

POTENTIAL VALUE OF C-REACTIVE PROTEIN AS INFLAMMATION MARKER AND IL-1 β , TNF- α AS PRO-INFLAMMATORY CYTOKINE ON DISEASE ACTIVITY OF RHEUMATOID ARTHRITIS

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ABSTRACT

A study on the role of C reactive protein as inflammatory marker and IL-1 β , TNF- α as pro inflammatory cytokine on disease severity of rheumatoid arthritis, was performed 40 patients comprising 33 females and 7 males. All patients had received treatment with single or combined therapy using corticosteroid, NSAID or DMARDs. The patients were group into 4 categories according to the degree of disease severity with Disease activity score (DAS 28) methods. 14 Patients showed severe disease activity (DAS > 5.1) 16 patients showed moderate disease activity (3.2 < DAS < 5.1). It was found that many patients with severe or moderate disease activity showed IL-1 β concentration, TNF and CRP concentrations had a significant correlation with the assessment of rheumatoid arthritis disease activity on 28 joint (DAS 28). This study did not show a significant correlation between IL-1 β concentration and CRP concentration ($r=+0.005$, $p= 0.981$), but have positive correlation between IL-1 β and DAS 28 ($r = +0.300$, $p = 0.048$) as well as between CRP concentration and DAS 28 ($r=+0.074$, $p=0.704$). The correlation between TNF- α and CRP did not showed significant, because the CRP concentration is influenced by genetic polymorphism of CRP gene, IL-1 β gene, short half life of CRP and the usage of drugs (NSAID, corticosteroid, DMARDs) between CRP concentration and DAS 28 ($r=+0.074$, $p=0.704$). The principle finding of this study concluded that there was a significant association between pro-inflammatory cytokine (IL-1 β and TNF- α) and disease severity of RA (DAS 28). It was shown that the plasma level IL-1 β has significant correlation with severe as well as moderate degree of disease activity, while the plasma levels TNF- α has significant correlation with severe disease activity as based on measurement by DAS28. The data found in this study indicates that screening of C reactive protein and the combined use of IL-1 β and TNF- α could be considered as a powerful approach to identify subjects at risk for rheumatoid arthritis, and need a large scale of population study which may be used as the basis for the design of new intervention strategies, especially in order to improve the degree of disease severity.

Keywords : Rheumatoid arthritis, C reactive protein, IL-1 β , TNF- α , DAS 28

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INTRODUCTION

The prevalence of rheumatoid arthritis (RA) as a chronic progressive autoimmune disease is increased every year in Indonesia and over of the world, this indicates that some specific factors may play pivotal role in the increment of the prevalence of the disease. Actually, RA has been well described since long ago, however advances in understanding the pathogenesis of RA limited. It was already published that RA as polygenic multifactor disease influence the pathogenesis of RA; such as genetics, autoimmune, infection of microorganism etc. Cytokine as immune mediator has already entered the mainstream of medical practice, particularly for autoimmune disease and play a role in

the pathogenesis and progression of RA as well as the severity of the disease.

In disease such as RA, where a specific causative agent and/or antigen has not been identified, on cytokine balance may represent a way to control autoimmunity and chronic inflammation. In general, the cytokine found routinely in the circulation during inflammatory process, however many cytokine are not commonly detected in the circulation, but act more in local or immediate microenvironment, ; these cytokine include proinflammatory cytokine such as interleukin-1(IL-1) and tumor necrosis factor- α (TNF- α). Studies of the contribution of cytokine to the pathogenesis of RA are focused largely on IL-1 β and TNF- α , characterized as

the most important in the pathogenesis as well as therapeutic targets of RA.

