4. DISCUSSION

Rheumatoid arthritis is characterized by chronic inflammation of the joints that causes structural and functional damage. Joint destruction occurs as the disease progresses which decreases the quality of life and increases the socioeconomic burden. There is growing evidence that therapeutic interventions early in the course of RA lead to disease control, less joint damage and a better prognosis. When Rheumatoid arthritis is clinically suspected, immunological studies are required, such as testing for the presence of RF and anti-CCP along with traditional markers of disease activity like ESR, CRP, joint count, based on these tests and clinical examination the physician would rule out other possibilities.

In the present study we have evaluated the usefulness of biochemical markers of joint tissue along with traditional markers of disease activity in rheumatoid arthritis patients at the time of study entry (phase I) and after one year of follow-up (phase II).

**Phase I**

**Traditional markers of disease activity in RA patients**

According to Table 3.1, a total of 140 study subjects participated in phase I which includes total 102 rheumatoid arthritis patients (27 male and 75 female) with disease duration (median age 2.6, range 1-6.25) and age matched 38 control healthy control subjects. Among the total RA patients, 50 patients were early RA with disease duration of less than two years and...
52 were late RA patients with disease duration of more than two years from the onset of disease symptoms (Table 3.2)

In our study we have observed the traditional markers of disease activity like swollen and tender joint count, physical activity, VAS and DAS-28 score, ESR and CRP were significantly increased in RA patients compared with controls (Table 3.1).

Sokka and Pincus have reported the joint count is the most specific clinical method to quantify abnormalities in patients with rheumatoid arthritis. The swollen joint count reflects the amount of inflamed synovial tissue and the tender joint count is associated more with the level of pain. In agreement with this study, we have also observed significant increase in both the swollen and the tender joint count in RA patients.

Rojkovich and Gibson had reported one of the most popular methods of self evaluation in RA patients is the 100mm visual analogue scale (VAS). It requires patients to indicate on a line the maximum level of pain intensity experienced during a prescribed period. They were asked to indicate their pain severity on 100mm horizontal VAS each labelled “no pain” at one end and “worst possible pain” at the other. In our study the median VAS score values ranges from 55(37-75) in RA patients and among subgroups we have observed 48(40-56) in early RA and 59(38-80) in late RA patients with statistical significance (Table 3.2).

Prevoo et al developed a disease activity score (DAS-28) based on the number of tender and swollen joints (n=28), ESR (or CRP) and the patient’s self estimated general health. DAS-28 has become an important
tool for rheumatologists to monitor disease activity. In our study, we have used ESR values for calculate DAS28 and it is significantly increased in RA patients than control (Table 3.1). Meanwhile, within subgroups of RA these values were increased in late RA than early RA patients with statistical significance reflecting the disease activity in later stages of disease (Table 3.2). In one of the recent studies Eman et al\textsuperscript{297} reported increased DAS-28 score in RA patients than control and using the DAS-28 score to evaluate RA disease activity, a score ≤ 2.6 (disease remission), 2.6–3.2(low disease activity), 3.3–4.9(moderate disease activity), and >5.1 as severe disease activity. In our study we have observed with moderate disease activity (3.86 -4.79) in the RA patients.

In our phase I study group RA patients shows marked inflammatory activity which is reflected in increased ESR and CRP values(Table 3.1) than controls suggesting the role of inflammatory synovium in RA pathogenesis\textsuperscript{40}. Though one of the recent studies reported normal ESR and CRP levels in most of the RA patients contrary to this, our study we had a majority of RA patients with high ESR and CRP levels (294) Acute phase reactants such as CRP and ESR are direct measure of inflammation rather than tissue destruction. C-reactive protein is a convenient and sensitive marker of inflammatory activity in clinical practice. It is a distinct entity as general inflammatory marker whereas the ESR depends on several variables such as immunoglobulin levels, sex, and abnormal size or shape of red blood cells apart from mirroring inflammation.\textsuperscript{298}
Although the CRP and ESR usually run similar courses, in our study we have observed CRP levels are markedly increased in RA patients than ESR. In agreement with another recent Indian study, we have observed the CRP level is increased in more than 80% of RA patients when compared with controls though it is not specific for RA and increased in any other inflammatory conditions. We have also observed both ESR and CRP increased in 52% of RA patients and raised ESR alone in only 58% of the patient group. Thus, measurement of both CRP and ESR is more helpful than either alone but CRP is probably more informative and better marker of inflammation whereas ESR adds information reflecting disease severity, and a combination of the tests may be worthwhile.

**Traditional markers of disease activity in subgroups of RA patients**

The duration of disease for early RA is around $1(0.87-1.45)$ years and for late RA is $6 (3-11)$ years respectively and the age of study subjects in early RA is $43 (36.75-50.5)$ years and for late RA it is $50.5 (41.25-58.5)$ years respectively compared with normal age of $50.5 (39-59.25)$ years, showing statistical significance (Table 3.2). Among the subgroups of RA we have observed significant increase in swollen and tender joint count in late RA patients than early RA suggesting more synovial inflammation and pain in later stages of disease.

According to Table 3.2, among the sub groups of RA there was a significant increase in ESR, CRP and DAS28 levels in late RA patients than early RA clearly indicate persistent inflammatory disease activity, a
characteristic feature of rheumatoid arthritis patients which is more prominent in later stages of disease.\textsuperscript{300}

But contrary to the studies by Eman \textit{et al}\textsuperscript{297} with increased DAS-28 in early stages of RA we have observed moderate to severe disease activity (4.27-5.28) in late RA patients than moderate disease activity (3.54-4.16) in early RA suggesting increased disease activity in later stages of rheumatoid arthritis. In our study, the CRP level correlates more than ESR with disease activity though there is no statistical significance thus it is a predictor of functional status/outcome and joint damage in rheumatoid arthritis.\textsuperscript{95} Another study also suggested serum CRP is the more sensitive measurement, but that CRP and ESR do not have identical clinical significance.\textsuperscript{28}

We could not establish any correlation within ESR, CRP, joint count and DAS 28 as with same magnitude as several previous studies. Although the ESR and CRP values were by no means interchangeable, these parameters seems to be better in terms of correlation with other markers like anti-CCP, RF and synovial inflammation marker hyaluronic acid which we have discussed in the later part of the study.

According to Table 3.3 the various biochemical markers level are increased in RA patients than control group. The auto antibodies like anti-CCP, RF and synovial inflammation markers like HA, YKL-40 and MMP-1 levels were significantly increased in RA patients than control group.
Autoantibodies status in Rheumatoid arthritis patients

Anti-CCP level in RA patients

Anti-cyclic citrullinated peptide is one of the most talked about autoantibodies in recent past when compared with rheumatoid factor. The presence of these antibodies may predict the development of RA and can be detected in healthy individuals who are in the risk of developing RA, years before the onset of clinical RA and are also used as a marker for more severe disease.

According to Figure.3.1, Anti-cyclic citrullinated peptide is significantly increased in total RA patients when compared with control proving this new biomarker plays an important role in RA pathogenesis. When we looked at the anti-CCP levels individually it is about 15-25 times more in RA patients than normal reference intervals (<30U/ml). We believe this sharp rise attributed to the disease activity mostly involving synovium in RA patients.\textsuperscript{301} In agreement with couple of other studies, we have observed 83% of RA patients were positive for anti-CCP antibodies.\textsuperscript{120,302} At the same time 17% RA patients with negative anti-CCP levels still diagnosed with rheumatoid arthritis.

Anti-CCP levels in subgroups of RA patients

In our study we have observed there is significant difference in the anti-CCP level among early RA, late RA and control group (Figure 3.1.1). Similarly, there is a significant increase in anti-CCP level in early RA than late RA as shown in Figure 3.1.2.
Studies reported prevalence of anti-CCP positivity is 60% in early RA patients and 77% in established late RA patients. But when we further assess anti-CCP levels in among early RA and late RA patients groups we found that 84% early RA and 73% late RA were anti-CCP positive in our study. Typical clinical symptoms of RA are not manifested completely in early disease course. Hence, the early diagnosis of RA is essential, as it has been observed that progression occurs within 2 years of disease onset and if not aggressively treated early, joint destruction occurs. Thus, a highly specific and early diagnostic marker is needed for the detection of rheumatoid arthritis. It has been demonstrated that anti-CCP antibodies occur years before disease onset suggesting that the induction of disease in anti-CCP-positive RA patients occurs years before presentation.

