6. SUMMARY AND CONCLUSIONS

Summary

Rheumatoid arthritis (RA) is an autoimmune, inflammatory disease which involves multiple synovial joints. RA causes significant disability and burden as a result of synovial inflammation and joint destruction. Patients with RA face difficulty in performing their daily activities. RA, like other autoimmune diseases is found to be more prevalent in females as compared to males. Female to male ratio in RA is 3:1. The differences in over occurrence and development of worst and aggressive disease in females is not clear but genetic and hormonal factors are suggested to be involved.

Higher disease activity of RA is controlled by various treatments either given singly or in combination and include DMARDS, NSAIDS, glucocorticosteroids and biologics. However patients report differential response to the same therapy and side effects of these on liver and kidney are reported. RA also affects hemoglobin levels in affected individuals.

In RA, high inflammation is observed with high oxidative stress. Probably inflammatory and oxidative stressors together orchestrate the onset and progression of complex disease as RA. Immune cells and cytokines play an important role along with oxygen radicals as superoxide and hydrogen peroxide released by activated macrophages in the progression of rheumatoid arthritis. Oxidative stress is the condition when concentration of ROS and RNS becomes deleterious and damage the cells and biological macromolecules. Oxidative stress occurs due to disturbed balance between body’s antioxidant mechanisms and oxidative stress production and has important role in the development of chronic disease as autoimmunity like RA, cancer etc. They 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 tissue is the main site which experiences very high activity during RA. Due to chemo-attractant property of synovial fluid, leukocytes accumulate with in the synovial tissue triggering a respiratory burst characterized by increased oxygen consumption and increased anaerobic glycolysis leading to generation of superoxide, hydroxyl, hypochloric radicals etc. Neutrophils have been shown to be very active in synovial fluid of patients with RA which leads to inflammation and damage. Studies show that enzymatic/non enzymatic antioxidant systems are highly deregulated and impaired in RA. Markers of protein and lipid oxidation have been found to be raised in arthritic animals. Therefore there are chances of free radical mediated damage to the body of RA patients due to their higher production or improper scavenging.

Epidemiological studies have shown an increased premature mortality in patients with RA compared with general population. Patients with RA have abnormal lipoprotein pattern, principally low levels of high density lipoprotein (HDL) and high levels of low density lipoprotein (LDL). The improvement in the lipoprotein profile in RA appears to be associated with suppression of inflammation. Dyslipidemia is often associated with normal or decreased LDL, HDL in a manner comparable to inflammatory and infectious diseases. Patients with systemic inflammation like Rheumatoid arthritis face a significantly increased risk of CVD compared with general population. Control of inflammation may have an effect on modifying cardiovascular risk.

There is little and conflicting data available for mineral content like Mg, Zn, Cu and P in RA patients. These are essential elements which are responsible for normal functioning of the body. However change in level of Mg, Zn and Cu have been implicated in pathogenesis of RA, a chronic inflammatory disease as they are the co-factor of important enzymes involved in collagen and bone metabolism, the
antioxidant defense system and the immune system. In the previous studies, the
development and progression of RA was suggested due to marginal deficiencies of Zn
and Cu based on their serum levels. The alteration of these trace elements has been
linked to inflammatory response.

As RA is a complex disease therefore it is very important to analyze the major
players involved in the pathogenesis of RA. The present study was therefore planned
to analyze i) demographic and serological parameters in the RA patients with liver
and kidney function monitoring; ii) the level of MDA which is product of lipid
peroxidation and activities of enzymes of free radical scavenger system like
superoxide dismutase (SOD), GR, catalase and levels of alkaline phosphatase (ALP)
in RA patients treated with MTX, Folic acid Vit-C and occasional corticosteroids, iii)
Analysis of mineral content in RA patients versus control, iv) evaluation of lipid
parameters in RA and control subjects, v) genetic association of RA with a few loci
for analyzing their association with our Indian RA patients, vi) NMR analysis of
metabolites for their possible role in RA. These may help in determination of possible
roles of these in damage to the RA patients and may open therapeutic opportunities
for better management of rheumatoid arthritis.

There was no significant difference in the BMI of female and male patients.
Females have more aggressive and painful disease than males. As female patients in
our study had less average age than males suggesting early onset of RA in them as
compared to males. There are differences in functional capacity in female and male
subjects with RA where females have more functional impairment than males. In our
study also DAS-28-CRP is higher in females as compared to males. These differences
may be due to general strength of bones and muscles, bone mineral density (BMD),
hormones etc.
The patients had reduced hemoglobin as compared to controls. Patient’s ESR, pain during holding and lifting of objects on VAS and C-reactive protein levels were significantly higher as compared to control. The values for liver function test, i.e., SGOT and SGPT of RA subjects were significantly lower than control. As compared to control, the uric acid of RA patients was significantly reduced.

In our study lipid per-oxidation in terms of MDA production was significantly increased in RA patients which may be due to increased ROS during chronic inflammation. 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. There are reports of raised levels of MDA in the serum, plasma and erythrocytes of RA patients. In our study SOD activity were highly increased. Superoxides anion (O$_2^-$) has important role in pathogenesis of many diseases. It is neutralize by SOD to hydrogen peroxide (H$_2$O$_2$). H$_2$O$_2$ is further quenched by activity of catalase and glutathione peroxidase. The patients showed significantly higher activity of SOD and ALP. There was strong positive correlation between SOD and ALP activity. They showed reduced activities for catalase and glutathione reductase. The GR activity was positively correlated to MDA, SOD and ALP.