Other studies showed that circulating levels of IL-1 β correlated with disease severity in patients with exacerbation of RA and that resident macrophage in the rheumatoid synovium constitutively expressed IL-1 β and TNF- α . A number of biochemical substance defined C-reactive protein is an acute phase reactant and has a function as a marker of inflammation and it presented and is presented within a higher concentration in persistent infection or inflammation of rheumatoid arthritis. It was shown in the study by Pearson et al (2003) that CRP was increased in chronic inflammatory process of RA patients, and many studies stated that CRP is better than erythrocyte sedimentation rate (ESR), especially for monitoring rapid alterations because it does not depend on the blood level of fibrinogen or immunoglobulin and is not affected by the number and shape of the red blood cell.

At the present time, we do not have significant data from the explorative study to find a biologic substance to be a target of the autoimmune process which precedes rheumatoid arthritis and is associated with the presence of multiple cytokines which is involved in the sequence of disease process and disease severity. This can be made possible by measuring the value of CRP which may be useful in determining disease progress. The aim of this study is to determine the role of IL-1 β , and TNF- α as a pro-inflammatory cytokine of RA and the correlation of the plasma level of C Reactive Protein as a marker of inflammation with disease activity or severity of rheumatoid arthritis.

MATERIALS AND METHODS

This observational study was carried out on 40 patient (33 female and 7 male) with RA from Rheumatology Clinic Dr. Sutomo Hospital, who had been diagnosed based on ACR revised criteria 1987. The age of patient are older than 16 years, and do not have symptoms of infectious disease, metabolic syndrome, and other immune compromised diseases. The method used for the determination of IL-1 β and TNF- α plasma level was solid phase Sandwich Enzyme Linked Immunesorbent Assay (ELISA) from Diaclone Research. The detect ability of IL-1 β and TNF- α is < 5 pg/ml. Plasma level of CRP as acute phase protein with a molecular weight with 23 kDa was determined using solid-phase chemiluminescent immunometric assay/method with IMMULITE® reagent. Disease Activity Score 28 (DAS 28) as an instrument to measure disease activity in patient with rheumatoid arthritis or the measurement index on 28 joint. There are 4 components of DAS 28 score/ measurement : tender joint count on 28 joint (TEN 28/TJC), swollen joint count on 28 joint (SW28/SJC), erythrocyte sedimentation rate (ESR) in mm and self assessment/patients 'global assessment of disease activity/general health. (Fansens 2003). The aim of self assessment of general health in RA patient in order to know : the disease activity of rheumatoid arthritis during the last 7 days. The formula for DAS 28 calculation : $DAS\ 28 = 0.56 \sqrt{TJC} + 0.28 \sqrt{SJC} + 0.70 \ln ESR + 0.0014\ SA\ GH$ (Note : The normal value ESR in males : 0 – 13 mm/hour, and in females : 0 – 15 mm/hour (Wallach 1992).

Table 1. The Evaluation of DAS 28

Current DAS 28	DAS 28 : Difference to initial value		
	> 1.2	> 0.6 and \leq 1.2	\leq 1.2
≤ 3.2 Low/mild	Good improvement	Moderate improvement	No improvement
$> 3.2 \leq 5.1$ Moderate	Moderate improvement	Moderate improvement	No improvement
> 5.1 High activity/ severe	Moderate improvement	No improvement	No improvement

RESULTS

The results of DAS 28 varied from 2.002 to 7.860. Patients were classified into 4 categories according to the DAS 28 values found in this study i.e RA patients showing severe disease activity (range : 4.153-7.860), moderate disease activity (range: 3.278 – 5.090), mild disease activity (range: 2.601-2.607) and the patients with the remission category (range: 2.002-2.295). The plasma levels of IL-1 β range from 1.2 to 27.2 pg/ml and

it was found out that the highest plasma level of IL-1 β was found in moderate and severe Diseases Activity (DAS 28) as shown on Figure 1. Plasma samples from RA patients with remission/inactive serve as control and were compared to plasma samples from patients with moderate and severe/high disease activity. The difference of plasma level of IL-1 β between the remission and mild with the moderate and severe/high activity was statistically significant ($p < 0.01$).