**Rheumatoid factor level in RA patients**

Rheumatoid factor (RF), a circulating autoantibody exists as different isoforms and it forms an immune complex with IgG and contributes to the pathogenesis of rheumatoid arthritis. In our present study RF (IgG) level is significantly increased in RA patients compared with control (Figure 3.2). One of the earliest studies Fye et al reported the specificity of RF (IgG) in RA patients is between 40-65% suggesting, the pivotal role of RF in the pathogenesis of RA and its clinical utility in the diagnosis of the disease. In our study we have observed 59% of RA patients were positive for RF antibodies (Table 3.4) and our finding is in concordance with other
published data, which confirmed that IgG RF frequency ranges from 43-68% in RA patients.\textsuperscript{125,245}

**Rheumatoid factor in subgroups of RA patients**

In several studies Rheumatoid factor has been shown as the most important blood test used to detect RA and to distinguish it from other types of arthritis and other inflammatory processes, but the sensitivity and specificity of RF are not agreeable for all situations and also researchers have reported anti-CCP antibodies as a powerful serologic marker for differential diagnosis of RA especially because of their high sensitivity as well as high specificity.\textsuperscript{307}

In our present study, when we analyzed the levels of RF in early stages of RA found to be increased in more than 74% of early RA patients and among late RA patients only 46% were RF positive in phase I. In fact we have observed a significant difference in serum RF levels among early RA, late RA and controls (Figure 3.2.1) but unlike anti-CCP level, there is no significant difference in RF level within early and late RA groups (Figure 3.2.2).

**Combined role of anti-CCP and RF in RA patients**

As per the new 2010 ACR/EULAR RA classification, high positive for both RF and anti-CCP considered to be rheumatoid arthritis.\textsuperscript{37} When we analyzed serum anti-CCP and RF levels together in our study we have observed that in early RA patients 80% were positive for anti-CCP and 68% were positive for RF autoantibodies. Among late RA patients it was 73% positive for anti-CCP and 46% positive for serum rheumatoid factor.
Interestingly, another recent Indian study Rajiva et al\textsuperscript{308} also reported 72\% of early RA positive for anti-CCP and 64\% positive for RF antibodies. At the same time only 57\% of late RA was positive for anti-CCP and 61\% late RA with high RF levels. Another south Indian (Chennai) study by Narayanan et al\textsuperscript{309} reported that RF was positive in 36\% in late stages of RA (46\% in our study) concluding multiple factors responsible for less specificity of RF in later stages of disease. RF levels in these patients would have been increased earlier since they were under treatment for all these years which could have decreased RF level in the blood. These observations made us to conclude that anti-CCP, a better diagnostic marker in the early stages of disease in Indian RA patients similar in line with other studies on Indian RA patients.

In concurrence with one of the recent Malaysian study by Gomez et al\textsuperscript{310} our study group has shown that 55\% RA patients were positive for both the serological markers (Table 3.4) concluding that anti-CCP and RF may be recommended for evaluation of patients with suspected rheumatoid arthritis.

In a recent south Indian study by Alghuweri et al\textsuperscript{311} reported the incidence of anti-CCP and RF is 79 \% and 82 \% respectively but 24\% of patients had positive for both serological markers. Another Indian study by Oommen et al\textsuperscript{312} demonstrated anti-CCP sensitivity is 82\% than 76\% RF in RA patients. In our study as well, we detected around 41\% RF (IgG) negative RA patients with symptoms of rheumatoid arthritis (Table 3.4).
Munthe and Natvig\textsuperscript{222} reported that 15-35\% RF negative patients with classical symptoms of rheumatoid arthritis. More than that a recent German study provides enough evidence that measurement of anti-CCP levels are a better sensitive marker in RA patients with negative RF status.\textsuperscript{313} Interestingly in our study 31\% RA patients showed increased anti-CCP levels but with negative RF (Table 3.4) suggesting the importance of anti-CCP in RA pathogenesis and its usefulness as a traditional marker of disease activity in rheumatoid arthritis. Though there is no significant correlation between RF and anti-CCP at baseline levels, anti-CCP emerged as a better marker than RF with many previous studies supporting our observations.\textsuperscript{314,315} Based on the findings from our study which are in agreement with the above mentioned studies we conclude that anti-CCP antibody represents a superior serological marker for rheumatoid arthritis as it is highly specific for the disease.

**Correlation between autoantibodies with traditional markers of disease activity**

In accordance with studies by Annette et al\textsuperscript{316} our study observed no significant differences in baseline total joint count or in the DAS28 score between RA patients with and without anti-CCP antibodies. In agreement with same study most of the anti-CCP positive patients in our study group have more inflamed joints than anti-CCP negative patients based on swollen and tender joint count evaluation and once there is more inflammation there is more likelihood for rapid joint destruction. But different
from this study, we recorded the anti-CCP levels showed a significant correlation with CRP, a marker for generalized inflammation.

According to Table 3.5 we have observed a significant positive correlation between anti-CCP with both inflammatory markers, CRP and ESR suggesting their role in RA pathogenesis. A Russian study supports our view that a positive correlation exists between autoantibodies and inflammatory markers in RA patients. Anti cyclic citrullinated peptide antibodies are locally produced in inflammatory RA joints and these citrullinated proteins identified as citrullinated fibrins in RA synovial tissue. In contrast to our studies Serdaroglu et al reported no correlation between anti-CCP with serological markers of disease activity (ESR, DAS 28, CRP). Furthermore, in our study, we could not find any significant correlation between anti-CCP levels with age of RA patients; joint count and DAS score in RA patients.

According to studies by Winchester et al rheumatoid factor was detected in 70% of RA patients with established disease and there is no association of RF with the level of disease activity markers like ESR, Joint count, DAS28 but RF is an integral part of the definition of this disorder. Though we have observed a weak correlation between RF with CRP but there is no association with levels of other disease activity markers (Table 3.5). In fact the correlation between RF with CRP levels though weaker than anti-CCP, but unlike anti-CCP there was no correlation at all between RF and ESR in RA patients.
Thus, according to Table 3.5 both anti-CCP and RF showing correlation with general inflammatory marker CRP, suggesting their involvement in the pathogenesis of rheumatoid arthritis. Our findings strongly suggest a possibility that local citrullination of intra-articular proteins might be the initial event leading to autoantibody production in rheumatoid arthritis.

**Synovial markers in Rheumatoid Arthritis**

**Hyaluronic acid**

In accordance with couple of studies by Sasaki *et al*[^318], Sudha *et al*[^9] we have also demonstrated that serum HA concentration in RA patients is significantly increased than control (Figure 3.3). Synovial inflammation is a characteristic feature in rheumatoid arthritis, and hyaluronic acid is being produced by synovial tissue and escapes out from inflamed synovium and raises its level in blood. High levels of plasma HA in the absence of any hepatic dysfunction can be considered as indication of an increased input from the synovial joint tissues. This makes it a natural choice of biomarker in synovial inflammation in rheumatoid arthritis.^[320] In RA patients it has been reported that HA acts as an anti-inflammatory substance by inhibiting the adherence of immune complexes to neutrophils through the Fc receptor or by protecting the synovial tissues from the attachment of inflammatory mediators.^[321]

Another study from Poland by Czeczuga and Zajkowska[^322] reported that HA concentration in RA patients is higher than any other forms of arthritis proving a better marker for rheumatoid arthritis. Goldberg *et al*[^182]
concluded that plasma HA may be unique as a marker, in that it may be a reflection of synovial involvement and inflammation, rather than only of inflammation, in arthritis.

Fraser *et al* has observed that increase in serum HA level in RA can only be a result of lowered catabolism or excessive production and endothelial cells of hepatic sinusoids are mostly responsible for catabolism of hyaluronic acid. However, RA patients of our study group had no laboratory signs of liver affection, therefore the increased HA values found in the present study are presumably not a consequence of impaired elimination or degradation of this polysaccharide but rather a consequence of stimulated synthesis or outflow from the connective tissue.

In general, another reason for increased serum HA level in RA patients could be physical activity as well as production of large volume of synovial fluid which thereby increases serum HA level. A study reported the effect of physical activity which was recorded in HAQ based on the mobility of joints on serum HA is more pronounced in RA patients than in healthy individuals.

