Correlation of Tumor Necrosis Factor-Alpha, High Sensitivity C-Reactive Protein, and Disease Activity Score 28 (DAS28) in Rheumatoid Arthritis Patients

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Abstract

Inflammatory markers such as tumor necrosis factor-alpha (TNF-α) and high sensitivity C-reactive proteins (hs-CRP) have been suggested as markers disease activity (severity) in patients with rheumatoid arthritis (RA) but the data are limited. The aim of this study was to determine the correlation between both TNF-α and hs-CRP levels and disease activity of RA patients. A cross sectional study was conducted at Dr. Soetomo Hospital, Surabaya, Indonesia. Plasma levels of TNF-α and hs-CRP were determined by ELISA and solid-phase chemiluminescent immunometric assay, respectively and the degree of disease activity (severity) was measured using disease activity score 28 (DAS28). The levels of TNF-α and hs-CRP between the level of disease severity were compared using Anova test and the correlations of TNF-α or hs-CRP level and DAS28 were tested using Pearson's or Spearman correlation as appropriate. Thirty-one patients were enrolled in this study, where most of them (87.1%) were women, with mean of age 45.61 years. Based on DAS28 score, 45.2% of the patients were categorized as high disease activity (severe). The level of TNF-α was statistically significant between disease activity groups (high vs. moderate with p=0.012; and high vs. low with p=0.036). There was a significant positive correlation between the TNF-α and DAS28 score (r=0.417, p=0.02). No correlation between hs-CRP and DAS28 score (r= -0.117, p=0.532). The level of TNF-α is associated with DAS28 score, suggesting its potential as a marker of RA disease activity or severity. Further studies are therefore warrant to validate this finding in order to provide more robust evidence.

Keywords: *Rheumatoid arthritis, tumor necrosis factor-alpha, high sensitivity C-reactive protein, DAS28*

Introduction

Rheumatoid arthritis (RA), characterized by persistent synovitis, is a systemic autoimmune inflammatory disorder typically found in women.[1] RA may affect all ages and ethnicity, but the prevalence of the disease increases with age.[2] It affects 0.5-1% adults in industrialized countries with 5-50/100.000 new cases every year.[3] Left untreated, RA could cause joint damage, disability, and decreased quality of life.[1,3] Disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate, hydroxychloroquine, prednisone, leflunomide, and sulfasalazine have been shown to reduce systemic inflammation, slow joint damage, and improve function in RA patients.[1]

Tumor necrosis factor-alpha (TNF-α) plays a vital role in the development and progression of RA as a driver of inflammation.[4,5] Previous studies suggested that high level of TNF-α was positively correlated with disease activity, and TNF-α blocking therapy significantly affected disease activity and improved clinical outcome

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in patients with RA.[4,6] TNF-α and C-reactive proteins (CRP) have close correlation since inflammation could induce the production of CRP.[5] A study found that the production of CRP was induced by pro-inflammatory cytokines and could be potential inflammatory marker in RA patients.[7,8] Another study found the levels of high sensitivity CRP (hs-CRP) and TNF-α were higher in RA patients compared to healthy population.[6]

DAS28 is a modified Disease Activity Score (DAS), the scoring system used to evaluate the disease activity of RA, which includes 28 joint counts.[9] The modified DAS was proven as valid as the original version and more convenience to be used in clinical settings.[10] The calculation of DAS28 score is composed of the number of tender and swollen joints, patient's assessment of disease activity on a visual analogue scale (VAS), and erythrocyte sedimentation rate (ESR).[9-12] The recent guideline for RA recommends the use of composite measurements such as DAS28 to evaluate disease activity in combination with inflammatory markers such as CRP and TNF-α for better prognostic power.[13] However data on correlation of the plasma level of TNF-α, hs-CRP and DAS28 in RA patients is limited. The objective of this study was to evaluate the correlation of plasma level of TNF-α and serum level of hs-CRP with DAS28 in order to determine their diagnostic potency as markers of RA disease activity.

Methods

Study design and patients

A cross sectional study was conducted at Dr. Soetomo Hospital, Surabaya. Patients at the Rheumatology Outpatient Clinic who were diagnosed with RA based on ACR 1987 criteria[14] and aged >16 years were recruited. Patients who had local or systemic inflammation (based on history, physical examination, and had white blood count more than $11x10^3/\mu l$ or less than $4x10^3/\mu l$), autoimmune disease, malignancy, irritable bowel disease, severe heart disease, diabetes, or end stage renal disease were excluded.