Correlation between plasma levels of IL-1 β and CRP of RA patients

It was shown that many patients with severe or moderate disease activity showed IL-1 β and CRP concentration much lower than the average of total samples. This study did not show a significant correlation between the concentration of IL-1 β and CRP ($r=+0.005$, $p=0.981$, Spearman test) as shown in Figure 2.

The Correlation Plasma Levels IL-1 β and DAS 28 in RA patients admitted to this study

A significant positive correlation was found between IL-1 β plasma concentration and DAS 28 ($r=+0.300$; $p=0.048$, Spearman's test).

Distribution of TNF- α and DAS 28 in RA Patients admitted to this study

The results of TNF- α concentration in RA patients admitted to this study were classified into 4 categories according to the DAS 28 values as followed : in severe disease activity the concentrations ranged from 12.13 to 79.30 pg/ml ; (46.49 ± 14.93), and in moderate disease activity the concentration ranged from 11.64 to 55.68 (32.55 ± 5.090), in mild /low disease activity the

concentration ranged from 7.39 to 40.03 (19.15 ± 18.12) and the remission group the concentration ranged from 7.40 to 31.261 (29.50 ± 31.26).

The plasma levels of TNF- α were found only in severe of diseases activity (DAS 28) as shown in figure 4. Plasma samples from RA patients with remission/inactive serve as control and were compared to plasma samples from patients with severe disease activity. The difference of distribution TNF- α plasma concentrations was only significant ($p < 0.01$), in the group of patients with severe disease activity.

Correlation between the plasma levels TNF- α and CRP in RA patients admitted to this study

This study did not show a significant correlation between plasma levels TNF- α and CRP concentration ($r=+0.100$, $p > 0.05$, Spearman test).

Correlation between plasma levels TNF- α and DAS 28 on RA patients

Statistically analysis of the correlation of plasma levels of TNF- α and DAS 28 was found significant ($r=+0.417$, $p < 0.001$)

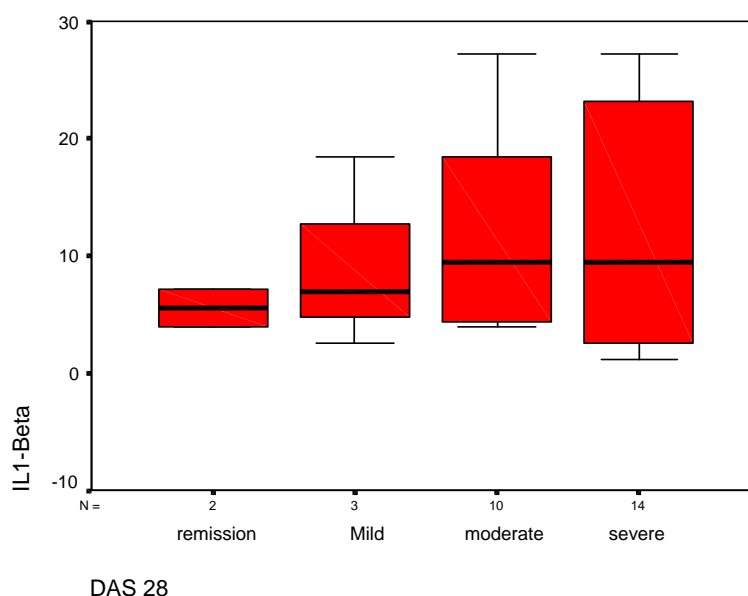


Figure 1. Plasma levels of IL-1 β and DAS 28 in RA patients admitted to this study

