee for Standardization in Hematology (ICSH) recommends the use of the Westergren method (Thomas et al. 1993). The Erythrocyte Sedimentation Rate is an indication of the inflammation in the knee joint. ESR levels are used to monitor disease activity. Many studies reveal that associations of inflammatory markers (C-reactive protein (CRP) and ESR) with muscle strength changes. The probability of disease at any age increases with increased ESR and becomes more significant when the ESR exceeds 50 mm/hr (Stevens et al. 1995). It
appears that age alone has only a marginal effect if any, on the ESR. In blacks normal values of the ESR are at least 2 mm/hr to 13 mm/hr higher even after correcting for age, hemoglobin concentration and certain chronic diseases (Gillum 1993 and Bester et al. 1993).

1.4. Serum enzymes of Matrix Metalloproteinases (MMPs)

This diagnostic relevance of enzymes was put into practice as early as the 1900s. One of the earliest reported enzyme measurement in body fluids was that of amylase in urine by Wohlgemoth in 1908. The use of serum as the diagnostic fluid for measuring enzyme activity started in 1920s and 1930s. Among various biological markers associated with Osteoarthritis, matrix metalloproteinases (MMPs) play a primary role in cartilage degradation in human joint disease and function downstream of Osteoarthritis signalling pathways (Benedetti et al. 2010 and Ahmad et al. 2009). The study shown that matrix metalloproteinases (MMPs) play an important role in the degradation of the matrix in Osteoarthritis (OA) and rheumatoid arthritis (RA) (Vincenti et al. 1994). MMPs is one of the predominant proteinases belong to a zinc-dependent proteases family, they are responsible for the characteristic matrix degradation in Osteoarthritis (Murphy and Nagase 2008). Matrix metalloproteinases are a family of structurally-related, zinc-containing enzymes that have the ability to breakdown connective tissue. MMPs are a large family of calcium-dependent zinc-containing endopeptidases which are responsible for the tissue remodeling and degradation of the extracellular matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins and proteoglycan.
MMPs are categorized into the following groups (Sapolsky and Howell 1992):

- Collagenases (MMP-1, MMP-8, and MMP-13);
- Gelatinases (MMP-2 and MMP-9);
- Stromelysins (MMP-3, MMP-10, and MMP-11);
- Matrilysin (MMP-7);
- Metalloelastase (MMP-12); and
- Membrane-type matrix metalloproteinases (MT-MMP 1, 2, 3, and 4).

Several studies dealing with the MMPs of -1, -7,-8 etc. referring to Osteoarthritis, at present a very few number of investigation have pointed out the Stromelysin-1 (MMP-3) and Collagenase-3 (MMP-13) especially for Knee Osteoarthritis patients in India as well as in foreign countries. The present research summarizes state of knowledge of MMP-3 and MMP-13 role in Knee Osteoarthritis.

1.4.1. MMP-3 or (Stromelysin-1)

MMP 3, a proteoglycan degrading enzyme, can be localized to the zones of cartilage with active proteoglycan depletion (Okada et al. 1992). Stromelysin-1 can degrade aggrecan, denatured collagens and interhelical collagen domains, as well as aggrecan and link protein. Importantly, stomelysin-1 can cleave the aggrecan molecule at the MMP site, at the Asn341-Phe342 bond, to liberate the G1 domain from the remainder of the molecule (Flannery et al. 1992). It has been shown that stromelysin-1 can activate the pro forms of collagenases and that this activation is a key step in cartilage degradation (Suzuki et
In osteoarthritic cartilage, stromelysin-1 is localized in chondrocytes of the superficial and transition zone (Okada et al. 1992) and its strongest RNA expression is found in early degenerative articular cartilage (Bau et al. 2002). (Mehraban et al.1998) the study indicating that both cell types can produce stromelysin-1. It has been shown that in humans, the plasma level of stromelysin-1 was a significant predictor of joint space narrowing in Knee Osteoarthritis (Lohmander et al.2005).

1.4.2. MMP-13 or (Collagenase-3)

This collagenase is mostly expressed by chondrocytes surrounding osteoarthritic lesions (Shlopov et al. 1997) and can be found in superficial (Wu et al. 2002) and deep layers of osteoarthritic cartilage (Freemont et al. 1999; Moldovan et al. 1997). Many different in vivo studies have shown the importance of MMP-13 in Osteoarthritis. Administration of specific MMP-13 inhibitors to animal models of Osteoarthritis has shown a significant reduction in the severity of the pathology (Baragi et al. 2009; Johnson et al. 2007; Settle et al. 2010). Recently, MMP-13 knockout mice have been developed and surgical induction of osteoarthritis by destabilisation of the medial meniscus in these animals demonstrated that structural cartilage damage is dependent on MMP-13 activity (Little et al. 2009). MMP-13 is one of collagenases that responsible for MMP-TIMP balance is shifted towards MMP result in an degradation of collagen II and other substrates such as excess of activated MMPs leading to cartilage Col I,III,IV,IX,X,XIV, gelatin (Visse and Nagase 2003).

