5. DISCUSSION

*Rheumatoid arthritis* (RA) is an inflammatory autoimmune disease which affects symmetric and multiple joints of the body. RA causes significant morbidity and mortality as compared to general population. The disease has 0.8% prevalence with annual incidence of 0.5-1% in the world (Makhdoom *et al.*, 2009, Gubler *et al.*, 1953). Like other autoimmune disease RA is more prevalent in females as compared to males (1-4). The difference in the occurrence and development of aggressive disease in females is not clear but genetic and hormonal factors are suggested to be involved (6-11). Joint destruction occurs as the disease progresses. The study was planned to study the changes occurring in RA patients in their activity score (DAS-28), pain on visual analog scale (VAS), inflammatory changes and changes related to oxidative stress. Their general well being and risk of patients developing cardiovascular disease was also analyzed by evaluation of their response to therapy and analysis of lipid parameters.

The sample collection was done from hospitals, local clubs, neighbourhood patients and control were recruited after verifying inclusion and exclusion criteria as discussed in material and methods. The study was approved by Institutional Ethical Committee and written informed consent was obtained from all the participants.

In our study, there was no significant difference in the BMI and systolic blood pressure of patients and control (Table-2 and figure 7) and male and female RA subjects. The haemoglobin of the patients was significantly decreased in RA patients as compared to control. Previous studies have reported (Ganna 2014) low hemoglobin levels in RA which was related to disability and impairment. Choy and Panayi, 2001 have also reported the severity of the disease accounts for systemic manifestations with effects on blood. Anaemic syndrome has been reported to be a common manifestation which may result in increased disease activity (Bloxham *et al.*, 2011).
The patients showed increased ESR, pain on visual analog scale and CRP as compared to control. Though patients are on medication, still raised values indicate ongoing inflammatory process in their body leading to increased production of CRP. Female patients in our study had higher DAS and VAS as compared to Male RA subjects. Higher CRP is suggestive of reoccurrence of active disease sometimes along with ongoing medication or after withdrawal of the drug. DAS was significantly increased in patients along with the ESR and CRP level. Pain on visual analog scale (VAS) was significantly increased in patients (Table-4) with pain in females as compared to male RA subjects.

Our previous report has shown improves CRP, MDA, DAS-28 and VAS in RA patients treated with methotrexate at 6 months of followup (Patel et al., 2015). The aggressive disease as RA is controlled by DMARDs, NSAIDS, glucocorticoid and biologics wich target TNF-alpha, IL-1 and IL-6 receptors (23.39). There is heterogeneity in the response of the drugs and various side effects on liver and kidney are reported. In our study we could not observed adverse effects of MTX therapy on liver or kidney function.

Serum uric acid was lower in RA patients as compared to control. Uric acid is the end product of purine metabolism and potent antioxidant. It has protective effect against oxidative stress as an intracellular free radical scavenger. Choe and Kim, 2015 has reported reduced serum uric acid concentrations with leflunomide and methotrexate combination and methotrexate treatment alone with more pronounced changes with leflunamide treatment. Leflunamide may possibly affect urate transporters in renal epithelial cells (Emery et al, 2000) and as MTX is also showing similar response thus it may also have effects similar to leflunamide. Lower uric acid may be suggestive of increased urinary secretion of uric acid but may not be related to disease activity status in RA patients (Choe and Kim, 2015).
The values for SGOT and SGPT which indicate functional status of liver are significantly lower in RA patients. Our treated patients showed significantly reduced levels of SGOT and SGPT as compared to control. In patients the values for SGOT positively coorelated with SGPT ($r=0.575; p<0.01$). Study by Iannone et al, 2014 has shown that RA patients may be successfully treated with MTX without increasing the risk of hepatotoxicity. However, Curtis et al, 2010 have reported abnormal SGOT or SGPT levels in their patients with RA on DMARD therapy (methotrexate $>10$mg/day). Our findings did not show elevated levels of these enzymes probably because of methotrexate administration with folic acid and vitamin C supplementaion with drug dose of 15mg/week (intramuscular or subcutaneous) (Patel *et al.*, 2015). However, patients refractory to low-dose MTX therapy may require larger doses of oral MTX. Several previous studies have demonstrated variability in bioavailability of oral MTX at high doses. This warrants a subsequent switch to parenteral MTX subcutaneous (SC). The pharmacokinetics of SC MTX is similar to intramuscular MTX but SC MTX may be preferred by most patients (Yadlapati & Efthmiou 2016). Our patients are also on SC or intramuscular MTX, which has better potential and effective at a dose of 15 mg/week.