In the present study, we found decreased level of serum magnesium in female and male RA subjects as compared to reference range, though no significant difference was observed between the two sexes in serum magnesium levels. Decreased Mg level is considered as marker for RA. The level of phosphorous and copper was non-significantly higher in male RA patients as compared to females. In female RA patients, phosphorous showed inverse correlation with copper. There was strong association between elevated phosphorous and Ca and phosphorous products and the development of calciphylaxis. Both female and male RA patients had higher
serum copper levels as compared to reference values. RA patients are shown to have high levels of copper. Their levels have been shown to increase in all inflammatory processes including RA.

We found decreased level of serum magnesium in female and male RA subjects as compared to reference range, though no significant difference was observed between the two sexes in serum magnesium levels. Chronic inflammatory conditions in RA may alter the levels of magnesium and possible mechanism of reduced magnesium may be due to chronic inflammation and autoimmune injury. Our results are in accordance with other studies, suggesting that RA, is associated with serum magnesium disturbances. Mg is one of the essential nutrient of the body and studies suggest its role in reducing chronic inflammation. Decreased Mg level is considered as marker for RA. Magnesium is an activator of sodium potassium ATPase, is antiarrhythmic and is associated with cardio vascular disease susceptibility. Inflammation trigerrs its deficiency in animal models. In humans, low serum magnesium concentrations have been associated with high C-reactive protein (CRP) levels.

The patients show dyslipedemia with significantly higher total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein as compared to control but changes were within or at borderline of reference range. The HDL of the patients was significantly reduced as compared to control. Inflammation may be the primary cause for systemic alterations in the levels of minerals and enzymes which further modulate acute phase plasma proteins. Negative correlation are being reported 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.
Genome wide association study (GWAS) revealed association of RA with many genes. In our study PADI alleles RS188_2 and PADI_102 showed some association with RA. But these allelic combination of SNP was also observed in controls suggesting the need to recruit a much larger cohort for the analysis. Significant association was found with A/G SNP of TIMP4 with RA.

NMR study showed higher citrulline formation in RA which correlates well with presence of anti citrullinated antibodies in RA patients. Lipid parameters were also deranged in RA.

Therefore RA in the present study has been shown to have marked oxidative stress, high inflammation, deranged minerals particularly hypomagnesium, dyslipidemia and lower hemoglobin. Magnesium supplementation and oxidative stress management may be considered important therapeutic option for RA along with DMARD.

Rheumatoid arthritis (RA) is chronic inflammatory autoimmune disease with unknown etiology. RA affects various symmetric joints of the body. The disease is more prevalent in female as compare to male. Women tend to have worst disease than male. The study was done on age and sex matched control and patients.
CONCLUSIONS

The final conclusions are

1. RA patient showed significantly lower haemoglobin and higher ESR, DAS, CRP and VAS as compared to controls. Significant differences were observed in ESR, DAS, CRP and VAS in RA female versus males.

2. RA patient had normal uric acid and SGOT and SGPT. As the patients are on methotrexate therapy supplemented with folic acid and vitamin C. The results show that the therapy with supplements is not worsening liver or kidney function in our patients.

3. RA patients had high oxidative stress as they had high MDA. They had significantly higher activity of superoxide dismutase and alkaline phosphatase but lower activity of catalase and glutathione reductase. RA females experienced more oxidative stress however, they also have better activities of antioxidant enzymes as compared to RA males. Positive correlation was observed between GR and SOD and SOD and ALP suggesting their dependence to quench excessive free radicals in the body.

4. The serum level of zinc, copper and phosphorous where significantly elevated in RA patients as compared to control where as serum magnesium was significantly decreased in RA patients as compared to control.

5. RA patients showed dyslipidemia which may be due to ongoing process of inflammation and oxidative stress. Patient showed higher level of total cholesterol, very low density lipoprotein, triglycerides, low density lipoprotein and lower level of high density lipoprotein as compared to control. Dyslipidemia may increase their risk for arteriosclerosis and subsequent cardiovascular disease (CVD).
6. In our study peptidylarginine deiminase (PADI) alleles, RS 188_2 and PADI_102 showed some polymorphism in RA versus control. However allele occurrence was also seen in controls therefore it is difficult to predict their association with susceptibility to RA. Another gene PTPN-22 was found to the non-polymorphic in our population. However significant association of AG SNP of TIMP4 was observed in our patients.

7. NMR analysis showed that lipid parameters are deranged in RA patients as compared to control.

Therefore our study shows that RA is more aggressive in females. Onset of RA in females is probably early as compared to males. RA patient had increased risk of oxidative stress, dyslipedemia, deranged minerals and higher inflammation as compared to controls. Their antioxidant enzymatic activities were compromised as compared to control. They showed some association with PADI (RS 188_2) and PADI_102 and TIMP4 (AG SNP). The serological changed were confirmed by NMR analysis. Probably including magnesium in therapy and inclusion of antioxidant along with regular medicine may help to control disease better than present treatments.