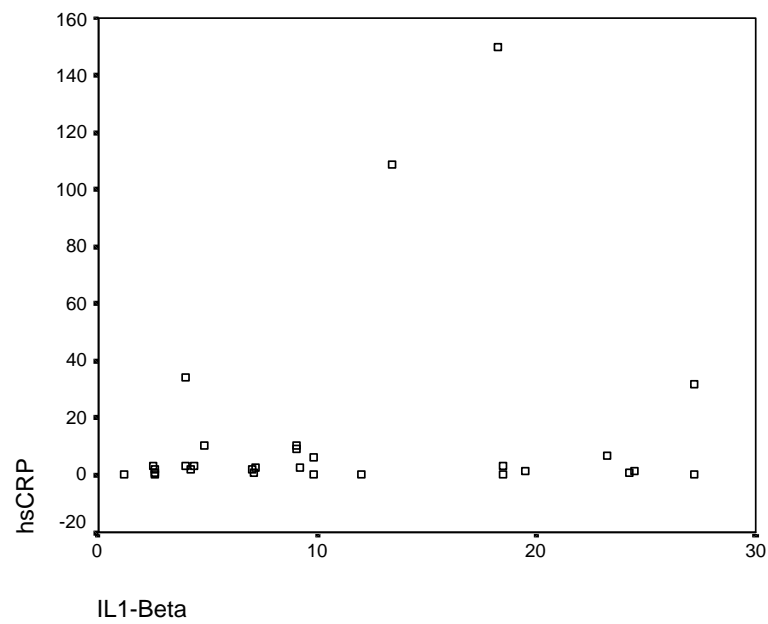


Figure 2. Correlation between IL-1 β and CRP concentration in RA patients admitted to this study

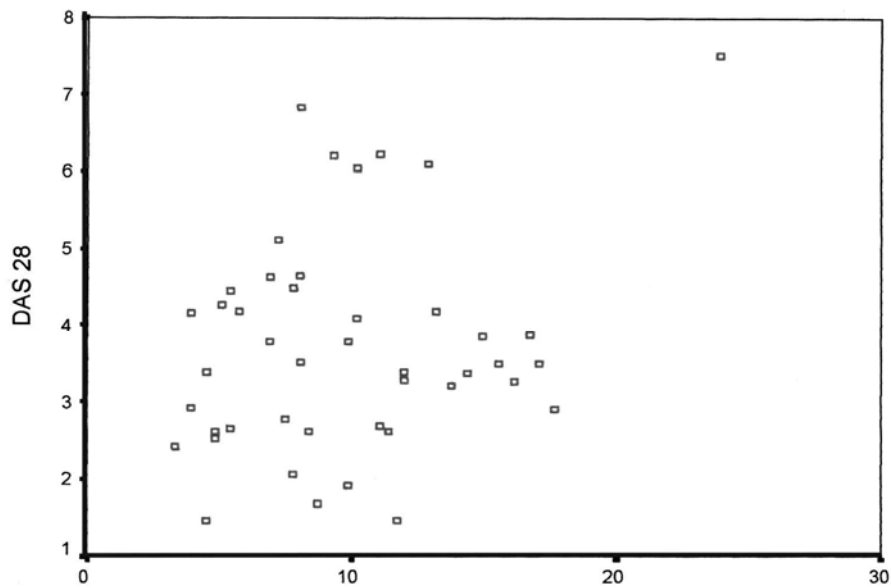


Figure 3. Correlation between plasma level of IL-1 β and DAS 28 in RA patients admitted this study