**Hyaluronic acid level in early and late RA patients**

We have also noted a significant change in serum HA among the subgroups of RA and control group (Figure 3.3.1). Though we could able to shows an increase in serum HA in both subgroups there was a significant increase in serum HA in late RA than early RA indicating involvement of synovium in the pathogenesis of rheumatoid arthritis more prominent in later stages of disease (Figure 3.3.2).
In contrast, Majeed et al\textsuperscript{282} and Paimela et al\textsuperscript{72} also reported increased serum level of HA in early RA patients concluding serum HA levels may reflect ongoing joint destruction and may even predict subsequent joint damage in early stages of rheumatoid arthritis. There is no published study to assess the level of HA in later stages of RA but one study on osteoarthritis patients had been reported that increased serum HA level in late stages of erosive OA suggesting HA as a surrogate marker with the predictive value for further radiographic progression in osteoarthritis.\textsuperscript{326}

**Correlation between HA with traditional markers of disease activity**

In agreement with Majeed et al\textsuperscript{282} serum hyaluronic acid level correlates with clinical and laboratory measures of disease activity like ESR, CRP in RA patients. In our study also a weak correlation exists between serum levels of HA with ESR and CRP (Table 3.6) in RA patients suggesting synovial inflammation leads to RA pathogenesis. At the same time we could not able to establish any significant correlation between HA with other markers of disease activity such as swollen and tender joint count, DAS-28, VAS scores in the phase I study.

One more study by Luiz et al\textsuperscript{327} also reported that serum HA levels are elevated in RA and correlated with serological markers of RA such as ESR, CRP and joint count in RA patients. A study by Poole et al\textsuperscript{328} also reported that a direct correlations between the number of tender joints, ESR and the number of joints with effusions with serum HA in RA patients. It was reported that the factors stimulating an increased production of
hyaluronic acid in RA have not been identified, but evidently the serum concentrations of rheumatoid factor or immune complexes do not influence the circulating HA levels but the significant positive relationship between acute phase plasma proteins like CRP and HA concentrations in RA suggests that an increased synthesis of HA might be linked to the intensity of the inflammatory process.\textsuperscript{325}

In another study Emlen et al\textsuperscript{147} reported increased serum HA in RA patients than in healthy controls and also significant correlation with patient age, number of swollen joints concluding advanced age and inflammatory joint status are directly influencing serum HA level. But we could not establish any correlation between serum HA and these factors in our study.

**YKL-40 in RA patients**

Serum YKL-40 is a relatively new marker of synovial joint inflammation in RA and we have studied there role in RA patients which happened to be first of its kind on Indian population. In accordance with couple of other studies\textsuperscript{329,153} we found that in our study, serum YKL-40 were significantly increased in RA patients compared to control (Figure 3.4) suggesting that YKL-40 is released locally by arthritic joints followed by a secondary increase in serum. Its level may reflect more local aspect of inflammatory process in RA unlike acute phase reactants like ESR, CRP which represent non-specific distant response by hepatocytes for inflammation with the help of mediators.

Researching the role of YKL-40 in RA pathogenesis was difficult as details were hard to come by as very few studies reported their role in
rheumatoid arthritis. Several studies on YKL-40 in osteoarthritis patients were done but not in rheumatoid arthritis but it is already established as a surrogate marker of for inflammatory and degenerative joint diseases. Another study by Kawasaki et al reported YKL-40 reflects the degree of inflammation rather than cartilage metabolism and it may be a useful inflammatory marker of hip joint diseases.

Couple of other studies by Hakala et al and Hu et al also reported increased YKL-40 level in serum of RA patients. In one such study by Knorr et al suggested that increased expression of YKL-40 by connective tissue cells such as activated synovial fibroblasts or synoviocytes present within joints of rheumatoid synovium reflects the level of YKL-40 in serum. It is also reported that YKL-40 is abundant in articular cartilage but down regulated and not up regulated in osteoarthritis. But there are no published studies on regulation of YKL-40 synthesis in rheumatoid arthritis patients.

**YKL-40 in subgroups of RA**

In our study we found significant changes in the serum YKL-40 level in early RA, late RA and control (Figure 3.4.1) but there was no significant changes within early and late RA groups(Figure 3.4.2). Very few published studies related to elevated serum YKL-40 leading to progression in joint destruction in early and late RA patients are available. However, studies by Peltomaa et al provide evidence about increased serum YKL-40 as an inflammatory marker correlating with disease activity in early RA but we could not prove the correlation in our early RA patients.
Another study on OA patients by Johansen et al\textsuperscript{153} reported that patients with late stage OA had significantly increased YKL-40 when compared to early stages of osteoarthritis indicating its serum level reflect human articular cartilage degradation and the degree of synovial inflammation in the knee joint.

**Correlation between YKL-40 with traditional markers of disease activity in RA**

Contrary to the studies by Kazakova et al\textsuperscript{333} indicated that serum YKL-40 levels were showing positive correlation with CRP and ESR in Bulgarian RA patients but we could not establish any relation between these markers (Table 3.6). Johansen et al\textsuperscript{261} suggested that serum YKL-40 might be a new biochemical marker for joint injury and it reflects the degree of inflammation rather than cartilage metabolism. We completely agree with the above statement as in our study population since HA levels are significantly increased in RA patients but with normal or low serum COMP levels.

Kaspar et al\textsuperscript{334} reported serum levels of YKL-40 in RA patients found to be not associated with gender suggesting age and genotypes are all strong independent factors affecting serum concentrations of YKL-40. We could not establish the effect of age, gender on serum YKL-40 level as this was not our focus of study.

In another study Ju Dong et al\textsuperscript{335} proved that although the exact mechanism is not known, alcohol consumption and smoking have also been associated with serum YKL-40 level. But in our study all the subjects
are non smokers and non-alcoholics as per the exclusion criteria we have no explanation for this. Same time we could not prove any correlation between YKL-40 with any of the biochemical markers in RA patients.

Our findings suggest that YKL-40 is implicated in the pathogenesis of the rheumatoid arthritis and could indicate the level of joint inflammation indirectly.\(^{336}\) The potential of serum YKL-40 to provide new information on disease activity and pathophysiology of the synovitis in RA is apparent at any stages of disease. Moreover, we could not establish any correlation between YKL-40 with HA or any other biochemical markers as well as traditional markers of disease activity in RA patients and hence its specific role in synovial joint inflammation alone could not be proved (Table 3.6). Though with all evidence about role of YKL-40 in RA pathogenesis, we are unable to conclude about its importance in diagnosis and prognosis of RA suggesting further studies needed to ascertain its significance in rheumatoid arthritis.

**MMP-1 in RA patients**

Rheumatoid Arthritis is characterized by destruction of extracellular matrix around joint tissue primarily mediated by family of proteolytic enzymes like matrix metalloproteinases.\(^{337}\) We have studied MMP-1 an attempt to link with RA pathogenesis. The outcome of our observation has in effect allowed us to achieve the purpose of using MMP-1 as a marker of synovial tissue degradation, a marked feature in rheumatoid arthritis.\(^{274}\)

When we shifted our focus from YKL-40 to MMP-1, another marker of synovial inflammation the observations are almost similar among RA
patients and control i.e. from our studies it is demonstrated that serum MMP-1 level is significantly increased in systemic circulation of RA patients compared with controls (Figure 3.5). There are no published Indian studies to support our view. Mahmoud et al\textsuperscript{338} has reported that Serum MMP-1 was significantly elevated in patients with RA and OA, compared to healthy controls. Meanwhile, Clark et al\textsuperscript{339} reported increased MMP-1 in RA patients in serum but not in synovial fluid.

**MMP-1 in subgroups of RA**

In our study we have observed significant changes in serum MMP-1 level like YKL-40 in early RA, late RA patients and control group (Figure 3.5.1) proving synovial inflammation in RA patients. Rengel et al\textsuperscript{340} reported that the importance of MMP-1 in early diagnosis of RA is insignificant though they are detected in very little in systemic circulation due to short half life still it is proved to be involved in RA pathogenesis. In agreement with this study we also observed no significant difference in serum MMP-1 levels between early and late RA patients (Figure 3.5.2)

**Correlation between MMP-1 with traditional markers of disease activity**

In our study we could not establish any significant correlation between MMP-1 with any traditional markers of disease activity (Table 3.7). Cunnane\textsuperscript{341} has reported no significant correlation between serum levels of MMP-1 with ESR in early RA patients. But another study Green et al\textsuperscript{275} has reported significant correlation between MMP-1 with ESR in early RA patients. It is therefore understandable that markers of local joint damage
such as MMP-1 would be particularly useful at this early stage of disease and prior to the onset of an elevated acute-phase response. Shovman et al.\textsuperscript{223} has reported that MMP-3 and MMP-1 level in serum showing no significant correlation with anti-CCP and RF in RA patients. Another study by Myers et al.\textsuperscript{342} reported there was no correlation with age of the patients involving rheumatoid arthritis and psoriatic arthritis.