In our study lipid per-oxidation in terms of MDA production was significantly increased in RA patients (Table 1) which may be due to increased ROS during chronic inflammation. Female patients had higher MDA as compared to male patients. Lipid peroxides are generated at the site of tissue injury due to inflammation and diffuses into blood and can be estimated in serum or plasma(Gutteridge JM (1995)) . Several studies (Ali *et al.*, 2014, Kamanli *et al.*,2004, Sarban *et al.*, 2005, Hassan *et al.*, 2001) have reported raised levels of MDA in the serum, plasma and erythrocytes of RA patients. In our study SOD levels
Superoxides anion ($O_2^-$) has important role in pathogenesis of many diseases. It is neutralized by SOD to hydrogen peroxide ($H_2O_2$). $H_2O_2$ is further quenched by activity of catalase and glutathione peroxidase. Transformation of $O_2^-$ to $H_2O_2$ prevents the formation of aggressive compound as peroxynitrile (ONOO) and hydroxyl radical (OH) (Afonso et al., 2007). The patients showed significantly higher activity of SOD and ALP (Table 8). There was strong positive correlation between SOD and ALP activity (Table). They showed reduced activities for catalase and glutathione reductase.

Reactive oxygen species and oxidative stress have a role in the pathogenesis of RA (Kamanli et al., 2004). Free radicals and other reactive species play an important role of super oxidant leading to oxidation of biomolecules like proteins, amino acids, lipids and DNA (Mirshafiey et al., 2008), which are ultimately responsible for cell injury and death (McCord 2000). Prime targets of ROS attack are the polyunsaturated fatty acids in the membrane (Table 8) lipids causing lipid peroxidation (LPO) which may lead to disorganization of cell structure and function. Further decomposition of peroxidized lipids yields a wide variety of end-products, including malondialdehyde (MDA) (Gambhir et al., 1997). Malondialdehyde (MDA) is one of an important lipid peroxide which is high in RA patients (Mishra et al., 2012, Patel et al., 2015). Measurement of MDA is widely used as an indicator of LPO.

Many studies have reported high MDA in the serum, plasma and synovial fluid of RA patients (Kamanli et al., 2004, Gambhir et al., 1997, Pallinti et al., 2009). MDA has an important role in pathogenesis of RA. There is growing awareness that reactive oxygen species and free radicals may play an important role in mediating cellular injury and tissue damage in rheumatoid arthritis. Thiele et al.
(2015) has reported malondialdehyde-acetaldehyde (MAA) adduct formation is increased in RA. They appear to result in robust antibody responses which are strongly associated with anti citrullinated protein antigens (ACPAs) suggesting that MAA formation may be a cofactor that drives tolerance loss, resulting in the autoimmune responses characteristic of RA.

Higher levels may be the result of respiratory burst triggered by leucocytes. A study has shown activation of neutrophilic myeloperoxidase-hydrogen peroxide system in RA synovial tissue which may contribute to cyclic self-perpetuating inflammation (Nurcomb et al. 1991). Methotrexate treatment has been reported to increase Zn-SOD activity but it has no effects on GSH-Px in rats (Armagan et al., 2008, Al-Saleh et al., 2009).

But possibly increased activity of SOD (Vijayakumar et al., 2006, Cimen et al., 2000) may be attributed to increased $O_2^-$ production by hyperactive cells leading to SOD induction (Gregory & Fridovich 1973). Another possibility may be excessive free radical production through the xanthine-xanthine oxidase system is the primary factors in RA, rather than an impaired antioxidant system (Cimen et al., 2000). Else higher SOD levels may be a change to nullify excessive free radical production. Post treatment the antioxidants are increased which lead to lower plasma MDA and increased total antioxidant capacity (TAC) (Nourmohammadi et al., 2010).