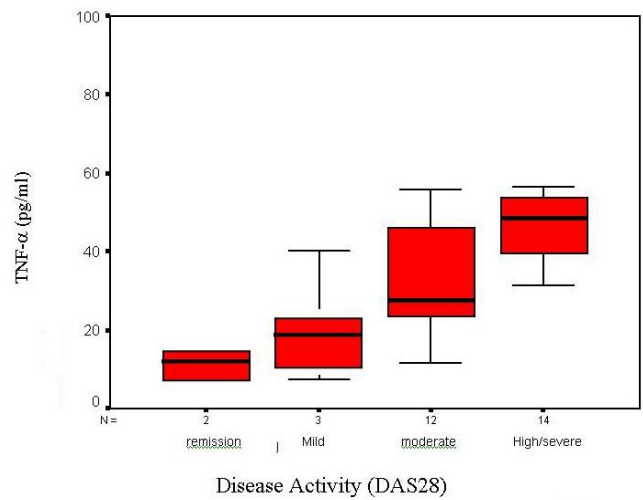


Figure 4. Plasma levels TNF-α and DAS 28 in RA patients admitted to this study

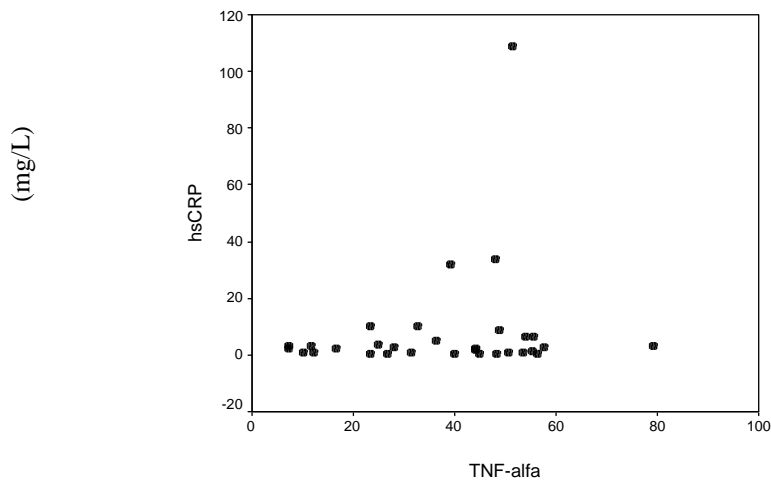


Figure 5. Correlation Plasma Levels TNF-α and CRP in RA patients admitted to this study

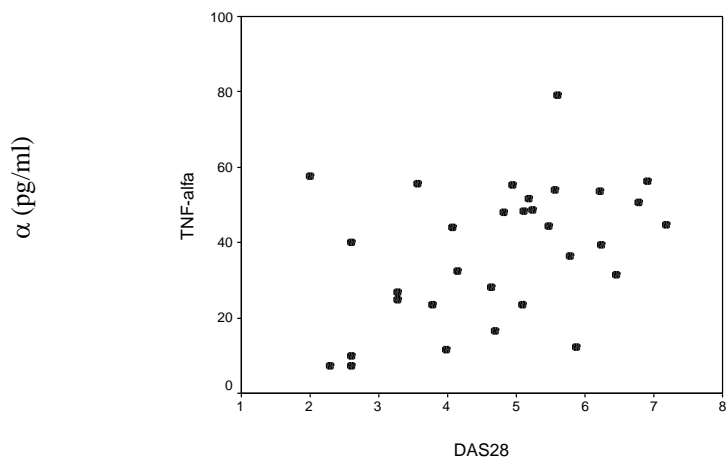


Figure 6. Correlation plasma levels of TNF-α and DAS 28 in RA patients admitted to this study

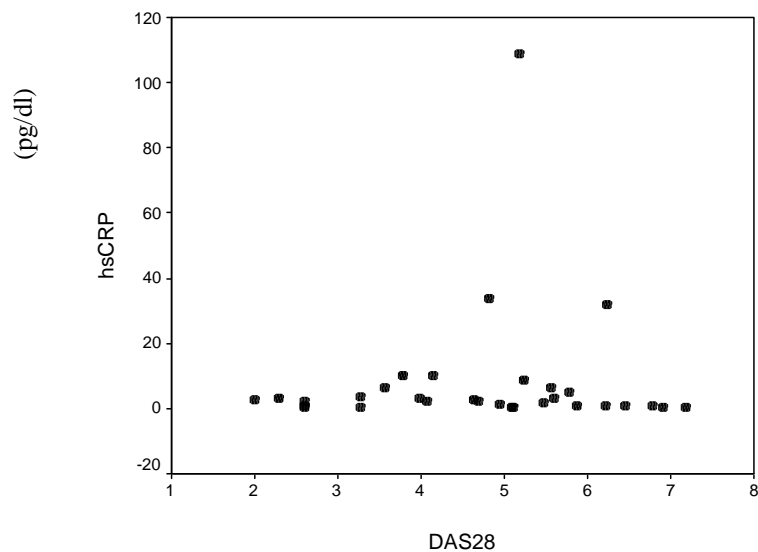


Figure 7. Correlation between CRP and DAS 28

Table 1. Distribution of samples with various degree of Disease Activity (DAS 28), ESR and CRP