According to Figure 3.5.3 in our study serum MMP-1 level is significantly increased in both anti-CCP and RF positive RA patients when compared with anti-CCP negative and RF positive RA patients revealing that this connective tissue destructive enzyme is more in RA patients who are positive for both auto antibodies.

**Markers of cartilage degradation**

**Cartilage oligomeric matrix protein (COMP)**

According to Figure 3.6 in our present study there is no significant difference in serum COMP level between RA and controls. The serum COMP is one of the most extensively studied cartilage marker so far and researchers have found an association between increased COMP & progression in rheumatoid arthritis. Apparently, serum levels of COMP are known to reflect increased cartilage turnover and the study by Manek and Lane\textsuperscript{79} shows that serum COMP may be used as a prognostic marker of cartilage degradation in patients with established rheumatoid arthritis.

The studies by Andersson et al.\textsuperscript{343} reported that there is considerable diurnal variation in serum COMP, with the lowest values at
night during bed rest and serum COMP levels are stable in daytime that is, when serum samples are normally obtained.

Oldberg et al\textsuperscript{344} reported that COMP in serum is a biomarker for the cartilage turnover and is elevated in RA patients concluding inflammatory synovium has been considered as a potential tissue source of serum COMP since the molecule has been detected in the synovium in rheumatoid arthritis. But indifferent from these observations in our study, there is not much significant change in the levels of COMP in RA patients when compared with controls, shows the degree of uncertainty on the role of COMP in RA pathogenesis.

Several previous studies provide contradicting reports about serum COMP—either decrease or increase or no change in RA patients. Despite the fact that, COMP is a biomarker of cartilage degradation in RA and other rheumatic and joint diseases, we have found that it is less significant unlike other biomarkers in predicting the disease progression and from our observations it is clear that serum COMP values are almost similar in RA patients and control. In agreement with one such study we have also propose that there is no changes in serum COMP levels in RA patients signifying its limited role in RA pathogenesis.\textsuperscript{240} Another study by Volck et al\textsuperscript{159} has reported that serum COMP in higher in osteoarthritis than RA patients and level of synovial inflammation determines the serum cartilage level concluding the expression of COMP in other tissues other than cartilage explains its tissue specificity and raised its first concerns for its use as a destructive cartilage degradation marker.
COMP in subgroups of RA

Unlike other biomarkers, the levels of serum COMP are not much different in subgroups of RA and control (Figure 3.6.1). At the same time we could not able to establish a significant correlation between early as well as late RA groups (Figure 3.6.2). Contrary to our observations another recent Japanese study showed a decrease in serum COMP and HA level in RA patients with anti-TNF therapy in early and late RA patients advocating the usefulness of therapeutic efficacy of biological therapy, for determining the degree of ongoing structural joint deterioration.\textsuperscript{345}

However, in our study around 16% of early RA patients with more affected joints showed an increase in COMP values suggesting cartilage involvement. Though there is no noticeable correlation, 10% RA patients with more inflamed joint i.e. with more swollen and tender joint count turnout exhibit very high values for serum cartilage oligomeric matrix protein.

Correlation between COMP with traditional markers of RA

From our study we were unable to establish any kind of significant relationship between COMP with traditional disease activity markers of RA such as ESR, CRP, swollen and tender joint count in our study group. But we have observed a weak but significant relation between serum levels of COMP with age of RA patients (Table 3.7). This could be the fact that as age advances cartilage and related tissues tend to breakdown in RA patients as a normal physiological process resulting in increased levels of breakdown products in systemic circulation. Lindqvist \textit{et al}\textsuperscript{225} reported that
serum COMP showed a weak correlation with ESR, CRP in RA patients and increased serum COMP levels may occur early in the course of rheumatoid arthritis as a sign of cartilage involvement.

Hummel et al\textsuperscript{246} confirmed that the serum COMP levels are highly specific markers for the cartilage degradation process in RA and not related to non-specific inflammatory process in arthritic joint.\textsuperscript{249} But in our study the serum COMP levels were not different in RA patients and controls. Moreover, COMP is not showing any of statistical significance with none of the traditional markers of disease activity suggesting less significant association with disease activity in rheumatoid arthritis.\textsuperscript{239}

**Markers of bone formation**

**Osteocalcin**

Rheumatoid arthritis is associated with severe progressive joint destruction and erosion of bone. So we have studied serum osteocalcin, a bone specific protein and marker of bone formation in rheumatoid arthritis patients and control. In our study there was no statistical significance in serum osteocalcin level between RA patients with controls (Figure 3.7). Deodhar and Woolf\textsuperscript{86} reported that there is no unanimity about the levels of markers of bone formation in RA patients and as we evaluated osteocalcin level found there is no significant difference between RA and controls suggesting normal bone turnover rate in RA patients.

In general, serum osteocalcin levels are elevated in patients with diseases characterized by a high bone turnover rate and serum levels echoes the expected changes in bone formation following surgical and
therapeutic intervention. Hence serum osteocalcin is considered a specific marker of osteoblasts function, as its levels correlate with the bone formation rate. In our study serum osteocalcin concentrations in patients with RA were not statistically different from those of control subjects, indicating a normal rate of bone formation. But these data disagree with studies in which both increased and decreased serum osteocalcin concentrations in patients with RA were reported. In contrast, Gevers et al found increased serum osteocalcin concentrations in patients with RA.

Normal osteocalcin levels in rheumatoid arthritis patients could be due to various reasons. Studies reported severe RA produce low amounts of active osteocalcin and higher than expected amounts of inactive osteocalcin in the synovial fluid as well as in serum. Another study by Hassager et al reported serum osteocalcin levels follow a circadian rhythm characterized by a decline during the morning, a low around noon, and a gradual increase to a peak after midnight. Markers of bone formation are either enzymes or other proteins secreted by osteoblasts /by products of collagen breakdown. Variation in diurnal and day to day changes affects the bone forming and bone-resorbing activities the serum markers of bone formation appear to be vary less day to day. Jacobs et al has reported that diurnal variation in serum OC level with high values in the night and very low values in early afternoon. This could also be the reason for normal OC level in RA patients.

Another study Frederick and David reports that improper collection, handling of specimen, using different laboratories for serial
measurements (even if they use identical methods) can seriously affect assay precision. Moreover, optimal time to collect samples would be in the morning and careful sample storage is also particularly important in measuring osteocalcin levels in serum. But in our study these possibilities are minimal since we have strictly followed the protocol and procedures as per instruction manual. Hall et al reported with slightly increased OC level in premenopausal RA patients than normal subjects suggests that generalized bone loss occurs in active RA and is characterized by an evident bone resorption correlated with the high levels of inflammation. In another study Suzuki et al reported in premenopausal RA, bone formation was equal to that in premenopausal normal controls, but bone resorption was increased. In postmenopausal RA, bone formation was lower while bone resorption was higher than in postmenopausal normal controls.

Osteocalcin in subgroups of RA

At the same time osteocalcin levels found to be not different within subgroups and control group (Figure 3.7.1) and no significant difference between early and late RA patients (Figure 3.7.2). Even though some late RA patients are reported slightly higher serum osteocalcin levels may be due to increased erosion of bones, interestingly the median serum osteocalcin level is just a little increase in early RA patients than control. Marhoffer et al reported increased OC levels correlate with disease activity in older patients with active RA, suggesting impaired bone turnover concluding the heterogeneity in RA patients with late onset
patients displaying high bone turnover. But in our study we have noticed no significant changes in OC level in both early and late RA patients of all age groups.

We had anticipated at least reasonable change in serum osteocalcin levels in RA subgroups, in particular late RA patients since bone turnover rate is more evident than early RA patients but to our surprise there is no marked difference in their levels any subgroups of rheumatoid arthritis. On close examination we have also found 3 controls were alarmingly high levels of osteocalcin than RA patients. They otherwise found to be normal as other parameters are within reference range but still made us to exclude them from our further study.