TNF-α and hs-CRP measurements

Peripheral blood samples were collected for measurements of TNF-α and hs-CRP and ESR, a component of DAS28 assessment. The level of plasma TNF-α was measured by quantitative Enzyme-Linked Immunosorbent Assay (ELISA) kits (Diaclone SAS, France). Serum level of hs-CRP was measured by solid-phase chemiluminescent immunometric assay using an IMMULATE® High Sensitivity CRP by DPC®.

Disease Activity Assessment

The degree of RA disease activity (severity) was measured using DAS28 questionnaire, which includes the 28 tender and swollen joint counts, ESR, and the patients' assessment of disease activity.[15] The 28 tender joint count (28TJC) and 28 swollen joint count (28SJC) range from 0 to 28, erythrocyte sedimentation rate (ESR) ranges from 0 to 150, and patients' general health assessment (GH) ranges from 0 to 100. The formula for DAS28 score calculation has been explained elsewhere.[15] The DAS28 scores range from 0 to 9.4, and was categorized into four: (1) DAS28 <2.6 for remission; (2) DAS28 ≥2.6 and ≤3.2 for low disease activity; (3) DAS28 >3.2 and ≤5.1 for moderate disease activity; and (4) DAS28 >5.1 indicates high disease activity.[15]

Statistical Analysis

All the descriptive variables were presented as the mean ± standard deviation (SD). The levels of TNF-α and hs-CRP between disease activity groups were compared using Anova followed by post hoc analyses. The correlations of TNF-α and hs-CRP levels and DAS28 were calculated using Pearson's or Spearman correlation as appropriate. Statistical Package for the Social Sciences (SPSS 20 version) was used for all statistical analyses.

Results

Participants' characteristics

The present study included new 31 RA patients, recruited from the Rheumatology Outpatient Clinic of Dr. Soetomo Hospital, Surabaya, Indonesia. Majority of the patients (87.1%) were women, with mean of age 45.6 years (**Table 1**). The mean of disease onset was 66.1 months and approximately half of the patients (48.4%) had AR for 6 months to 2 years. Twenty-two patients (70.9%) were treated with disease modifying anti-rheumatic drugs (DMARDs) such as chloroquine and methotrexate, where half of them (50%) had taken DMARDs for 6 months to 2 years (**Table 1**).

Disease severity and the levels of TNF-α and hs-CRP

Based on DAS28 score, 14 (45.2%) patients were categorized as high disease activity (severe) and 12 (38.7%), 3 (9.6%) and 2 (6.4%) patients were categorized as moderate activity, low activity and remission stage, respectively (**Table 1**). One of the DAS28 variables is ESR and the ESR was varied between 15 to 137 mm/hour among patients in this study. The highest mean level of TNF-α was among those with high disease activity, while the lowest mean level was recorded in patients with remission and low disease activity. The mean level of TNF-α in patients with high disease activity was 46.4 pg/ml and this level was significantly higher compared to those with moderate (32.5 pg/ml) and low disease activity (19.1 pg/ml) (**Table 2**). The level of TNF-α levels were significant different among the disease activity groups (p=0.034) (**Table 2**). Post hoc analyses also suggested that the level of TNF-α levels were significant different between disease activity stratifications (high *vs.* moderate with p=0.012 and high *vs.* low with p=0.036).

The levels of hs-CRP in the present study ranged from 0.20 mg/L to 109 mg/L. Those with high activity of RA had the highest level of hs-CRP compared to other groups. One-way Anova indicated that the levels hs-CRP were not different among the groups of disease severity (**Table 2**). Post hoc analyses also suggested that the levels of hs-CRP were not different between disease activity group.

Table 1. Participants' characteristics (n = 31)

Characteristics	Frequency (n, %)
Gender	
Male	4 (12.9)
Female	27 (87.1)
Age (mean, year)	45.61
Onset of disease (mean ± SD, months)	66.14 ± 73.67
Duration of disease	
< 6 months	4 (12.9)
> 6 months – 2 years	15 (48.3)
> 2 – 5 years	4 (12.9)
> 5 years	8 (25.8)
Treatment	
DMARD	22 (70.9)
Non-DMARD	9 (29.1)
Disease activity	
High	14 (45.2)
Moderate	12 (38.7)
Low	3 (9.6)
Remission	2 (6.4)

Table 2. Comparation of ESR, TNF-α and hs-CRP among different disease activities of rheumatoid arthritis