Disease Activity	N	DAS 28 (mean, SD)	ESR (mm/hr) (mean, SD)	CRP (mg/L) (mean, SD)
Remission	4	2,004 - 2,295 (2,14±0,20)	15 - 25 (19±8,4,)	2,71- 3,15 (2,93±0,30)
Mild	6	2,601 - 2,607 (2,60±3,46)	25 - 35 (30±5)	0,24 – 2,27 (1,15±1,05)
Moderate	16	3,278 – 5,09 (4,19±0,63)	27 - 62 (47,16±13,3)	0,20-34 (6,37±9,33)
High	14	4,153-7,860 (5,97±0,67)	15 - 137 (46,5±14,93)	0,25-137 (12,2±29,06)

Calculation of the mean and standard deviation from a frequency table 1

The results of this study as illustrated in table 1, showed that the ESR of RA patients had the greatest effect on DAS 28 and was statistically significant ($r = +0.354$; $p < 0.05$; Spearman's test). DAS 28 value vary from 2.004 until 7.860 i.e 4.153 – 7.860 in 14 patients with severe disease activity, 3.278 – 5.090 in 16 patients with

moderate disease activity, 2.601-2.607 in 6 patients with low/mild disease activity, and 2.002-2.295 in 4 patients in remission status.

DISCUSSION

Two cytokines are studied in this research, namely Interleukin 1- β and TNF- α , the correlation with C

reactive protein as inflammation marker and association with disease activity (DAS 28) were also evaluated.. This study was performed comprising on 40 RA patients (33 females and 7 males). The result of plasma levels of IL-1 β was between 1.2 to 27.2 pg/ml (11.28 ± 8.3). The concentration of IL-1 β in the group of patients with severe disease activity was 3.30 to 23.92 (11.80 ± 5.67), in the group of patients with moderate disease activity was 3.90 to 17.05 (10.36 ± 4.4), in the group with mild disease activity was 3.90 to 17.65 pg/ml (8.75 ± 4.53) and in the group of patients with remission was 3.30 to 11.67 pg/ml (7.23 ± 3.11). A significant positive correlation was obtained between IL-1 β plasma levels and DAS 28 ($r = + 0.300$; $p = 0,048$; Spearman test). Using the one way Anova test, for the difference of the mean, it was found that in the group of patients in this study there were not significant ($p = 0.226$) difference among these groups. The cut-off value in each category could not be determined by ROC curve because the p values were not significant.

The CRP concentration of the patients in this study ranged from 0.20-150 mg/L (13.7 ± 33.54). The CRP concentration in the group of patients with severe disease activity ranged from 0.20 to 150 mg/L (23.32 ± 46.44), in the group of patients with moderate disease activity ranged from 0.20 to 34.0 mg/L (6.37 ± 10.16), in the group of patients with mild disease activity ranged from 0.24 to 2.27 mg/L (1.15 ± 1.03) and in the remission group of patients, ranged from 2.72 to 3.15 mg/L (2.93 ± 0.30). In this study there were no significant correlation between IL-1 β and CRP concentration ($r=+0.005$, $p= 0.981$), between IL-1 β and DAS 28 ($r=+0.098$, $p=0.613$) as well as between CRP concentration and DAS 28 ($r=+0.074$, $p=0.704$). It was found that many patients with severe or moderate disease activity showed IL-1 β concentration as well as CRP concentration much lower than the average total samples. There are several reasons which can explain this condition such as specific inhibitors (IL-1R α , IL-1Rs, IgG against IL-1 β), nonspecific inhibitors (binding protein, β_2 macroglobulin), protease, excessive consumption, lack of synthesis, immune suppressant drugs (corticosteroid, DMARDs), production of anti inflammatory cytokines (IL-10, IL-4, IL-13, IFN- γ , TGF- β), the role of other pro inflammatory cytokines (IL-6 and TNF- α), genetic polymorphism of IL-1 β gene, and incorrect sample handling because the half life of cytokines is very short (in minute).

