1. INTRODUCTION

Immune system is the defence system of our body which protects us from invading pathogens. It is able to differentiate self from nonself components. However sometimes this mechanism fails resulting in inappropriate targeting of self components leading to a condition called auto (self) immunity (defence). Autoimmunity is a biological phenomenon which activates self reactive B and T lymphocytes. Autoimmune reactions due to the abnormal auto reactive T cells and auto-antibodies can cause organ specific or systemic diseases. Autoimmune diseases (AID) are characterized by the failure of immune-tolerance against self antigens that are regulated by complex genetic factors or infectious agents and/or environmental factors. Autoimmune diseases can be divided into two categories, organ-specific diseases like diabetes mellitus (DM) and intestinal bowel diseases (IBD), that only affect specific organ systems, and systemic diseases such as systemic lupus erythematosus (SLE), Rheumatoid arthritis, systemic sclerosis (SS) and Sjogren’s syndrome (SJS) which involve the whole body.

Rheumatoid arthritis

According to a 2014 report of the UN non communicable diseases account for about 2/3rd of all deaths. Rheumatoid arthritis (RA) is a non communicable inflammatory disease which leads to progressive destruction of synovial joints (Bodman & Roitt, 1994). The worldwide prevalence of RA is 0.8% with an annual incidence of 0.5-1% in both developed and developing countries (O’Dell et al., 2007; Scott et al., 2010). Prevalence of RA in Indian population was reported to be about 0.75% (Malaviya et al., 2003). Rheumatoid arthritis (RA) is diagnosed on the basis of American College of Rheumatology (ACR) criteria, presence of anti-cyclic citrullinated antibody (anti-CCP). ACR criteria involve radiological, clinical and serological parameters, where presence of three or four criteria confirms the diagnosis of RA. Measures of inflammation as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) elevation are also considered important for diagnosis of inflammatory disease as RA.
The inflammation in RA is measured by acute phase reactants. The disease activity measures of RA are expressed in disease activity score-28 (DAS28) which measures swelling and tenderness in 28 joints of affected person according to ACR criteria (Felson et al., 1993; Prevoo et al., 1995). In RA the most widely used inflammatory markers are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) evaluations. The level of inflammation is measured by acute-phase plasma protein levels in blood that is indirectly measured by ESR. Since the inflammation causes the red blood cells to settle more promptly (Firestein et al., 2009). ESR is a non-specific acute phase reactant of systemic inflammation, but elevated levels has many reasons therefore only rheumatic disease may not be attributed for high ESR. ESR levels can be greatly influenced by malignancies, infections or abnormal size and shape of red blood cells (Kushner 1991). In affected rheumatic patients ESR tend to be higher in females as compared to males (Nestel AR, 2012; Radovits et al., 2008; De Silva et al., 2008). It also increases with age (Nestel AR 2012; Radovits et al., 2008; De Silva et al 2008; Ranganath et al., 2005) and body mass index (BMI) (Firestein et al., 2009). To overcome the limitations of ESR, another important component as CRP has been suggested as a substitute inflammatory marker for the disease activity evaluation in Rheumatoid arthritis (Fransen et al., 2003). The use of CRP over ESR in assessing Rheumatoid arthritis inflammation estimation has been emphasized by (Crowson et al., 2009).

In Rheumatoid arthritis elevated production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and depletion of antioxidants are being implicated. The intracellular redox state may be an important determinant leading to the progression of the disease. The enhanced imbalance between oxidants and antioxidants may be enough to induce structural and functional changes in cells and tissues. This state is typically termed as oxidative stress. These ROS and RNS have both beneficial and toxic effects. Feldmann et al, 1996 reported that the generation of ROS (SOD, H₂O₂) in large amount by activated macrophages in RA leads to oxidative stress (Feldmann et al., 1996).
These ROS are capable of damaging membrane lipids, connective tissue and nucleic acids of the cell. Free radicals and their byproducts are essential mediators of inflammation. Synovial cavity is the main target of immune attack in RA which may be due to chemo-attractant property of synovial fluid. This leads to accumulation of leukocytes within the synovial tissue and causes respiratory burst characterized by increased oxygen consumption and increased anaerobic glycolysis leading to generation of superoxide, hydroxyl, hypochloric radicals etc. (Marshells & Bangert, 1995). Neutrophils are very active in synovial fluid of RA patients which augment inflammation and enhance damage of joint components (Vasudevan and Sree Kumaris, 2001; Nurcomb et al, 1991).

Free radicals generation and enzymes degrading them are in tight homeostasis in the body, which prevent damage. However, imbalanced activity may be lead to free radical mediated tissue injury. The study by Blake et al. 1981 showed that enzymatic/non enzymatic antioxidant systems are highly deregulated and impaired in RA (Blake et al., 1981). Thus, there are chances of free radical mediated damage in the body of RA patients due to their higher production and impaired quenching. The analysis of activities of different antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) may have effective therapeutic potential (Blake et al., 1981; Mazetti et al., 1996; Shah & Vohora, 2002).