1.4.3. Enzyme-Linked Immunosorbent Assays (ELISA)

Enzyme-linked immunosorbent assays (ELISA) are used for quantification of substances of immunogenic properties. The assays typically involves at least one antibody specific
for a particular antigen. The antibody is directly or indirectly linked to an enzyme catalyzing a biochemical reaction. The most commonly used enzyme is horseradish peroxidase (HRP), which catalyzes a shift in colour which is detected as absorbance in a spectrophotometer. Based on the strategy used for detection, ELISAs are commonly classified in four groups; direct, when the antigen is reacted directly with the antibody, indirect, when the detection is via a secondary antibody specific for the antigenic antibody, sandwich, when one antibody adsorbed to the solid phase is used to capture the antigen and a second antibody is used for detection, and competition ELISA, when two reactants are competing to bind to a third (Crowther 1995).

1.5. Justification of the study

Osteoarthritis (OA) is the second most common rheumatological problem and is the most frequent joint disease with prevalence of 22% to 39% in India (Chopra et al. 2001). In this background there are not many studies on Osteoarthritis especially Knee Osteoarthritis in India. This study mainly focuses on demographic, socio-economic profile and serum enzymatic influence on Knee Osteoarthritis patients. All are aware of the diseases like hypertension, diabetes mellitus, obesity etc so people are taking the precautionary measurements like morning walk, jogging and concentrating on diet also. But this osteoarthritis is age related disease and people are ignoring to take precautions and also have less awareness about the disease. In patients with Knee Osteoarthritis serum enzymes of Matrix metalloproteinases in particularly Matrix metalloproteinase-3 (MMP-3) and Matrix metalloproteinase-13 (MMP-13) enzymes are involved in the degeneration of the articular cartilage. This research output would be more significance
for the people working on Knee Osteoarthritis, medical practitioners and Knee Osteoarthritis patients.

1.5.1. National significance

There are not many studies on enzymes of Matrix metalloproteinases (MMP-3) and Matrix metalloproteinases (MMP-13) in India, this study would help to research community, drugs producers to inhibit the levels of MMPs in Knee Osteoarthritis patients.

1.5.2. Potential risks and benefits

The measurement of MMP-3 and MMP-13 enzymes in human joints can distinguish diseased and healthy joints. This study may help to reduce the risks of primary Osteoarthritis patients to take precautionary measures and it would be benefited to alter the load bearing of the articular cartilage.

1.6. Anthropological Corroboration

Although Osteoarthritis has been part of the history of mankind from the very earliest times – the “Java man”, a Homo erectus whose 500 000 to 700 000 year old fossilized femur show signs of OA (Copeman 1964) – it was not until 1888 it was first mentioned as a separate arthritic disease by John Kent Spender of England (Spender 1888). As a physician at the Royal Mineral Water Hospital in Bath, he noted that most cases of Osteoarthritis could be divided into slow and quick forms based on the velocity and tension of the heart’s action. Research on Osteoarthritis has evolved over the past 122 years, and has shown that the disease is considerably more complex. Another paradigm that has been shifted due to recent advances in the research is the traditional view of Osteoarthritis as a non-inflammatory arthritis; the inflammatory pathway has been shown
to be up regulated, at least in some patients and in some phases of the diseases (Van den Berg et al. 2003; Abramson 2004).

In the same year that Osteoarthritis was first described by Spender, Wilhelm Conrad Rontgen became Professor of Physics at the University of Wurzburg. Some years later, in 1895, he discovered what still today the most commonly used tool in diagnosing Osteoarthritis is: X-rays (Rontgen 1895). Bertha Rontgen, his wife, volunteered her hand for the first x-ray image – upon examination today, presence of osteophytes indicates that she in fact had hand Osteoarthritis in 1901; Wilhelm Conrad Rontgen received the Nobel Prize in Physics for his discovery. Today, radiographic changes described by Kellgren and Lawrence (Kellgren and Lawrence 1957) form the basis for radiographic diagnostic criteria for Osteoarthritis. Cardinal radiographic features of Osteoarthritis, such as joint space narrowing (JSN), and the formation of marginal osteophytes (Altman et al. 1995; Altman and Gold 2007), however appear late in disease and the search for early signs and mechanisms leading to advanced disease has been ongoing for many years. Spender himself, lacking radiography as a tool, tried to find early signs of Osteoarthritis and found that “pigmentation which goes by the common name of ‘freckles’ is a frequent accompaniment of early Osteoarthritis” (Spender 1888). It was first introduced to refer to the condition presently understood as Osteoarthritis and differentiated from rheumatoid arthritis by Archibald Garrod in 1907.