However lower SOD has also been reported in patients with RA on MTX therapy in comparison with RA without MTX therapy (Al-Youzbaki et al., 2013). It has also being observed that MTX can suppress directly or indirectly the generation of active oxygen metabolites induced by IL-6, which is produced in response to TNF-α stimulation in synovial cells of RA(Sung et al., 2000) as well as in polymorphnuclear cells. The increased levels serum Cu/Zn SOD may support the hypothesis of radical-mediated injury.
Over expression of extracellular SOD leads to dismutation of superoxide resulting in \( \text{H}_2\text{O}_2 \) accumulation. Analysis of \( \text{H}_2\text{O}_2 \) in different settings is being done and authors conclude, more SOD does not mean more \( \text{H}_2\text{O}_2 \) (Lin et al., 2006). The formation of \( \text{H}_2\text{O}_2 \) due to dismutation of superoxide is limited by the amount of superoxide, not by the rate it is converted to \( \text{H}_2\text{O}_2 \). Accumulation of superoxide leads to the oxidation of NO forming peroxynitrite. There more \( \text{H}_2\text{O}_2 \) is unlikely to be toxic as this would amount to substituting a very mild cytokine (\( \text{H}_2\text{O}_2 \)) for a potent (peroxynitrite) (Zaghloul et al., 2014).

Decreased activity of SOD in RA patients has also been reported (Mohamad A 2011). However our study is in line with (Kamanli et al., 2004) who have reported increased SOD levels in RA patients. Mazetti et al. (1996) have reported higher serum copper/Zn superoxide in patients with RA. Igari et al. (1982) have reported correlation between the overall synovial SOD activity and both the clinical severity of the disease and the CRP levels. Mazetti et al. (1996) have concluded that exercise induced hypoxic reperfusion mechanism within the inflamed joint in RA may lead to increased production of Cu/Zn SOD. Mateen et al. (2016) have shown that increase of oxidative stress increases with the progression of RA.

\( \text{H}_2\text{O}_2 \) formed due to activity of superoxide dismutase need to be detoxified by glutathione peroxidase and catalase activity. Catalase plays an important role in preventing ROS mediated damage by using \( \text{H}_2\text{O}_2 \) and converting it to water and oxygen. In our RA patients, catalase activity is significantly decreased as compared to control. Lower catalasae activity may be due to interaction of catalase by hydrogen peroxide(Mohamad et al., 2011). Lowered activities of their enzymes may lead to conversion of \( \text{H}_2\text{O}_2 \) to hydroxyl radical by iron released from hemoglobin of lysed erythrocytes (Taysi et al.,2002). However unaltered catalase activity in RA patients has been reported (Veselinovic et al., 2014).
Catalase activity was not found in serum of RA patients. Decreased erythrocytes catalase activity is also being reported (Taysi et al., 2002). Our study is in accordance with and shows lower catalase activity in serum of RA patients. Catalase expression affects expression of genes which influence inflammation (Benhamou et al., 1998). Lower levels of catalase may be responsible for high inflammation in RA. Cimen et al. (2000) have reported higher SOD activity and MDA levels and unchanged catalase and GSH-Px activities in RA patients. The study by Gonzalez et al. (2015) observed the positive correlation between antioxidant GPx and lipid peroxidation levels. Their results suggest that GPx activity is involved in the primary mechanisms against oxidative stress in RA patients. Both GPx and catalase use H2O2 as substrate where catalase acts in the presence of high concentration of the substrate while GPx acts at lower concentrations. They also suggested that H2O2 concentration may be lower than in other chronic inflammatory diseases, with oxidative damage being mediated possibly by HO (Prego et al., 1997).

Glutathione reductase (GR), an oxidative stress inducible enzyme, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes. Glutathione reductase is a flavoenzyme dependent on NADPH that catalyzes the reduction of GSSH to GSH. Feijoo et al. (2010) observed that myeloperoxidase levels are elevated in patients with chronic inflammatory disease, especially those with active disease, and that high myeloperoxidase levels are related to an increase in oxidative damage and the inflammatory response, for myeloperoxidase and GR seem to show a similar activity pattern based on the availability of NADPH. Erythrocyte GSH and glutathione reductase levels rise in healthy individuals exposed to chronic oxidative stress(Evelo et al., 1992). These findings suggest that GSH levels may be
inappropriate in patients with active rheumatoid arthritis, perhaps reflecting impaired glutathione reductase activity as observed in our study. The study by Aryaein et al. (2011) showed that GR, vitamin E, Beta-carotene was lower and MDA was higher in the patient group than in controls. Kamanli et al. (2004) observed significantly lower GSH-Px, catalase, levels of GSH in plasma of RA patients. However higher GR activity have also been reported in RA (Bazzichi et al., 2002). Kerimova et al. (2000) also reported decreased catalase and unaffected GR activities in RA subjects. Low GR activities in the red blood cells and polymorphonuclear leucocytes of patients with RA was reported by Mulherin et al. (1996). Vanella et al. (1987) described reduced EGR activity in 15 patients with rheumatoid arthritis and Tarp has reported a similar finding in nine patients with rheumatoid arthritis (Tarp 1992).