The higher inflammation and oxidative stress not only affects joints but also affects various other systems leading to important systemic manifestations. Cardiovascular disease (CVD) is one of them. The higher mortality rate of RA patients is due to their exposure to greater risk for cardiovascular diseases (CVD), myocardial infarction (MI) and stroke (Wolfe et al., 1994). There is dyslipidemia observed in RA patients (Mishra et al, 2012) that may increase their morbidity and mortality from CVD. In recent years tremendous data reported the role of biochemical
parameter which play an important role in RA (O’Dell, et al., 2007; Lucia et al., 2011; Pallinti et al., 2009; Fischman et al., 2010; Magnus et al., 2010; Chavan et al., 2015).

As RA has genetic predisposition and many loci have been implicated which are associated with the disease. The heritability of RA has been predicted in approximately 60% populations (Mac Gregor et al., 2000, Deighton et al., 1989). Genetic susceptibility for this disease is very high where (i) according to R.M. Nakamura, first degree relatives of the RA patients are at four to six times higher risk for developing RA (Nakamura RM, 2000). The presence of some MHC-II encoding alleles in HLA-DR molecules as (HLADRB1 *0401 and HLA-DRB1*0404) are found to be associated with more severe disease condition (Mattey et al., 2007, Van Gaalen et al., 2004).

There are some gene which may play important role in the disease susceptibility to RA (van der D et al., 2010, Szodoray et al., 2010). Linkage disequilibrium studies revealed susceptibility loci for RA within several chromosomes, with one consistently implicated the HLA-DRB1 gene. Peptidyl arginine deiminase type IV (PADI4) (EC 3.5.5.15) is one of member of PADI gene family located on chromosome 1p36.13. It encodes the enzyme which is responsible for the posttranslational conversion of arginine residues into citrulline in many mammalian tissues (Zhou & Menard 2002). A previous cloning analysis report has shown the presence of five isoforms in rodents: PAD I (Ishigami et al 2001), PAD II, PAD III (Nishijyo et al., 1997), PAD IV (Ishigami et al., 1998) and ePAD, which was provisionally named as PAD VI. PAD I has been identified in the epidermis (Guerrin et al., 2003), PAD II in sweat glands, PAD III in the hair follicle, while PAD IV has been detected in the precursors of neutrophils and macrophages (Vossenaar et al., 2004) and PAD VI mRNA has been detected in the testis, peripheral blood leucocytes and ovary.
Another gene PTPN22 is located on chromosome 1p13 which encodes the lymphoid-specific tyrosine phosphatase (LYP), which is involved in the suppression of T cell activation and thereby in T cell dependent antibody production (Gregersen et al., 2006). The R620W polymorphism in PTPN22 gene affects a proline-rich motif of LYP, involved in the protein–protein interactions.

Begovich et al. (2005) first described the association between a functional single-nucleotide polymorphism (SNP) in the coding region of the gene PTPN22 and RA, and the study has been replicated by several other groups in the UK, Spanish and Dutch RA populations (Lee et al., 2005; Hinks et al., 2005; Orozco et al, 2005). Risk of RA is increased 2 fold in the presence of the PTPN22 polymorphism R620W or(1858 T/T genotype) (Harrison 2006).

The actual agents involved in joint degradation or the matrix metalloproteinases (MMPs). The matrix metalloproteinases (MMPs) are a family of zinc dependent endopeptidases, that regulate the breakdown of extracellular matrix (ECM), which is necessary for physiological processes of tissue remodeling, morphogenesis, embryonic development and resorption but it very importance in the pathological conditions including tumor growth, inflammation and metastasis (Lambert et al., 2004). Extracellularly, the activity of MMPs is regulated by tissue inhibitor of metalloproteinases (TIMPs) (Visse and Nagase 2003). The TIMP family consists of four different members (TIMPs 1 to 4). All of these other than TIMP-4 are expressed in body fluids and most tissues. TIMP-4 has a tissue-specific distribution, being present in striated muscles, brain and ovaries. The expression of TIMPs is typically induced by external stimuli such as certain inflammatory cytokines (IL-6, IL-1β) and by certain growth factors.

Extracellularly, TIMPs forms high affinity noncovalent bonds with MMPs and inhibits its activities. Proteolytic activities of MMPs are inhibited by the binding of the amino-terminal domain of TIMP to the active site of MMPs. The carboxy-
terminal domain of certain TIMPs, may also form complexes with proenzymes of MMPs (proMMPs) (Visse and Nagase 2003). However, limited differences in TIMPs' specificities have been recognized. Indeed, TIMP-1 is a special inhibitor of soluble MMPs, while TIMP-2 and TIMP-3 are efficient inhibitors of the membrane-bound MMPs. TIMP-3; inhibitory activity is enhanced by some members of the ADAMTs (a disintegrin and metalloproteinase with thrombospondin motifs) family which inhibits TNF-α-converting enzyme and aggrecanases. TIMPs have been shown to regulate the cell survival and apoptosis, stimulate cell proliferation participating in mitosis and tissue differentiation, and inhibit angiogenesis.