Markers	Disease activity				p-value a
	Remission Mean ± SD (Range)	Low Mean ± SD (Range)	Moderate Mean ± SD (Range)	High Mean ± SD (Range)	
ESR (mm/hour)	19.00±8.40 (15-25)	30.00±5.00 (25-35)	47.16±13.30 (27-62)	46.50±14.93 (15-137)	0.052
TNF-α (pg/ml)	29.50±31.26 (7.40-51.61)	7.39±40.03 (19.15-18.12)	32.55±14.77 (11.64-55.68)	46.49±14.93 (12.13-79.30)	0.034*
hs-CRP (mg/L)	2.93±0.30 (2.72-3.15)	1.15±1.03 (0.24-2.27)	6.37±9.33 (0.20-34)	12.20±29.06 (0.29-109)	0.787

[a] Analyzed using one-way Anova

* Significant at p=0.05

Correlation between TNF-α and hs-CRP with DAS28

Pearson's correlation test showed a significant correlation between the level of TNF-α and DAS28 score (*r*=0.417, p=0.02) (**Table 3**). This study found no significant correlation between the level of hs-CRP and DAS28 score in RA patients (r= -0.117, p=0.532). The present study also found no significant correlation: between TNF-α and hs-CRP (*r*=0.100 and p=0.592), between TNF-α and ESR (*r*=0.118, p=0.532), and between hs-CRP and ESR (*r*=0.256, p=0.161).

Table 3. Correlation between TNF-α, hs-CRP and DAS28 in RA patients

Correlation	r	p-value
TNF-α-DAS28	0.417	0.020 a,*
hs-CRP-DAS28	-0.117	0.532 b
TNF-α-hs-CRP	0.100	0.592 b
TNF-α-ESR	0.118	0.532 a
hs-CRP-ESR	0.256	0.161 b

[a] Analyzed using Pearson correlation

[a] Analyzed using Spearman correlation

* Significant at p=0.05

Discussion

Majority of the RA patients in this study (87.1%) were female with mean of age 45.6 years. Previous studies showed that RA may affect all ages, but is usually seen in young women aged 25-45[1,2,16] and only about 2% of the geriatric population.[17] RA mostly affects women, where the sex ratio is about 3:1 with women having higher odds to get the disease.[1,16] Many autoimmune diseases, including RA, show a striking imbalance between the genders, with females having higher prevalence. Studies suggest that genetic factors (X-linked), hormonal factors, and different exposures are likely to influence the prevalence of autoimmune diseases and their severity.[16,18,19]

Our data suggested that the level TNF-α was significantly different among disease activity groups in which the higher disease activity the higher TNF-α level. A previous study also found a correlation between TNF-α levels and disease activity that measured by DAS28.[6] CRP and TNF-α are two of important inflammatory markers in RA and TNF-α plays an important role in activating inflammation cascade such as pro-inflammatory cytokines and then induce the production of CRP[4,5].

When we calculated the correlation between TNF-α and DAS28, our data suggested a significant positive correlation between the TNF-α and DAS28 score, supporting previous studies which suggested high level of TNF-α was positively correlated with DAS28 score.[4,6] TNF-α could induce the release of prostaglandins, reactive oxygen, and neutral proteinases such as collagenases and stromelysin that could degrade proteoglycans, resulting in destruction of cartilage.[6] Furthermore, TNF-α stimulates the release of tissue-destroying matrix metalloproteinase and inhibits the production of metalloproteinase inhibitors which also lead to joint damages.[20]

Although previous studies found that the higher levels of TNF-α and CRP reflected more rapid progression of the disease,[4,5] our data suggested a weak correlation between hs-CRP and RA disease activity. This finding is different from previous studies that reported a significant correlation between hs-CRP levels and disease activity in RA patients.[21-23] Studies found that the serum level of CRP was correlated with subjective (pain, morning stiffness and fatigue after walking), semi-objective (articular index, grip strength), and clinical parameters of RA disease activity.[5,24] Moreover, CRP level also demonstrated a good prognostic value of progressive joint damage, functional status, and outcome of RA.[5,24] One of the possible reasons we found a weak correlation probably because a small sample size in our present study.

This study has some limitations. The sample size in this study was relatively small leading to less proportional for each disease activity. A study with higher sample size and more proportional samples for each disease category is therefore necessary to be conducted in the future. The present study only assessed TNF-α and hs-CRP. Further study with other pro-inflammatory cytokines such as IL-6 and IL-10 is warranted, since those cytokines could also affect disease activity in RA. Similar study using TNF-α and hs-CRP from synovial fluid needs to be done in the future, to further evaluate the role of pro-inflammatory cytokines as local mediators in causing joint damages.

Conclusion

The level of TNF-α was statistically significant between RA disease activity groups (high vs. moderate; and high vs. low). There was a significant positive correlation between the TNF-α and DAS28 score and the level of TNF-α is associated with DAS28 score, suggesting its potential as a marker of RA disease activity or severity.

Acknowledgement: We thank Narra Studio Jurnal for its assistance during manuscript preparation.