In our patients alkaline phosphatase (ALP) activity is higher relative to control. ALP showed strong positive and significant relationship with SOD. ALPs role is implicated in osteoid formation and mineralization and expression of its isoform is in osteoblasts, leucocytes, liver, kidney, breast and brain (Weiss et al., 1986, Gum et al., 1990). The bone formation markers are measured in serum and about half of ALP in serum comes from bone. Several studies (Thompson et al., 1990, Nanke et al., 2002, Spooner et al., 1982) have reported high serum ALP levels in RA patients. The increased activity may be due to inflammatory cytokines as interleukin-1 (IL-1) which has been correlated with the acute phase reactants (Thompson et al., 1990) and CRP levels. The role of T-cells is well documented in the pathogenesis of RA. Raised ALP may be due to its leakage from injured or killed cells.

Alkaline phosphatase has been implicated as marker in RA patients. It can provide diagnostic information by determination of isoform of ALP derived from
liver or bone (Vaithialingam et al., 2013). Thus MDA and antioxidants systems work reciprocally to keep oxidative stress mediated damage in control. An inverse association between serum antioxidant levels and inflammation have been reported (Paredes et al., 2002).

Study by Jalili et al. (2014) showed that antioxidants may significantly improve disease activity but do not affect the number of painful and swollen joints. Thus antioxidants may be helpful in control of clinical outcomes and oxidative stress in RA patients. In conclusion oxidative stress management may be considered a therapeutic option for RA along with DMARD. Supplementation of antioxidants along with catalase and/or GPX may confer more protection. In recent studies RA since to have derangement of mineral contents as magnesium copper, zinc, phosphorous, boron etc. They are required in optimum concentration in the body. However changes in their levels as Mg(17), Zn (18) Cu (copper).

There is scarcity of data related to minerals and their role in RA. Magnesium levels in our treated RA patients were lower as compared to controls. Mg (Talal 1992) is one of the essential nutrient of the body and studies suggest its role in chronic inflammation (Weisinger & Bellonn, 1998). Decreased levels of Mg is considered as marker for RA (Lucia et al., 2011 Linos et al., 980). Magnesium has important functions in cardiovascular system, as an activator of sodium potassium ATPase, antiarrhythmic and is associated with cardio vascular disease susceptibility (Weisinger & Bellonn, 1998; Chiuve et al., 2011; Mahalle et al., 2012; Makhdoom et al., 2009). In humans, low serum magnesium concentrations have been associated with high C-reactive protein (CRP) levels (Guerrero & Rodriguez 2002; Rodriguez & Guerrero 2008). Several cross-sectional studies have reported inverse relationships between magnesium intake and some inflammatory markers, including high sensitive CRP (hs-CRP) and IL-6 (King et al., 2005; Song et al., 2005; Durazzo et al., 2006; Song et al., 2007; Chacko et al., 2010).
The level of phosphorous was significantly higher in RA patients as compared to controls (6.13± 0.101 Vs 4.08± 0.122; p<0.05). In RA patients phosphorous showed positive correlation with catalase (r=0.396; p<0.05) and zinc levels (r=0.344; p<0.05) and negative correlation with copper (r=-0.412; p<0.05) and MDA (r=-0.345; p<0.05). The studies suggest strong association between elevated phosphorous and Ca and phosphorous products and the development of calciphylaxis. Phosphorous influences a number of pathways involved in vascular calcification. It also has a role in induction of differentiation of vascular smooth muscle cells into osteoblast-like cells capable of extraskeletal mineralization which is important process in development of vascular calcifications. Thus phosphorous may have a role in augmenting inflammation.