Statistical analysis to evaluate the correlation plasma levels TNF- α and DAS 28 was significant ($r = + 0.417$, $p < 0.001$), but there was no significant correlation between plasma levels of TNF- α and serum CRP concentration ($r = +0.100$, $p > 0.05$, Spearman test). The

data obtained in this study had shown that IL-1 β and TNF- α play a very important role in the pathogenesis of RA, it act synergistically on the same target tissue in the joint of RA patients and were significantly correlated with disease severity but as inflammatory cytokines, they had no significant correlation with CRP as inflammatory marker. CRP is a protein whose level is increased in response to injury but the diagnostic value was not specific for a certain disease. Thus, for the confirmation of clinical diagnosis, it has only limited value. According to the literature, CRP rise up to 50000 fold in acute inflammation such as infection. Usually it is rising above its normal limits within 6 hours and peaks at 48 hours after tissue injury. Its half life is constant and therefore its level is mainly determined by the rate of production (and hence the severity of the precipitating cause). There are several reason that serum levels CRP is influenced by many factors like genetic polymorphism of CRP gene, IL-1 β gene. The levels of CRP is not influenced by corticosteroid or other drugs, except colchicin.

Measurement of the serum level of CRP values is proved useful in determination of the disease progress or the effectiveness of treatments. The increasing levels of serum CRP indicate the progress of RA disease, and the serum levels more than 10 mg/l was observed in mild degree of disease's activity, serum levels 30-40 mg/l was observed in moderate disease activity and serum levels more than 100mg/l (William 2000) was observed in severe degree of disease's activity. Based on data shown in figure 7, in this study no significant correlation was found between CRP concentration and DAS in RA patients ($r = +0.005$, $p = 0.981$, Spearman's test). The serum level of CRP also indicates the degree of inflammation or tissue injury present in RA patients. It has been stated the determination of CRP is a better test than the ESR. In patients with rheumatoid arthritis, a high serum CRP level is a good parameter of the presence of significant inflammation or injury in the body. At the present time, serum levels of CRP and ESR value are used to monitor disease activity and the results of treatment. The above mentioned finding gave rise to the question regarding the role of ESR as one of the components of the total DAS28 score.

Several studies had been done to know the role of erythrocyte sedimentation rate (ESR) as one of the component of Disease Activity Score 28 (DAS28) on total DAS 28 in RA patients. It has been studied in Japan by Toshihiro Matsui (2007), to evaluate disease activity and improvement by comparing DAS26 using ESR and by DAS 28 using CRP in Japanese patients with RA. It was concluded that DAS28-CRP significantly underestimates disease and overestimate the improvement of the disease compared with DAS28-

ESR. It was recommended that DAS28-CRP should be evaluated using different criteria of DAS28-ESR.

CONCLUSION

Based on the data obtained and discussed in this study, it can be concluded that there was a significant correlation between pro-inflammatory cytokines IL-1 β with severe and moderate disease activity in RA patients based on measurement by DAS 28. There was a significant correlation between TNF- α with severe disease activity in RA patients based on measurement by DAS28. CRP is very useful in monitoring disease activity in certain condition of RA specific treatment and as prognostic marker, but its use as a diagnostic tool is limited. This research results also have a note that even in known case of inflammatory disease such RA disease, a low CRP level is possible, but it is not indication that the RA had not inflammation.

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