Ethical Clearance: The protocol of the study was approved by the Institutional Ethics Committee, Faculty of Medicine, Universitas Airlangga. Written informed consents were obtained from all patients prior to the enrolment.

Source of Funding: Self

Conflict of Interest: Nil

References

1. Milman N, Karsh J, Booth RA. Correlation of a multi-cytokine panel with clinical disease activity in patients with rheumatoid arthritis. *Clin Biochem.*

2010;43(16-17).

2. Kobak S, Bes C. An autumn tale: geriatric rheumatoid arthritis. *Ther Adv Musculoskelet Dis.* 2018;10(1):3-11.
3. Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. *Lancet.* 2010;376(9746):1094-1108.
4. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res.* 2001;89(9):763-771.
5. Emery P, Gabay C, Kraan M, Gomez-Reino J. Evidence-based review of biologic markers as indicators of disease progression and remission in rheumatoid arthritis. *Rheumatol Int.* 2007;27(9):793-806.
6. Shrivastava AK, Singh HV, Raizada A, et al. Inflammatory markers in patients with rheumatoid arthritis. *Allergol Immunopathol (Madr).* 2015;43(1):81-87.
7. Nielen MMJ, Schaardenburg Dv, Reesink HW, et al. Increased levels of C-reactive protein in serum from blood donors before the onset of rheumatoid arthritis. *Arthritis Rheum.* 2004;50(8):2423-2427.
8. Singh HV, Shrivastava AK, Raizada A, et al. Atherogenic lipid profile and high sensitive C-reactive protein in patients with rheumatoid arthritis. *Clin Biochem.* 2013;46(12):1007-1012.
9. Prevoo ML, Hof MAvt, Kuper HH, et al. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum.* 1995;38(1):44-48.
10. Matsui T, Kuga Y, Kaneko A, et al. Disease Activity Score 28 (DAS28) using C-reactive protein underestimates disease activity and overestimates EULAR response criteria compared with DAS28 using erythrocyte sedimentation rate in a large observational cohort of rheumatoid arthritis patients in Japan. *Ann Rheum Dis.* 2007;66(9):1221-1226.
11. Gestel AMv, Prevoo ML, Hof MAvt, et al. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis Rheum.* 1996;39(1):34-40.
12. Prevoo ML, Gestel AMv, Hof MAvT, et al.. Remission in a prospective study of patients with rheumatoid arthritis. American Rheumatism Association preliminary remission criteria in relation to the disease activity score. *Br J Rheumatol.* 1996;35(11):1101-1105.
13. Hirata S, Dirven L, Shen Y, et al. A multi-biomarker score measures rheumatoid arthritis disease activity in the BeSt study. *Rheumatology.* 2013;52(7):1202-1207.
14. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 1988;31(3):315-324.
15. Fransen J, Stucki G, Riel PLCMv. Rheumatoid Arthritis Measures. *Arthritis & Rheumatism.* 2003;49(5S):S214-224.
16. Vollenhoven RFv. Sex differences in rheumatoid arthritis: more than meets the eye. *BMC Med.* 2009;7(12).
17. Laiho K, Tuomilehto J, Tilvis R. Prevalence of rheumatoid arthritis and musculoskeletal diseases in the elderly population. *Rheumatol Int.* 2001;20(3):85-87.
18. Olsen NJ, Kovacs WJ. Hormones, pregnancy, and rheumatoid arthritis. *J Gend Specif Med.* 2002;5(4):28-37.
19. Wilder RL. Hormones, pregnancy, and autoimmune diseases. *Ann N Y Acad Sci.* 1998;840:45-50.
20. Rahman EMA, Ezzat H, Mohsen MMA, Yosef K. Interleukin-10 Interleukin-16 and Interferon- γ in serum of patients with rheumatoid arthritis and correlation with disease activity. *Egypt J Hosp Med.* 2005;20:46-57.
21. Dessein PH, Joffe BI, Stanwix AE. High sensitivity C-reactive protein as a disease activity marker in rheumatoid arthritis. *J Rheumatol.* 2004;31(6):1095-1097.
22. Skogh T, Gustafsson D, Kjellberg M, Husberg M. Twenty eight joint count disease activity score in recent onset rheumatoid arthritis using C reactive protein instead of erythrocyte sedimentation rate. *Ann Rheum Dis.* 2003;62(7):681-682.
23. Shrivastava AK, Pandey A. Inflammation and rheumatoid arthritis. *J Physiol Biochem.* 2013;69(335-47).

24. Machold KP, Stamm TA, Nell VPK, et al. Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. *Rheumatology (Oxford)*. 2007;46(2):342-349.