In our study the levels of zinc and copper are higher in RA patients as compared to controls. This clearly shows that RA patients are not deficient in Zinc or copper (Milanino et al., 1993). As zinc is considered anti-inflammatory with studies showing negative correlation between zinc and levels of IL-1 and TNF-α. Our study is in accordance with findings of Mierzecki et al., 2011 who have reported nonsignificant but higher levels of zinc in serum. Though zinc levels should have been lower considering the role of proinflammatory cytokines as IL-1 and TNF-α inhibit albumin synthesis in liver and lower their zinc-binding capacity, which should in turn reduce the plasma zinc levels. However lower values of zinc in other studies may be due to pharmacological treatments or other effects which also need to be considered. Serum zinc levels have been shown to decrease during acute-phase response of inflammation and with treatment with NSAIDS (Balogh et al., 1980). It is suggested that these may be due to different disease activity and treatments. Probably alterations in inflammation may have some role in the levels of essential minerals.
In our study levels of copper are also higher. Their levels have been shown to increase in all inflammatory processes including RA. Our findings are consistent with Scudder et al 1978 and Tuncer et al., 1999. The studies have shown that hypercuperemia was associated with inflammatory response is due to oxidative stress (Ford 2000) as they found positive correlations between serum Cu levels and inflammatory markers serum CRP and ESR in RA patients (Liuzzo et al., 1994; Salomen 1991). In contrast to many other studies, we found inverse correlation between Cu and CRP level ($r=-0.419$, $P<0.01$). Cu is an environmental bioelement which play a key role in the cell’s physiology, as a cofactor or component of the enzymes, participating in anti-oxidative process, or in detoxification of oxygen free radicals. RA patients have higher levels of copper as compared to control (Rainford 1982). Later on it was found that Cu complexes were effective in treating arthritis. Cu complexes have anti inflammatory properties and antarthritic drug in their active form are complexed with copper (Rainford 1982). The hypercuperuria that develops was suggested to be the outcome of dyslipidemia (Aaseth et al., 1978) or the cytokines have been reported to enhance the release of Cu thioneins during the oxidative burst of polymorphonuclear cells (Balogh et al., 1980). As many studies have reported higher levels of copper in active RA, thus copper may be used as additional biochemical marker.

RA causes significant morbidity as a result of synovial inflammation, joint destruction and disability. Patients with RA have abnormal lipoprotein pattern (dyslipidemia). They have low level of HDL-c and high level of LDL-c in a pattern similar to inflammatory and infectious diseases (Rantappa et al., 1991, Filippatos et al., 2013). Systemic inflammation in RA leads to significantly increased risk of cardiovascular diseases as compared to general population (Turesson et al., 2004; Solomen et al., 2006). Therefore control of inflammation may have beneficial effects on cardiovascular risk and improvement in the lipoprotein profile.
The patients showed dyslipidemia with high total cholesterol, LDL-cholesterol, VLDL and low HDL cholesterol. Patients with RA have abnormal lipoprotein pattern. In our study also though dyslipidemia is observed as compared to control, but the values of lipid parameters analyzed are either within permissible limits or show borderline variations. These values may not be predictor of CVD risk in our RA patients. Several other studies did not show any variation in lipid levels in RA patients with respect to healthy population (Dessein et al., 2002). Some other observed an overall reduction in all lipid subfraction in case of active disease (Boers et al., 2003). The existing data has wide heterogeneity in the reporting of associated dyslipidemia. Studies have shown that in established RA, total cholesterol levels were only marginally raised irrespective of disease activity (Noumohamad 2007). High cholesterol induces oxidative stress leading to free radical generation that promotes lipid peroxidation (Prasad 2003). In hypercholesterolemia, high levels of lipids and phospholipids are accumulated resulting in increased production of arachidonic acid and prostaglandins with the help of phospholipase A2 and cyclooxygenase enzyme (Laurence et al., 2001). MDA is the end product of lipid peroxidation; therefore its measurement gives indirect evidence of LDL oxidation. Under intense oxidative stress, aldehyde level increases and take part in numerous pathological conditions such as cancer, arthritis, atherosclerosis, and cardiac disease (Uchida et al., 2003). Patients with RA showed higher accumulation of MDA.

