INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 239
2023 July; 29(3): 239 - 244
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

SAA as Inflammatory Marker in Rheumatoid Arthritis: Study on Standard Therapy and Moringa Extract

Yan Ajie Nugroho, B. Rina A. Sidharta, Lusi Oka Wardhani, JB. Suparyatmo, MI. Diah Pramudianti

Department of Clinical Pathology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi General Hospital, Surakarta, Indonesia.
E-mail: dj_ajie354@yahoo.com

ABSTRACT

Rheumatoid arthritis is a chronic systemic inflammatory autoimmune disorder characterized by persistent joint inflammation leading to cartilage and bone damage, disability, and systemic complications. The levels of APR such as SAA serum increase during synovitis. Previous studies have demonstrated the anti-inflammatory effect of *M.oleifera* leaf extract in the treatment of RA in animals; however, research data on humans remain limited. An experimental study on pre- and post-treatment of 40 RA patients was carried out by dividing subjects into 2 groups, including a standard therapy group and a standard therapy group added with *M.oleifera* leaf extract. The research was conducted at Dr. Moewardi Hospital, Surakarta from October 2020 to January 2021. The SAA levels were measured using ELISA. Paired T-test was used to analyze the differences in mean SAA levels before and after treatment. There was a significant difference between pre-treatment (346.57±54.40 ng/mL) and post-treatment (314.77±37.40 ng/mL) SAA levels in the standard therapy group added with *M.oleifera* leaf extract with p=0.01. Pre-treatment and post-treatment SAA levels in the standard therapy group were 322.68±87.01 ng/mL and 302.93±86.51 ng/mL, respectively with p=0.04. The mean of delta SAA in the standard therapy group added with *M.oleifera* leaf extract (-31.81±4.04 ng/mL) was greater than delta SAA in the standard therapy group (-19.75±4.07 ng/mL) with p=0.26. There was a significant decrease in SAA levels in RA patients on standard therapy and *M. oleifera* leaf extract.

Keywords: Rheumatoid arthritis, *Moringa oleifera*, SAA

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic systemic inflammatory autoimmune disease characterized by persistent joint inflammation, which causes cartilage and bone damage, disability and can lead to systemic complications. The development of this disease can cause functional loss, reduced quality of life, and increased morbidity and mortality.[1] According to Basic Health Research (Riskesdas) data in 2018, the prevalence of RA in Indonesia and Central Java was 7.30% and 6.78%, respectively.[2] There were 1180 cases of RA in the Rheumatology Outpatient Clinic at Dr. Moewardi (RSDM) Hospital Surakarta in 2019.

Although the specific cause of RA remains unknown, several hypotheses have been put forward, involving autoimmune mechanisms, super antigen-driven disease, and infectious stimuli. The etiology and pathogenesis of RA remain a mystery; however, more attention has been focused on the cellular and molecular mechanisms of the disease. The pathogenesis of RA involves the infiltration and activation of various cell populations and the release of many inflammatory and destructive mediators including cytokines, prostaglandins, and metalloproteinases.[3]

Various contents of *M.oleifera* have been reported to have anti-inflammatory and anti-rheumatic activities, one of which comes from its glycoside content. Anti-inflammatory activity in *M.oleifera* leaves is mediated by the inhibition of inflammatory signaling pathways. The role of leaf, root, and pod extracts in the treatment of inflammation such as cancer, asthma, allergic rhinitis, atopic dermatitis, and RA has also been studied.[4] *Moringa oleifera* exhibits inhibitory effects on Nitric Oxide (NO), Vascular Endothelial Growth Factor (VEGF), Tumor Necrosis Factor a (TNF-α), Interleukin (IL) 2, IL-1ß, IL-6, glucose-6-phosphate, insulin resistance, leptin, resistin and cholesterol.[5]

Serum levels of Acute-Phase Reactants (APR) such as CRP and serum amyloid A (SAA) are increased during active synovitis. Elevated APR levels are significantly associated with joint damage and disability on long-term follow-up. The measurement of serum APR is used as an indicator of disease severity, progression, and prognosis in RA. Serum

amyloid A and CRP have many advantages compared to ESR, which is strongly influenced by age and gender and has a slow response to changes.[6]