Garnero et al.\(^67\) reported that in late RA that bone formation is reduced in both patients with and without joint destruction, whereas resorption is increased only in patients with joint destruction in relation to disease activity. Contrary to this we have observed no significant difference in OC levels in both subgroups. Goodship\(^351\) reported that uncoupling of bone formation and resorption markers with normal or reduced bone formation but increased bone resorption markers in RA patients in comparison to healthy controls suggesting reduced bone formation at the remodeling unit level is the predominant mechanism in RA patients. Though we have not studied bone resorption marker there was normal OC, bone formation marker in RA patients and control groups.

van der Wiel et al.\(^652\) reported that bone formation is impaired in patients with RA, in whom as a consequence of the disease, physical
ability, mobility and muscle strength are impaired, and therefore the bone load-bearing strain is reduced. Next to impaired bone formation, immobilization also increases bone resorption.

From the results of our study we are suggesting a normal osteoblastic activity in patients with RA, more probably due to an increased bone resorption than to decreased bone formation.

**Correlation between OC and traditional markers of RA**

In our study there was no correlation serum OC with traditional markers of rheumatoid arthritis (Table 3.8). Kroger et al. has reported OC concentrations were significantly decreased in RA patients compared with the controls, suggesting reduced bone formation and bone remodeling in RA. The lowest values were found in early RA patients. In the same study they have found that serum OC is not correlated with ESR and CRP.

**Correlation within the biochemical markers in RA patients**

In our present study, we have observed a significant positive correlation between anti-CCP with hyaluronic acid in phase I suggesting involvement of synovium in RA pathogenesis (Table 3.9) Simultaneously, anti-CCP showing significant negative correlation with cartilage destruction marker, COMP in phase I study group (Table 3.9) There was no correlation between RF with any of the biochemical markers in our study. In our study none of the other biochemical markers showed any kind of correlation with autoantibodies.

In our study, it is clear that both HA and anti-CCP shows relatively good positive correlation in phase I. We have also observed a significant
increase in serum levels of HA in RA patients with both RF and anti-CCP positive antibodies compared with both negative RA patients (Figure 3.3.3). According to, Figure 3.3.4 there was significant increase in serum levels of HA in RA patients with RF negative and anti-CCP positive antibodies compared with both negative RA patients. To conclude Figure 3.3.4 explains the relation between HA and auto antibodies. This strengthen our views about the role of both anti-CCP and HA in the pathogenesis of rheumatoid arthritis.

Studies by Dahl and Husby\textsuperscript{354} showed articular HA synthesized by the synovial membrane reaches the circulation by lymphatic drainage and the rheumatoid synovium secretes a considerable amount of HA as demonstrated by several studies of rheumatoid synovium cultures at various cellular stages. As we have discussed earlier that both anti-CCP and HA in serum originates from rheumatoid synovium and both being products of rheumatoid synovium playing an important role in RA pathogenesis. Faaber \textit{et al}\textsuperscript{355} explained that Hyaluronic acid concentration is higher in synovial fluid and it is shown to initiate formation of IgG-IgM RF complexes in RA patients. Despite this we observed no correlation between HA and RF (IgG) in the serum of RA patients suggesting that they are involved independently in the pathogenesis of rheumatoid arthritis.

We were able to establish a substantial relationship between HA and COMP levels in RA patients (Table 3.9). In a Danish study by Lottenburger \textit{et al}\textsuperscript{356} pointed that diurnal variation may play a significant role on serum HA and COMP levels. Considerable efforts have been taken to collect data
in support of our arguments but we could not gather any information as non-availability published studies about other possible reasons for increased serum hyaluronic acid level. Further, except anti-CCP, a traditional disease activity marker in rheumatoid arthritis the serum HA levels are not associated with any of the biochemical or traditional markers of disease activity in RA patients signifying that each marker has an independent role in the pathogenesis of rheumatoid arthritis.

This is an important observation from our study on Indian patients with rheumatoid arthritis. We could able to establish a reasonable correlation between Hyaluronic acid with ESR, CRP and anti-CCP all of which are directly involved in RA pathogenesis (Table 3.9). According to conventional thinking, hyaluronic acid and anti-CCP is more associated with synovial degradation and ESR and CRP with inflammation it is clear that synovial inflammation is a distinguishing feature of RA pathogenesis suggesting the role of above mentioned markers in RA pathogenesis.

In our study there was no significant correlation between YKL-40 with any other biochemical markers in RA patients. Though serum YKL-40 found to be involved in the pathogenesis of RA indirectly there was no studies reported its correlation with biochemical markers of rheumatoid arthritis. Couple of studies like Schiavon et al.\textsuperscript{357} and Saitou et al.\textsuperscript{359} reported correlation between YKL-40 with HA in liver diseases but not in RA or any other type of rheumatic disease. In another baseline and follow-up study on early RA patients by Syvergen et al.\textsuperscript{215} concluded that YKL-40 and MMP-3 are just markers of joint inflammation with no correlation between
themselves. Again there were no reports about correlation between serum MMP-1 and YKL-40 in RA patients.

We had observed no significant correlation between MMP-1 with auto antibodies as well as any other biochemical markers in our phase I study. A study by Houseman et al.\(^{359}\) reported no significant correlation between MMP-3 with anti-CCP in RA patients again any studies on correlation between MMP-1 and anti-CCP in RA have not been reported. No studies explaining any correlation between MMP-1 with RF in RA patients have been reported elsewhere. Ishiguro et al.\(^{278}\) reported that in OA patients there was no correlation between MMP-1 including other types of MMPs with hyaluronic acid but no published reports available in RA patients. Study by Luo et al.\(^{360}\) reported there is no correlation between MMP-1 and osteocalcin in RA patients but a positive correlation between MMP-2 with serum OC levels in RA patients. Kullich et al.\(^{361}\) reported a weak correlation exists between serum MMP-1 and COMP level in RA patients concluding synovial inflammation followed by cartilage degradation in RA patients. But in our study there was no significant correlation at all between these two markers.

We have found no significant correlation between serum levels of COMP with RF in our study and have also observed a negative correlation between serum COMP and anti-CCP levels in RA patients (Table 3.9). Figure 3.6.3 shows serum COMP level was significantly decreased in anti-CCP (+) and RF (+) RA patients than anti-CCP (-) and RF (+) patients.
According to Christensen et al\textsuperscript{362} serum COMP level is decreased in anti-CCP positive patients with RA giving evidence that anti-CCP antibody may be implicated in the disease process by modifying cartilage metabolism. Though we agree with this study we have also observed the serum COMP level is decreased in a small number of anti-CCP negative RA patients indicating its independent involvement in disease process. Soderlin et al\textsuperscript{363} has reported that no significant differences were found between anti-CCP and COMP and raised serum COMP levels in RA patients indicating cartilage involvement in early stages of disease.

In our study we have found a weak association between serum COMP and Hyaluronic acid in RA patients. Lottenburger et al\textsuperscript{256} has reported that diurnal variations affects the serum level of HA and COMP in RA patients. Jong et al\textsuperscript{242} reported that generally early RA is often unnoticed by the patient and the physician, there was a weak positive correlation exists between COMP and HA simultaneously weak negative correlation with anti-CCP antibodies suggesting though in early stages of RA, cartilage destruction is not necessarily very prominent, the serum COMP might be very much useful in the early diagnosis of this joint disease but not found to be a reliable predictor of future joint damage in this RA population. Concurrently, in our study group also there is no correlation between serum osteocalcin levels with any of the biochemical markers.

Peter et al\textsuperscript{364} has reported that serum osteocalcin levels were increased in RA patients with high disease activity with increased anti-CCP and RF levels. But in our study we could not establish any correlation
between OC and auto antibodies in RA patients. In RA patients bone metabolism is related to disease activity and patients with active RA had significantly higher OC levels than patients with inactive RA suggests that in active disease, bone resorption is increased more than bone formation, which may lead over time to bone loss.\textsuperscript{256} A German study by Hermann \textit{et al}\textsuperscript{365} reported that no correlation was determined in the RA patients between serum OC levels and RF of the all types (IgA, IgG, IgM) along with CRP, ESR levels. Similarly we also found no correlation between serum OC and RF(IgG) along with inflammatory markers.