Although lipid levels are important risk factor, especially high density lipoprotein, other studies have observed changes in TC, LDL-c and HDL-c after MTX monotherapy and combination therapy (Pincus et al., 2003) and improvement in HDL-c levels post DMARD therapy (Watson et al., 2009). In our previous report (Patel et al., 2015) we observed increased HDL levels after 24 weeks of followup in MTX treated patients, but long term therapy does not have any favourable effects on HDL, nor does it increase TC, VLDL, TG and LDL. In RA there are reports showing either
increased, decreased or similar levels for TC, LDL-C and HDL-C in comparison to control subjects (Heldenberg et al., 1983; Lorber et al., 1985; Lakatos & Harsagyi 1988; Kavanaugh 1994; Asanuma et al., 1999). Larger cross-sectional study in 204 patients with RA, demonstrated an inverse association between elevated CRP and HDL-C-levels (White et al., 2006). Studies have shown presence of dyslipidemia at least ten year before the onset of clinical symptoms of RA (Van Halm 2006). In our study boarder line dyslipedemia is present in subjects with RA who are on DMARD treatment. Our patients are given local corticosteroid when they complain of severe pain and swelling. Prednisolone rapidly improved the atherogenic index (total/HDL cholestrol), an important prognostic CVD risk factor and it appears that the use of corticosteroid is not a risk factor for cardiovascular disease (Wallberg et al., 1997). Conventional DMARD (including corticosteroids) treatment has favorable effects on the lipid profile, as there is mounting evidence for favorable effects of DMARD treatment on the cardiovascular risk in RA (Van Halm et al., 2006) this might be (partially) mediated by favorable effects on the lipid profile..

**Genetic Polymorphism**

PADIs are involved in the post-translational deimination of arginine in proteins; the resulting citrullination partially un folds proteins via loss of the positive charge of the arginine moiety (Ikari et al., 2005, Yamada et al., 2005, Yamamoto et al., 2005). PADI4 is non-HLA genetic factors involved in RA by citrulline formation, which have been implicated in RA pathogenesis (Anzilotti 2010). Our results (table-16) showed significant association of PADI_102 C/T polymorphism in RA. These result were similar to the data of Somia (2012) who have reported the significant association between RA and control. PADI gene has been suspected in the prognosis, activity and severity of RA (Suzuki et al., 2013, Ceccarelli et al., 2012). Ikari et al., (2005) was also reported the significantly differences in frequencies of RA and
control. In Asian population some variants of PADI4 genes have been reported in susceptibility of RA (Ikari et al., 2005, Suzuki et al., 2003, Hoppe et al., 2006). No significant association was observed between RS188_2 C/G polymorphism of PADI in RA and control. Many studies have failed to confirm this association in various European populations (Martinez et al., 2005, Burr et al., 2010).

PADI4 haplotypes have been demonstrated to be associated with RA in several different populations, including Japanese, Korean and Chinese cohorts(Suzuki et al., 2003, Freudenberg et al., 2011, Fan et al., 2008). Potential explanation for the differences between different populations may be attributed to differences in the genetic variation in PADI4, or in gene-gene interaction or gene-environment

PTPN22 gene is negative regulator of signaling pathways of T and B cell receptor which encode the lymphoid protein tyrosine phosphatase (Lyp). (Cloutier & Veillette 1999 & Stanford et al., 2010) At the position of 620 in PTPN22 1858C>T polymorphism arginine changed to tryptophan residue and 1858T variants are found to be associated with diabetes. (Bottini et al., 2004) Protein tyrosine phosphatase non receptor 22 (PTPN22) has recently been recognised as a missense SNP, associated with RA. (Begovich et al., 2004) Both RA and type I diabetes (T1D) show strong association with Trp620 allele (rs2476601) polymorphism. (Lee et al., 2007, Smyth et al., 2008).

A replicated study of PTPN22 revealed the association with juvenile idiopathic arthritis (JIA) and RA. (Anne et al., 2005). The different studies in Japanese and Russian populations reported that there was no direct association of PTPN22 R620W polymorphism with RA. (Ikari et al., 2006 & Zhebrun et al., 2011) An Australian case-control study, recently reported the association of PTPN22
rs2476601 polymorphism with JIA in females only. (Chiaroni et al., 2015) In European population other locus of PTPN22 (rs3789607, rs12144309, rs3811021 and rs12566340) were genotyped and they found that was not associated with risk of RA. (Wan R. Wan Taib et al., 2010) Independent of HLA, PTPN22 1858C>T gene polymorphism is best described genetic risk factor for RA. (Lee et al., 2012) In central India a research finding revealed the association between PTPN22 polymorphism and RA while there was no association of Vitamin D receptor (VDR) polymorphism with RA susceptibility. (Shukla et al., 2014).