Several studies have shown that SAA is a more sensitive marker of inflammation compared to CRP because elevated SAA can sometimes be detected even when CRP levels are normal. Serum amyloid A can reach higher levels than CRP and decrease rapidly, making it a more accurate indicator of disease activity.[7] Serum amyloid A levels can increase rapidly within 8–12 hours, reaching a peak level after 24 hours with a short half-life of 24–36 hours.[8,9] In contrast to LED or CRP, little is known about SAA and its clinical use in RA. There have been no studies on the use of SAA to evaluate *M.oleifera* therapy. Previous studies have shown the anti-inflammatory effect of *M.oleifera* leaf extract in the treatment of RA in experimental animals; however, research data in humans remain limited.

METHODS

This research was an experimental study with a double-blind Randomized Controlled Trial (RCT) method; research subjects and authors did not know the treatment or intervention given. Initial measurements (pre-test) and final measurements (post-test) were carried out. The research was conducted at the Clinical Pathology Laboratory, Dr. Moewardi (RSDM) Hospital Surakarta from October 2020 to January 2021. Research related to providing therapy was not carried out in the laboratory but was carried out by Internal Medicine Doctors in the Internal Medicine Outpatient Clinic, Sub-Department of Rheumatology. The population was patients with a diagnosis of RA who sought treatment at the Internal Medicine Outpatient Clinic, Sub-Division of Rheumatology. The research subjects were divided into 2 groups, such as a group of RA patients with standard therapy and a group of RA patients with standard therapy added with *M.oleifera* leaf extract. Methotrexate at a dose of 7.5 mg/week was used as standard therapy. *M.oleifera* leaf extract in 500 mg capsules given 2x2 capsules/day for 28 days was given as additional therapy. The inclusion criteria for research subjects included age >18-72 years, meeting the 2010 ACR/EULAR criteria, and agreeing to participate in the study by signing informed consent. Exclusion criteria in this study were pregnant patients, post-surgery patients, patients taking non-steroidal anti-inflammatory drugs (NSAIDs), patients receiving intra-articular injections, patients with comorbidities: diabetes mellitus, liver disease, infection, malignancy, trauma, Alzheimer's, coronary heart disease and chronic kidney disease, patients whose history of *M.oleifera* allergy and hypotension. Drop-out criteria were patients who forgot to take the medication, patients who refused to continue the study, drug side effects or allergic reactions occurred and the patient died. The serum was used as a sample for laboratory tests. Serum amyloid A levels were measured using the ELISA method with the Rayto RT-2100C microplate reader instrument.

Characteristics of the research subjects were presented in a table containing the mean and standard deviation if data were normally distributed and in a median, minimum and maximum if data were not normally distributed. The Shapiro-Wilk test was used to determine the normality of the data. An unpaired T-test was used to analyze the mean difference between the control group (standard therapy) and treatment (standard therapy and *M.oleifera* leaf extract supplements). Paired sample T-test was used to analyze the difference between 2 mean SAA variables before and after treatment with *M.oleifera* extract. Data were processed using a computer with a significant $p < 0.05$. This research has been approved by the Biomedical Research Ethics Commission with number 1.331/XII/HERC 2020.

RESULTS AND DISCUSSIONS

A total of 42 patients who met the inclusion and exclusion criteria were divided into 2 groups by randomization of the treatment prior to measurement of pre-treatment and post-treatment SAA levels. There were 2 patients who dropped out because they were not willing to continue the study, resulting in a total of 40 patients. The basic characteristics of the research subjects are presented in Table 1.

Table 1 shows that the research subjects were dominated by females with a median age of 44.50 (20-64) years in the standard therapy group, lower than that of the standard therapy group added with *M.oleifera* leaf extract, with a median age of 49.50 (18-63) years. There were no significant differences in the characteristics of age, gender, ESR, and hs-CRP levels between both groups.

Table 2 shows that there was no significant difference in the mean pre-treatment and post-treatment SAA levels in the standard therapy group and a group of standard therapy added with *M.oleifera* leaf extract.