In another study by Salisbury and Sharif\textsuperscript{366} including both RA and OA patients reported that the OC concentrations were directly correlated with HA in the serum of OA patients but not in RA patients. In our study also as reported earlier there was no correlation between serum levels of OC and HA levels in RA patients. In our study we found there was no significant correlation between serum OC and COMP levels in RA patients. In a recent study Jonathan \textit{et al}\textsuperscript{367} reported that there was no correlation between serum OC and COMP levels in OA patients. But no reported studies were available in RA patients.

So based an all these studies we have to conclude that the serum osteocalcin, no doubt a good marker for bone turnover as reported by several established studies. Though it would be premature to assess, its role in the pathogenesis of RA is still ascertained as more future studies required.
Phase II

Rheumatoid arthritis is a lifelong disease, although patients can go into remission. Patients who do not respond well to a single disease-modifying drug may be candidates for combination therapy. It has no disease specific diagnostic features and patients present themselves with wide range of manifestations. In our study, we could able to follow-up 55 RA patients (14 male and 41 female) which include 25 early and 30 late RA patients after one year from the phase I study group of 102 RA patients. The results from these RA patients were compared with 30 age matched controls.

Traditional markers of disease activity in RA

In phase II of our study we have observed that the levels of disease activity measures like DAS28 score, swollen and tender joint count is significantly increased along with ESR and CRP in RA patients compared with control after one year follow up. This shows persistent high inflammatory activity and involvement of joints in the RA pathogenesis (Table 3.10). The same observation is also been noticed among the subgroups of RA and control individuals (Table 3.11).

Comparison of Traditional markers of disease activity in RA patients in phase I & phase II

In early RA patients the serum CRP level remains high in phase II in comparison with phase I and the markers of disease activity such as ESR, swollen and tender joint count was found to be decreased after one year follow–up (Table 3.13). These results suggest that there is some degree of
inflammation persists in early RA patients. Though we could substantiate with clinical improvement in terms of reduction in joint count, improved physical activity according to HAQ in these early RA patients as they were under drug therapy, we could not provide any evidence to support our view.

We had also noticed the persistent inflammatory activity in late RA patients which we have assessed by unchanged high ESR and CRP level in phase II same as phase I. But we found a significant decrease in swollen and tender joint count in phase II than phase I (Table 3.14). Rindfleisch and Muller\textsuperscript{39} reported ESR can be influenced by a number of unrelated factors, such as age, gender or plasma proteins and in several investigations that have used different study designs including RA patients reported with different outcome.

Study by Wolfe\textsuperscript{96} on RA patients suggested CRP measures the acute phase response only, but that ESR measures elements of chronicity and severity of RA in addition to the acute phase response. In the same study it is also reported, ESR correlates better than CRP with measures that are not acute phase proteins, such as immunoglobulin’s, RF and general measure aspects of severity of rheumatoid arthritis. In turn, in our study both the ESR and CRP are not showing any kind of correlation with clinical measures of RA disease activity in phase II similar to the studies by Brahn and Scoville\textsuperscript{368} which concludes that inflammatory status during sampling stage reflects in the levels of ESR and CRP in RA patients.

Devlin et al\textsuperscript{100} reported that suppression of elevated CRP in patients with active RA is associated with improvement in functional score, whereas
persistent elevation of CRP is associated with functional deterioration. Once abnormal CRP is suppressed, no functional deterioration is likely to occur without re-elevation in C-reactive protein. Another study Dawes et al. stated serum CRP level has better prognostic value in terms of progressive joint damage and functional status and outcome.

Though in our study there is no significant changes in CRP level in both early and late RA patients in phase II compared with phase I since CRP level showing better correlation with HA, marker of synovial inflammation than ESR in phase II proved that CRP has better prognostic value than ESR in rheumatoid arthritis patients.

**Auto antibodies status in RA patients in phase II**

**RF level in RA patients**

In the present study we have observed significant increased levels of serum RF (IgG) in total RA patients when compared with controls after one year follow-up. (Figure 3.10) Rheumatoid factor level is increased in 65% (35/55) of total RA patients and decreased or low in remaining 35% of RA patients. In sub groups of RA there are no significant changes in RF level in early, late RA and control (Figure 3.10.1) also within early and late RA patients in phase II (Figure 3.10.2).

**Comparison of RF level in RA patients in phase I and phase II**

In our study when comparison of RF values between phase I and phase II it is observed that serum RF values are increased in total RA patients after one year follow up than baseline levels (Figure 3.14). Moreover, the steady increase in serum RF which is more prominent in
early RA with statistical significance (Figure 3.14.1) in phase II than phase I group. According to Figure 3.14.2 there was no significant change in RF values in late RA patients in phase II and phase I.

Among early Rheumatoid arthritis patients despite the relatively good clinical response which was reflected in the rapid mean decline of ESR, swollen and tender joint count and improved physical activity (based on HAQ) compared with baseline levels, high titers of RF considered to be a risk factor for the further pathogenic development of RA indicating an early preclinical stage of the disease and also a poor prognosis. Surprisingly in these early RA patients we have also observed elevation of CRP, but unchanged DAS 28, which are other indicators of poor prognosis of rheumatoid arthritis.\(^{370}\)

We have noticed 20% early RA patients had decreased RF levels after one year follow-up. Studies proved infliximab therapy could reduce the amount of synovium infiltrating cells, including plasma cells because RF-producing cells are present in inflamed rheumatoid synovium and the reduction in inflammatory lympho-plasmacytic infiltrate in rheumatoid synovium will lead to a reduced production of RF (IgG) early in the course of therapy\(^{371}\). In agreement with this, our study also suggests this could be one of the reasons for decreased RF (IgG) level in early RA after the follow up. About 25% late RA patients in our study showed decreased RF (IgG) level after the follow up. Study by Toubi et al\(^{372}\) with small group of RA patients published with decreased RF level with clinical improvement in rituximab-treated patients with rheumatoid arthritis.
Sune et al\textsuperscript{373} reported Rheumatoid factor levels are believed to increase with age in the general population and RF IgG isotype is a predictor for poor clinical response to treatment with anti-rheumatoid combination therapy concluding RF antibodies cannot be used for disease monitoring. Similarly, in our study also we have observed increased RF levels with age but no statistical significance. Hence from the data of our study it is clear that increased RF level in phase II than phase I suggesting poor prognosis and cannot be used for disease monitoring.

We have also observed 16\% of the RA patients with RF positivity at baseline turning RF negative after one year follow up. Study by Cordonnier et al\textsuperscript{374} reported that there is an association of RF with the severity of RA, and RF-positive patients can turn RF negative following therapy. De Rycke et al\textsuperscript{109} reported that an increased RF level within three years of the onset of symptoms was prognostic for a more severe disease outcome six years after the onset of symptoms Hence this short follow up period (one year) may not provide better picture of patient disease status in early stages of rheumatoid arthritis.

There are several studies including a very recent French study, reporting poor response from RA patients taking conventional combination therapy-DMARDs or one more TNF-alpha blockers or stable low dose of steroids in a short follow-up with not many significant changes in autoantibody level like RF and other disease activity measures.\textsuperscript{375} Though IgM (RF) is currently assessed in clinical practice however, the combined detection of additional isotypes like IgG and IgA may improve diagnostic
and prognostic value of RF in RA patients. The role of IgG (RF) as well as other isotypes as markers of RA in response to treatment is not yet fully understood.

Some studies reported a drop in RF level after effective treatment with both the traditional disease-modifying anti-rheumatic drugs (DMARDs) and anti-tumor necrosis factor (TNFα) treatment\(^{376}\) whereas another study on a small series of RA patients failed to show any reduction in the levels of RF as well as anti-CCP after treatment with DMARDS\(^{377}\). However, data confirming a definite relationship between decreased RF levels and clinical response are scarce. Even though different drugs or combination of drug therapies have dramatically changed the treatment of RA, almost one third of patients are still poor responders, and no definite serological predictors of lack of response have as yet been reported.

In our study group we have found high RF level persists in those patients who had high RF at baseline. Farragher et al\(^{378}\) reported that in RA patients who had high RF (IgG) level at baseline and follow-up are considered as non-responders to treatment whereas no obvious difference was found in patients with RF (IgG) low-positive and normal. These observations indicate that high levels of RF (IgG) are significantly associated with a poor response rate to treatment. The reasons for the different effect of treatment on RF and anti-CCP production in advanced RA are still elusive. Rheumatoid factor production could be at least partially dependent on inflammation whereas anti-CCP production might be more
constitutive even though local production of antibodies directed to citrullinated antigens has been reported in rheumatoid synovitis.\textsuperscript{379}

Altogether these observations made us to conclude Rheumatoid factor is not a very good prognostic marker in early stages of rheumatoid arthritis in the present series of patients with Indian origin.