Osteoarthritis (OA) is an ancient disease. It has been found in the skeletal remains of dinosaurs (Wells 1973) and Neanderthal as well as Cro-Magnon man (Dequeker and Luyten 2008). The disease has also been found in Egyptian mummies (Braunstein et al.
and was common among ancient Saxons in England (Rogers 1981). Osteoarthritis affects all races of man (Bremner et al. 1968) and is not confined to any particular geographic area (Roberts and Burch 1966).

1.7. Research Hypothesis

The research intends to test the recommendation to a better understanding. The following questions are framed to develop a discourse.

Research questions

1. Is there any significant association between ESR level and different sex population in Knee Osteoarthritis patients and controls?

2. Is there any significant association between serum of MMP-3 and MMP-13 in Knee Osteoarthritis patients and controls?

3. Does the serum enzymes of MMP-3 and MMP-13 directly correlated to articular cartilage degradation in Knee Osteoarthritis patients and controls?

4. Is there any significant association between WOMAC scores and MMP-3 and MMP-13 in Knee Osteoarthritis patients?

5. Is there any correlation among the variables of Weight, ESR level, MMP-3 and MMP-13 by sex and age?
1.8. **Aim of the study**

To ascertain the population structure, characteristics and Serum Enzymatic influence on Knee Osteoarthritis patients.

1.9. **Objectives**

1. To study the demographic profile and clinical features of Knee Osteoarthritis patients among the selected population in Mysuru city;
2. To study and compare the Serum Enzymatic profiles of normal and Knee Osteoarthritis patients;
3. To find out the association of Erythrocyte Sedimentation Rate and Serum Enzymes level of Matrix metalloproteinases (MMP-3) and Matrix metalloproteinases (MMP-13) in Knee Osteoarthritis patients; and
4. To bring out the prototype and positive prevalence for Knee Osteoarthritis.

1.10. **Scope and Limitations of the study**

The scope of this research is to find out demographic characteristics, clinical background and their serum enzymatic profile of the Knee Osteoarthritis patients. This study examines the Erythrocyte Sedimentation Rate (ESR) level and serum enzymes of Matrix metalloproteinase-3 (MMP-3) and Matrix metalloproteinase-13 (MMP-13) in Knee Osteoarthritis patients and controls.

This study is limited to selecting 150 Knee Osteoarthritis patients and 15 controls (normal) based on the various inclusive and exclusive criteria. The Enzyme- Linked Immunosorbent Assay (ELISA) were assayed 72 Knee Osteoarthritis patients and 8
controls (normal) for detection of serum enzymes of Matrix metalloproteinase-3 (MMP-3) and Matrix metalloproteinase-13 (MMP-13). Further, the study is confined to two Human ELISA kits of ab100607 MMP-3 and ab100605 MMP-13 from Abcam, USA.

1.11. A preview of the report

The doctoral thesis organized into six chapters.

The First chapter is the introductory and it brings out concept and prevalence of Osteoarthritis and Knee Osteoarthritis, Erythrocyte Sedimentation rate, serum enzymes of Matrix metalloproteinases, research hypothesis, aim and objectives.

The Second chapter describes the literature review pertaining to Knee Osteoarthritis, Erythrocyte Sedimentation rate and serum enzymes of Matrix metalloproteinases i.e., MMP-3 and MMP-13.

Third chapter is the study area and it brings out the Mysuru city profile with reference to history and its development, physical setting, topography, regional connectivity, tourist importance, population growth and its trends, population of scheduled castes and scheduled tribes and history and health infrastructure availabilities in Krishna Rajendra Hospital.

The Fourth chapter deals with the materials and methods and it brings out the materials were used for the study and methods followed by various steps, procedures, protocol of Biochemical estimation of ESR level, serum enzymes of MMP-3 and MMP-13 and statistical applications were applied for analyzing the data.

The Fifth chapter is the results and discussion of demographic profile of Knee Osteoarthritis patients such as sex, age, family structure etc. Followed by food habits and
dietary pattern, clinical background, WOMAC scores, pain of Knee doing for different activities of Knee Osteoarthritis patients, Body Mass Index, Erythrocyte Sedimentation Rate, Serum enzymes of Matrix Metalloproteinases-3, Matrix Metalloproteinases-13 of Knee Osteoarthritis patients and controls and Scatter plot matrix among the variables.

The Sixth chapter, the last one covers the summary and conclusions followed by suggestions.