A significant difference in pre-treatment and post-treatment SAA levels between the standard therapy group ($p=0.04$) and a group standard therapy added with *M.oleifera* leaf ($p=0.01$) (Table 3).

Table 1. General characteristics of research subjects

Variable	Standard Therapy n=20		Standard Therapy+*M.oleifera* n=20		P
	n (%)	Median	n (%)	Median	
Gender					
Male	2 (10%)		3 (15%)		0.5
Female	18 (90%)		17 (85%)		0.5
Age (years)		44.50 (20-64)*		49.50 (18-63)*	0.76
ESR (mm/hour)		29 (2-86)*		11.5(2-97)*	0.11
hs-CRP (mg/dL)		0.32 (0.01-2.98)*		0.11(0.02-5.86)*	0.13

Abbrev:*: abnormal distribution, [Median (min-max)], Mann-Whitney test, n: total of research subjects, min-max: minimum-maximum, ESR: Erythrocyte Sedimentation Rate, hs-CRP: high sensitive C-Reactive Protein, RA: Rheumatoid Arthritis, p<0.05 was significant, CI=Confidence Interval of 95%

Table 2. The difference in SAA Levels between a standard therapy group and a group of standard therapy with *M.oleifera* leaf extract

Variable	Group		P
	Standard Therapy Mean±SD	Standard Therapy+*M.oleifera* Mean±SD	
Pre-treatment SAA (ng/mL)	322.68±87.01*	346.57±54.40*	0.31
Post-treatment SAA (ng/mL)	302.93±86.51*	314.77±37.40*	0.58

Abbrev: Mean value* = normal distribution, unpaired T-test, SD: standard deviation, SAA: Serum Amyloid A, p<0.05 was significant, CI=Confidence Interval of 95%

Table 3. The difference in pre-treatment and post-treatment SAA levels in a standard therapy group and a group of standard therapy with *M.oleifera* leaf extract

Group	SAA levels (ng/mL)		p
	Pre-Treatment Mean±SD	Post-treatment Mean±SD	
Standard therapy	322.68±87.01*	302.93±86.51*	0.04
Standard therapy added with *M.oleifera* leaf extract	346.57±54.40*	314.77±37.40*	0.01

Abbrev: Mean value*= normal distribution, paired T-test, SD: Standard Deviation, SAA: Serum Amyloid A, p<0.05 was significant, CI=Confidence Interval of 95%

Table 4. Difference of mean delta SAA levels between the standard therapy group and a group of standard therapy added with *M. oleifera* leaf extract

Variable	Group		P
	Standard Therapy Mean±SD	Standard Therapy+*M.oleifera* Mean±SD	
Delta SAA (ng/mL)	-19.75±4.07	-31.81±4.04	0.26

Abbrev: Mean value*= normal distribution (mean±SD), independent sample T-test, min-max= minimum-maximum, SAA: Serum Amyloid A, SD: Standard Deviation, p<0.05 was significant, CI=Confidence Interval of 95%

Table 4 shows that there was no significant difference in the mean delta SAA in the standard therapy group and a group of standard therapy added with *M.oleifera* leaf extract.

Rheumatoid arthritis disease occurs at all ages, but it is most commonly found at the ages of 40-70 years.[10] Mean age of RA patients in this study was 45.20±10.13 years in the standard therapy group and 44.0±14.64 years in a group of standard therapy group added with *M. oleifera* leaf extract. The peak of RA incidence takes place during the fifth decade of life, around the age of menopause in females;

however, in ~ 50% of patients, the disease starts during the reproductive years.[11]

The research subjects in this study were dominated by females. According to Favalli *et al.*, the incidence of RA was much more common in females than males with a ratio of 3:1; however, the mechanism by which gender affects vulnerability to RA remains unclear.[12] It is suspected that differences in sex hormones are the main cause. The peak age of onset of RA is the fifth decade when hormonal changes occur in females.[11]