Tissue damage in RA is determined by the ratio of MMP:TIMP. According to a study TIMP gene polymorphism are also associated with Rheumatoid arthritis (Lee et al., 2003). All these factor affects the protein of RA patients. Therefore it is very important for analyse proteomic and metabolic changes occurring in RA.

NMR analysis of metabololites allow rapid identification of metabolic perturbations in biological systems and is routinely used to evaluate systemic response to any subtle pathophysiological stimuli or stress. Over the years, it has been increasingly recognized as a valuable complementary approach to genomics, transcriptomics, and proteomics to achieve a complete understanding of the disease mechanism. It also provides opportunities for developing diagnostic/prognostic biomarkers for the disease. and. Practically, metabolomics approaches relies on multivariate statistical analysis of data collected with advanced analytical techniques - such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy. Of them, NMR combined with multivariate analysis is an ideal platform and it has been extensively used in several metabolomic studies. Compared to other analytical and biochemical methods, NMR poses several advantages. First, it is applicable for a variety of biological and clinical samples, tissue extracts and even cell lines. Second,
it is rapid, quantitative and offers the potential for high-throughput (>100 samples per day). Third, it is non-destructive, non-invasive and nonselective i.e. multiple metabolites are detected in as little as 50 µl of peripheral blood plasma/serum. And last it requires virtually no sample preparation and provides highly reproducible results.

Therefore it becomes very important to understand the process of the disease which could help in identifying the factors which are dearranged in the disease. Identifying of these would not only help in the diagnosis but would help also help in providing more therapeutic targets. Thus which can be utilized for diagnosis and better management of RA.

Identification of these would help in determining the potential of already existing parameter on our Indian participants. Analysis of other factor would help clinicians plan more targeted treatment to same tissue destruction and prevent ongoing inflammation in RA patients. The patients investigation was therefore planned to i) Analyze the existing diagnostic parameters and confirmation of RA with help of clinical diagnosis, ii) Analysis of serological parameter possible changes, iii) evaluation of the status of oxidant-antioxidant pathway in RA patients iv) Genome wide association studies(GWAS) of PADI4, PTPN22 and TIMP4 genes for their role in susceptibility to RA.

**Objectives**

RA being systemic inflammatory disease involves attractions in many physiological components therefore the present study was planned to analyze biochemical parameter and genetic association of a few genes for their role in susceptibility of RA.

(I) To study the effect of RA on serum enzymes involved in inflammation.

(II) Genetic polymorphism of PADI4, PTPN22 and TIMP4 in the RA subjects and controls for their possible role in disease onset and/or progression of RA.

(III) Analysis and compilation of results obtained.
2. REVIEW OF LITERATURE

2.1 Rheumatoid Arthritis

In the 4\textsuperscript{th} century BC Rheumatic diseases were first recognized by Hippocrates. The term rheumatology has its origin from “rheuma,” which indicates flowing, and is mentioned in an article titled Hippocratic corpus in which he observed that podagra was related to opulent lifestyle and termed it as “arthritis of the rich.” (Pasero and Marsen, 2004). Goemaere et al., (1990) reported that the appearance and distribution of lesions in ancient skeletons and suggested that Rheumatoid arthritis may have existed in North America at least 3000 years ago.

\textit{Rheumatoid arthritis} (RA) is a systemic, chronic autoimmune disease, characterized by inflammation in synovial membrane (synovitis). The affected joints become warm and swollen with tenderness and stiffness in the final stage which causes functional disability (Vandana et al., 2012). Inflammation and systemic swelling of peripheral joints is the hallmark of RA (Michelle & Kahlenber, 2011). Inflammation leads to cartilage destruction, bone erosion and joint deformity. RA patients commonly report pain and stiffness in multiple joints. Although some of them experience symptoms at just one location but later the symptom emerge at other sites, accompanied by symptoms of dieting behavior, weakness, or fatigue. The joints which are always involved are wrist joints, proximal interphalangeal (PIP) and metacarpophalangeal (MCP) joints. The distal interphalangeal (DIP) joints and sacroiliac joints are not affected (Harris et al., 2005). RA preferentially affects women, in ratios around 4:1 (Wendy Marder, 2015).

2.2 Prevalence

Worldwide the estimated prevalence of RA is 1 to 2\% (Sudha et al., 2012). Another study of (Malemba et al., 2012) reported that the prevalence of RA in African population was 0.6 to 0.9\% in adults (Manish et al., 2011). The prevalence of male populations differs significantly among different areas of the world. This variation maybe related to lower occurrence of the disease in developing countries or due to differences in the age distribution between the populations studied.