\textbf{Anti-CCP level in RA patients}

Figure 3.11 explains that anti-CCP levels in serum are significantly increased in total RA patients. There is a significant change in serum anti-CCP level in sub groups of RA compared with control (Figure 3.11.1). But there is no significant difference in anti-CCP level within early and late RA patients in phase II (Figure 3.11.2). We have observed 75\% of RA patients with increased anti-CCP levels and other 25\% with low or normal anti-CCP levels. Since we had discussed about various studies on anti-CCP specificity and sensitivity in the earlier part of discussion, from our studies it is clear that anti-CCP is better than RF in the diagnosis of RA in both phase I as well as phase II.

\textbf{Comparison of anti-CCP level in RA patients in phase I and phase II}

According to Figure 3.15, there was no significant difference in serum anti-CCP level in RA patients in phase II compared with phase I. From Figure 3.15.1 it is clear that in early RA patients reported a decrease in anti-CCP level in phase II than phase I with no significance and late RA patients show no significant change in anti-CCP level after one year follow up compared with phase I level (Figure 3.15.2).
Studies by Alessandri et al\textsuperscript{380} reported that there is decrease in the levels of anti-CCP levels in early RA patients when compared with baseline with better clinical response in early RA patients after one year follow up based upon decrease in ESR, CRP and joint count was observed after treatment. Early RA patients may subject themselves for aggressive anti-rheumatoid therapy in early stages which may bring down the levels of anti-CCP levels in early RA patients.

Same study also concluded that no data are currently available on the prognostic importance of a quantitative evaluation of anti-CCP levels in patients with RA suggesting serial evaluation of these antibodies could be useful in monitoring the clinical course of the disease, at least in patients undergoing treatment with different DMARDs or combination drug therapies. Totally on agreement with this study, we have also reported a decrease in anti-CCP level although the differences were not statistically significant.

Studies by Annette et al\textsuperscript{316} have indicated that anti-CCP positive early RA patients may develop a more erosive disease than those without anti-CCP. Other investigators have confirmed this and suggested the superiority of anti-CCP over RF in predicting an erosive disease course. In our present study we have observed increased inflammatory status (increased ESR and CRP) in anti-CCP positive patients, but we could not establish any relation between these two autoantibodies. Study by van der Helm et al\textsuperscript{381} reported during follow-up, anti-CCP-positive RA patients have more swollen joints than anti-CCP-negative RA patients in spite of the
difference in magnitude of the disease characteristics concluding anti-CCP has been shown to be an independent predictor of radiological damage and progression. In our study 33% of anti-CCP positive patients had more swollen joints than anti-CCP negative patients in phase II, thus the data may be useful in predicting disease progression in RA patients.

Another study by Ronnelid et al concluded higher anti-CCP concentrations at baseline were associated with greater disease activity during follow-up. Studies have reported the effects on RA-associated markers, including anti-CCP, in patients treated with DMARDs though treatment resulted in a significant decrease in disease activity scores, no changes in the percentages of patients who were positive for anti-CCP or IgG-RF were observed in early rheumatoid arthritis376 But contrary to this observations, in our study we are reporting with significant increase in RF (Figure 3.14.1) and no significant change in anti-CCP levels in early RA patients after one year (Figure 3.15.1)

In accordance with Bruns et al we have also observed little change in anti-CCP as well as RF level in 43% of RA patients who are under combination therapy with DMARDS after one year follow up. These results were confirmed by several other studies, although follow-up periods varied between the individual studies. Visser et al reported that the anti-CCP autoantibody system is different from the RF system with respect to response to treatment. Clearly, investigating the functional importance of anti-CCP in the progression of rheumatoid arthritis demands other approaches specifically aimed at decreasing this humoral response.
In phase II, though there is overall decrease in anti-CCP level in early RA than phase I, 10% early RA patients are reported with increased anti-CCP levels in phase II compared with base line level. Salaffi et al\textsuperscript{301} reported positive anti-CCP values has been found in several studies, to have prognostic properties in early (and very early) arthritis, although anti-CCP antibodies may not be present at disease manifestation but may develop later in a percentage of RA patients. Recent reports confirm the prognostic significance of anti-CCP antibodies in early RA to be even greater than IgM and IgG-RF\textsuperscript{384}.

In our study 8% of RA patients that were anti-CCP-negative at phase I have become anti-CCP-positive after one year. A relatively low prevalence of anti-CCP antibodies in early arthritis patients turning positive for anti-CCP after follow-up has been described by Soderlin et al\textsuperscript{363}. Further, we could not establish any kind of correlation between RF and anti-CCP with any disease activity measures as well as with COMP, a cartilage breakdown marker in Phase II study. We attribute this for may be small number of follow-up patients and may be study of large scale RA patients will give better clarity for our observation.

One of the crucial aspect of our study is that we were able to demonstrate a positive correlation between anti-CCP and HA in the follow-up in both early and late RA patients (Table 3.17) Interestingly, the same observation was noticed in phase I thus indicating both anti-CCP and HA play an important role in RA pathogenesis.
Hyaluronic acid in RA patients

Studies have been conducted to examine synovial biopsy samples at various stages of the rheumatoid arthritis. The most important conclusion is that all features of chronic synovial inflammation can be observed in early rheumatoid arthritis. In one study on RA patients Emlen et al. stated that HA level is tended to correlate with ESR and CRP at baseline, but with the institution of drug treatment the correlation was not seen, as the HA level continued to be raised while the levels of other markers of inflammation decreased. From our phase II study we have observed serum HA level is significantly increased in RA patients (Figure 3.12) as well as in sub groups of RA compared with controls (Figure 3.12.1) showing persistent inflammatory activity in the synovium but no significant differences within early and late RA patients(Figure 3.12.2).

Comparison of HA level in RA patients in phase I and phase II

The most important finding from our present study is that serum hyaluronic acid level is significantly (Figure 3.16) increased in phase II than phase I in total RA patients. Though in both early and late RA sub groups, the serum HA level in marginally increased in phase II study than phase I, we could not establish any statistical significance. (Figure 3.16.1 and Figure 3.16.2)

Inger and Gunnar suggested that no arguments exist for the effect of therapeutic intervention on synovitis varying in different phases of rheumatoid arthritis and in end-stage of rheumatoid arthritis factors that are secondary to the disease may contribute to the perpetuation of synovial
inflammation. Since serum hyaluronic acid is product of synovial inflammation, its serum level reflects inflammatory status in synovium.

In another study van de Sande et al. concluded subclinical inflammation of the synovium does not coincide with the appearance of serum autoantibodies during the pre-rheumatoid arthritis stage. Thus, systemic autoimmunity precedes the development of synovitis, suggesting that a 'second hit' is involved. Probably, the serum HA level gradually increases even before appearance of autoantibodies though there is no published study to support our view. Studies explained the role of HA in RA synovitis through receptor mediated mechanism. One such receptor CD44 is a multi-structural cell-surface glycoprotein that interacts with many cell-surface and extracellular ligands like hyaluronic acid. Stimulation of CD44 with HA transmits the signal into the cells, which leads to activation of T cells and cytokine or chemokine release from monocytes. The growth and function of chondrocytes is by binding to CD44 receptors on the chondrocytes resulting in synovitis often seen in rheumatoid arthritis.

Butler et al. reported that a number of immune regulatory functions are associated with HA, notably its effects on T cells and in turn production rate of HA is stimulated by pro-inflammatory cytokines, such as IL1, IL6, and TNF-alpha. In RA, in which there are multiple joint effusions causing large quantities of HA to be released from the synovium into the serum. There is limited published evidence for the role of any DMARDs on HA levels in any stages of rheumatoid arthritis. Laurent and Hallgren provide proof supporting that HA levels are very much increased in the morning in
RA patients with minimal or moderate exercise than healthy controls than afternoon or evening. Since we collected the samples from these patients in the morning this could be the reason for high HA level in RA patients of our study population. It is proposed that HA, produced in the joint tissue structures and accumulated at rest, is carried by the lymph vessels to the general circulation during physical activity.