The median ESR value in the standard therapy group was 29 (2-86) mm/hour and in the standard therapy group added with *M. oleifera* leaf extract was 11.5 (2-97) mm/hour. In a previous study, Lezcano *et al.* reported a median ESR of 23 (14-36.5) mm/hour from 1.461 RA patients studied.[13] Erythrocyte sedimentation rate is the most widely used marker of inflammation in RA and is an indirect marker of inflammation, which indicates acute phase plasma protein levels in the blood (e.g. fibrinogen) due to its ability to cause rapid precipitation of red blood cells. However, this marker of inflammation has several limitations. Although this test is relatively easy and inexpensive to perform, the ESR responds slowly to inflammatory stimuli and changes in disease activity. The erythrocyte sedimentation rate is a non-specific acute phase reactant of systemic inflammation; high levels of ESR are not necessarily due to inflammation in rheumatic diseases alone. It has been shown that ESR levels can be greatly affected by infection, malignancy, abnormally shaped or sized red blood cells, and serum protein concentration. Erythrocyte sedimentation rate levels tend to be higher in females than males and increase with age and BMI.[6,14]

The median hs-CRP in the standard-therapy group was 0.32 (0.01-2.98) mg/dL and the median hs-CRP in the standard-therapy group added with *M. oleifera* was 0.11 (0.02-5.86) mg /dL. These results were similar to previous studies by Hwang *et al.*, which found that the average CRP level of RA patients was 1.4±2.31 mg/dL, and a study by Ito *et al.*, which found a median CRP of 0.09 (0.03-0.30) mg/dL.[9,15] C-reactive protein assay has been suggested as an alternative inflammatory marker of RA disease activity. Many studies tend to prefer CRP over ESR in assessing RA inflammation because it is presumed to provide a better indication of disease activity than ESR. C-reactive protein responds to increased or decreased inflammatory stimuli more rapidly than ESR. Another advantage is that CRP is less influenced by external confounding factors such as age and gender compared to ESR. The difference between ESR and CRP values may be due to the influence of blood constituents that are not associated with inflammation but can interfere with ESR.[6,16]

The average SAA in this study was higher than the average SAA of RA patients in a study by Chao *et al.*, which was 24.3 ng/mL.[17] The difference in these results might be due to differences in test methods and differences in population characteristics such as race, environment, and habits.[18] Increased SAA levels have been reported in a number of inflammations including RA. The main biological function of SAA is unknown, but a number of biological activities can be explained, including mechanisms of inflammation and tissue damage in arthritis. Serum amyloid A induces migration, adhesion, and infiltration of monocytes and polymorphonuclear leukocytes from circulation into the tissues. In addition, SAA can induce the release of IL-1β, IL-1 receptor antagonists, and the production of type II TNF receptors by monocytes and can stimulate the production of cartilage-destroying proteases. A significant correlation between SAA, diagnosis of RA, activity of RA, and assessment of treatment response in RA has been reported.[7-9,19]

Negative results in mean delta SAA levels in this study indicated that there was a decrease in SAA levels after treatment, thereby suggesting that both standard therapy and standard therapy added with *M.oleifera* leaf extract supplements can provide benefits in reducing inflammation in RA patients, which caused no significant difference between both groups. This might be caused by the heterogeneity in the severity of disease of the research subjects, refusal in consumption of *M.oleifera* leaf extract supplements, insufficient dosage of *M.oleifera*, or inadequate time of administration of *M.oleifera* therapy.

A decrease in SAA levels can be useful in predicting response to therapy. Hwang *et al.* in their study found that SAA levels were moderately correlated with CRP levels in RA patients (rho = 0.58, p <0.0001). There was a significant difference in SAA levels at the visit (p=0.0197) and after treatment (p=0.0130). RA patients treated with etanercept, and methotrexate had a greater reduction in SAA compared to patients treated with standard Disease-Modifying Antirheumatic Drugs (DMARD) therapy.[9] A study by Charles-Schoeman *et al.*, which investigated the effect of tofacitinib and other DMARDs on lipid profiles in rheumatoid arthritis found that the mean SAA concentrations decreased

significantly in 6 weeks of tofacitinib therapy.[20]