A recent study in Iran showed that only C allele is present and there was no association with autoimmune disease susceptibility including RA in the population (Ahmadloo et al., 2015) but a study in South West of Iran reported that PTPN22 may play an important role in susceptibility of autoimmune diseases. (Abbasi et al., 2016) The same SNP reported in JIA is sex specific where females are reported to be more susceptible (Goulielmos et al., 2016). The risk of RA in Asian populations is not associated with PTPN22 1858C/T polymorphism but a meta-analysis reported that susceptibility to RA is associated with PTPN22 1858C/T polymorphism in Caucasian populations. (Gowher et al., 2016).

**TIMP4**

TIMP-4 is belongs to the TIMP gene family and located on 3p25.2 chromosome. (NCBI) In general all mammalian TIMPs have two domains one with 125 amino acid residues of N’ terminal and one with 65 amino acids residues of C’ terminal, further three disulfide bonds provide conformational stability to the proteins. (Williamson et al., 1990) Matrix metalloproteinases (MMPs) are the major catabolic proteinases includes collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs which break ECM of joints. (Murphy & Nagase 2008) The degradation of
ECM is the characteristic feature of arthritic diseases in which the structural parts of the cartilage, proteoglycan aggrecan and type II collagen are degraded by MMPs (matrix metalloproteinases) and ADAMTSs (disintegrin and metalloproteinase with thrombospondin motifs). (Clark & Parker 2003) A previous study has reported that there were no significant differences of allele or genotypic frequencies of TIMP-4 C/T gene polymorphisms between RA and control. (Lee et al., 2008) An association study of TIMP-4 SNP provide no significant association to the development of schizophrenia and autism spectrum disorders (ASDs). (Yim et al., 2013).

Thus RA being an autoimmune disease is associated with disturbances in serum magnesium levels (Cortes et al., 2007). Inflammation is the primary cause for systemic alterations in the levels of metals and enzymes which is further modulating acute phase plasma proteins (Dean 2007). RA is associated with dyslipedemia as observed by higher LDL-c, TC, cholesterol and lower levels of HDL-c. The study by Mahalle et al., 2012; Chavan et al., 2015 have shown negative correlation between serum magnesium with TC, triglycerides, LDL-c and positive correlation with HDL-c. Thus lower serum magnesium may be associated with worsened lipid profile and increased CVD risk of RA patients. (Panoulas et al., 2007) reported increased serum uric acid is independently associated with CVD in RA patients but uric acid is lower in our patients as compared to controls.

Therefore RA is a complex disease which is associated with inflammation, dyslipedemia, hypomagnesia, higher levels of phosphorous, copper and zinc. Probably deregulated minerals are consequences of dyslipedemia and inflammation. We found lowed SGOT and SGPT and lower values for serum uric acid. Panoulas et al., 2007 reported increased serum uric acid is independently associated with CVD in RA patients but uric acid is lower in our patients as compared to controls. Thus
monitoring of kidney functions along with liver functions are recommended. Methotrexate is treatment of choice for management of RA in our patients.

NMR analysis of RA samples showed dyslipidemia and higher citrulline in RA patients as compared to control.

Lakatos reported lower TG and higher TC and LDL-c levels, and reported lower HDL-c levels (Lakatos & Hárságyi, 1988).

High lactate was observed in our study. Yang et al. and Lauridsen et al. suggested that an elevated level of lactate could be one of the biomarker for the diagnosis of chronic inflammatory conditions (Yang et al., 2015, Van 2012, Lauridsen et al., 2010). Indeed, lactate is reported to be associated with a pathogenic role (X.Y. Yang 2015, A. van 2012, M.B. Lauridsen et al., 2010). The higher lactate concentration in RA may be related to low oxygen levels prevalent in inflammatory environments (increased NAC—N-acetylated glycoprotein) and the induction of hypoxia, promoting anaerobic respiration (Yang et al., 2015, Lopez et al., 2012, Gu et al., 2012).

Citrulline synthesized from ornithine and carbamoyl phosphate is a key intermediate of the urea cycle. Citrulline is generated by posttranslational modification of arginine residues by peptidylarginine deiminase (Tarsca E 1996). Because citrulline is a major antigenic determinant recognized in the RA. Therefore, ACPAs have been used for the diagnosis of RA and have been established as a useful tool to discriminate RA from other arthritic diseases (Bas S 2002). NMR analysis showed the abundances of citrulline and ornithine in the RA group than those in the controls.