In rheumatoid arthritis, the more pronounced HA outflow is probably due to enhanced synthesis, and thus accumulation of HA in the inflamed joint tissue. This may be a cause of morning stiffness, because HA that has excessively accumulated in the joint tissue could immobilize water and mechanically hinder joint movements. This hypothesis was supported by the observed relationship between the transient increase of HA and duration of morning stiffness.387,325

Normal serum HA concentrations are age dependent with a decline from infancy to adulthood, and rise again in the elderly.388 In another study Guechot et al389 reported following major tissue injury, hyaluronic acid production increases as a rapid response survival mechanism and increased hyaluronic acid production and turnover are often associated with increased hyaluronidases activity, the enzyme that degrades hyaluronic acid. As we have not measured serum hyaluronidase activity in our study which could give better idea about increased serum HA in rheumatoid arthritis patients. One of the Thailand study by Louthrenoo et al390 reported increased HA level in RA in patients with corticosteroid
therapy although inflammation is subsided suggesting HA as useful marker for the activity and severity of disease in patients with rheumatoid arthritis.

Petersen et al\textsuperscript{391} observed in RA patients positive correlations were found between serum hyaluronic acid and anti-CCP levels though increased serum hyaluronic acid could not be explained by impaired renal or liver function or by drug therapy. In the same study it is observed that NSAIDs have no role on the serum hyaluronic acid concentrations as the levels were not significantly changed in these patients suggesting an increased production of hyaluronic acid in RA, and the increase seems to be related to the activity of the inflammatory process. We completely agree with this as in our study also there is significant positive correlation between HA and anti-CCP (Table 3.17) with high hyaluronic acid level in RA patients in phase II than phase I (Figure 3.16)

Ingeman et al\textsuperscript{392} reported that there was increased HA level in RA patients during follow-up, irrespective of drug therapy suggesting serum HA showed a delayed decline as compared with the clinical signs of synovitis. This probably reflects the presence of persistent subclinical chronic inflammation even though there is decline in other disease activity markers in RA patients. So it is clear that one year period is too short to assess the status of change in HA level in RA patients.

Study on osteoarthritis patients by Chen-Liang et al\textsuperscript{393} reported that intra-articular injection of HA is a well-documented treatment for knee osteoarthritis, one of the multifactorial mechanisms is that exogenous HA can stimulate endogenous HA production in OA patients. But no such study
reported on RA patients. Hyaluronic acid proving to be in a good positive correlation with anti-CCP in both phases signifying its role in RA pathogenesis. It is well known fact that exodus of HA and anti-CCP from RA synovium eventually into systemic circulation as a part of disease progress. So measurement of these two markers proved to be most crucial part of our study.

We also proved in our phase II study that ESR along with anti-CCP showing a significant correlation with Hyaluronic acid (Table 3.16) further suggesting synovial involvement in RA pathogenesis. Hence assessing HA, anti-CCP along with other traditional markers of RA in the early stages of disease may be useful for better understanding of pathogenesis of early rheumatoid arthritis.

COMP level in RA patients

We have found no significant changes in the levels of COMP in RA patients (Figure 3.13). At the same time there were no significant changes in early and late RA patients after one year follow-up(Figure 3.13.2) and their levels were not different from normal control group (Figure 3.13.1). We had reported the same observation at base line level study. Hence the role of COMP as cartilage breakdown marker in RA pathogenesis is still elusive.]

Comparison of COMP level in RA patients in phase I and phase II

According to Figure 3.17 there was no significant change in the levels of serum COMP in RA patients between phase I and II. Specter et al reported generally in RA the inflammatory parameters such as the
ESR and CRP may provide useful information about the general inflammation process. However, biochemical marker like COMP are not joint specific and are poorly correlated with cartilage damage at the individual level. On agreement with this view in our present study also we could not find any correlation between COMP with ESR and CRP in RA patients (Table 3.16) Andersson et al\textsuperscript{343} reported the estimated half life of COMP is surprisingly very short and the high levels observed in certain patient groups reflect a high level of influx of COMP to the circulation from tissues. The finding of lower serum levels of COMP in patients with RA may be due to patient selection, and does not reflect a different characteristic of these diseases in general. This could be one of the reasons for normal or low COMP level in RA patients from our phase II study.

Our study in concurrence with the study by Crnkic et al\textsuperscript{247} reported that although COMP is not unique to cartilage but clearly support a role for serum COMP as a marker reflecting processes not directly linked to the inflammation in rheumatoid arthritis. Serum COMP levels decreased during treatment in patients with radiographic progression but remained low and unchanged in patients with no progression. In the same study it is concluded that lower serum levels of COMP in patients with rheumatoid arthritis may be due to patient selection, and does not reflect a different characteristic of these diseases in general.

However in one of the studies Niki et al\textsuperscript{345} put forward, in early RA serum COMP levels at baseline was low, and remained unchanged over
the period of one year, despite fully exertion of the therapeutic effects of various ARA drugs. Given the evidence that serum COMP and HA levels elevate with increasing physical activity. The constant levels of COMP over time in early RA might theoretically be explained if the decrease in COMP levels induced by these drugs is balanced by increased physical activity there by increased HA as evidenced from decreased HAQ scores. This could be the reason for constant COMP level and increased HA level in early RA patients from our study group in phase II.

The recent studies reported by Pavelka et al. showed increased CRP level in early and late RA, significant decrease in HA in early RA and decreased HA and COMP in late RA after one year follow-up. Contrary to this report we found increase in HA in both early and late RA but no significant changes in COMP level. No previous studies have provided such insights. Studies previously shown that serum concentrations of COMP are increased early in RA patients who subsequently develop advanced large-joint destruction. A prognostic value for joint damage by serum concentrations of HA is also suggested by previous studies. In our study serum HA level is increased an COMP level remains unchanged after one year follow-up indicating that they may not be good prognostic markers.

Postmenopausal women with RA receiving hormone replacement therapy (HRT) had lower serum COMP level. Intra-articular glucocorticoid treatment for knee synovitis in RA patients reduces serum COMP, with a slightly larger decrease of serum COMP in the group
randomized to 24 hour bed rest instead of normal activity. This could be also one of the reasons for low serum COMP level in RA patient group.

Lipsky et al stated there is no significant difference in serum COMP levels in early RA as well as late RA patients and these observations taken together are consistent with the hypothesis that inflammation and tissue destruction are not directly linked, thus corroborating earlier published studies. It is notable that the estimated half life of COMP is surprisingly very short and the high levels observed in certain patient groups reflect a high level of influx of COMP to the circulation from tissues. Taken together, the findings support the use of COMP as a serum marker for joint diseases.

Normal baseline serum COMP levels may be due effect of drugs taken as part of therapy on RA patients since studies by Saxne and Heinegard proved that glucocorticoids and stable, low-dose prednisolone treatment tend to lower serum COMP levels. However, this effect does not seem to be due to the anti-inflammatory effect of glucocorticoids, since the lowering of serum COMP by glucocorticoids does not correspond to a decrease in inflammation (as measured by CRP or ESR). In this study we did not find any difference in baseline levels of COMP between early RA and late RA patients suggesting normal cartilage turnover in these patients (Figure 3.17.1 and 3.17.2).
Correlation between biochemical markers with traditional markers of RA in phase II follow-up study

In our study except hyaluronic acid, there was no significant correlation between other biochemical markers with traditional markers of rheumatoid arthritis. A weak positive correlation between HA and ESR was found in phase II but no correlation was observed with any other traditional markers of RA in these patients (Table 3.16). Interestingly similar correlation was also observed between HA and ESR as well as CRP in phase I. Therefore from the results of our study it is clear that synovial inflammation is a marked feature in RA and hyaluronic acid is directly involved in the pathogenesis of rheumatoid arthritis.

Though in phase I we observed a positive correlation between RF with CRP and also between anti-CCP with ESR and CRP in RA patients, a similar observation was not made in phase II study.

Correlation within the biochemical markers in RA patients in phase II

Among the four biochemical markers studied in phase II we have observed a weak positive correlation between HA and anti-CCP level in RA patients (Table 3.17) similar to that in phase I. But there was no correlation within any other biochemical markers in phase II. As we had discussed earlier, from our study it is proved that the serum HA and anti-CCP, both products of synovial inflammation are directly involved in the pathogenesis of rheumatoid arthritis.