The results of this study were also similar to previous studies by Shailaja *et al*., which determined the anti-arthritic activity of the ethanol extract of *M.oleifera* seeds in adult female Wistar arthritis rats. It was found that serum levels of inflammatory markers TNF-α, IL-1, and IL-6 decreased when compared to the control group. Treatment with *M.oleifera* altered oxidative stress in relation to anti-inflammatory activity.[5] Studies using animal models have demonstrated the anti-inflammatory activity of *M.oleifera*. Glucosinolates and isothiocyanates compounds are thought to play a role in exerting this anti-inflammatory effect. Glucosinolates are organic compounds containing sulfur and nitrogen derived from glucose and amino acids. This substance is known to have a strong inhibitory effect on the production of NO. The isothiocyanate content in *M.oleifera* was also found to reduce insulin resistance, leptin, resistin, cholesterol, IL-1ß, and TNF-α in rats. Isothiocyanate compounds are the main bioactive ingredients that have potent anti-inflammatory activity in *M.oleifera*.[5,21]

Pandey *et al*. observed the anti-arthritic potential of *M.oleifera* in Complete Freund's Adjuvant (CFA) induced arthritis animal model and found that oral treatment of *M.oleifera* at doses of 25, 50, and 100 mg/kg significantly ($p<0.001$) down-regulated joint inflammation as evidenced via a reduction in the joint diameter, arthritic score and inflammatory cell infiltration.[22]

A study by Mahdi *et al*., which analyzed several rheumatoid parameter tests suggested that *M.oleifera* leaf extract has a considerable effect in preventing the development or improving the severity of arthritis. *M.oleifera* leaf extract showed significant anti-nociceptive activity in normal and arthritic rats.[23]

This study found increased SAA levels as a marker of inflammation in RA patients and decreased SAA levels after therapy both with standard therapy and with supplements of *M.oleifera* leaf extract at a dose of 2x500 mg capsules for 28 days. The results of this study proved that SAA as an anti-inflammatory marker can be used to assess the effectiveness of RA patient therapy.

CONCLUSIONS AND SUGGESTIONS

There was a significant decrease in SAA levels in RA patients both with standard therapy and those with *M.oleifera* leaf extract supplements. Serum amyloid A as an anti-inflammatory marker can be used to assess the effectiveness of therapy in RA patients. It is necessary to emphasize the factors, which can affect the test results and SAA levels.

REFERENCES

1. Calabresi E, Petrelli F, Bonifacio AF, Puxeddu I, Alunno A. One year in review 2018: Pathogenesis of rheumatoid arthritis. Clinical and Experimental Rheumatology, 2018; 36(2): 175-184.
2. Kemenkes. Riset Kesehatan Dasar (Riskesdas). Jakarta, Balitbang Kemenkes RI, 2018; 176.
3. Rathore H, Rathore B, Singh S. Rheumatoid arthritis: A chronic inflammatory disease and its pathophysiology. Era's Journal of Medical Research, 2019; 6(1): 88-92.
4. Gupta S, Jain R, Kachhwaha S, Kothari SL. Nutritional and medicinal applications of *Moringa oleifera* lam-review of current status and future possibilities. Journal of Herbal Medicine, 2018; 11: 1-11.
5. Waterman C, Rojas?Silva P, Tumer TB, Kuhn P, Richard AJ, *et al*. Isothiocyanate rich *Moringa oleifera* extract reduces weight gain, insulin resistance, and hepatic gluconeogenesis in mice. Molecular Nutrition & Food Research, 2015; 59(6): 1013-1024.
6. Harrison, Michael. Abnormal laboratory results: Erythrocyte sedimentation rate and C-reactive protein. Australian Prescriber, 2015; 38(3): 93.
7. Hwang YG, Balasubramani GK, Metes ID, Levesque MC, Bridges SL, Moreland LW. Differential response of serum amyloid A to different therapies in early rheumatoid arthritis and its potential value as a disease activity biomarker. Arthritis Research & Therapy, 2016; 18(1): 108.
8. De Buck M, Gouwy M, Wang JM, Snick JV, Opdenakker G, *et al*. Structure and expression of different Serum Amyloid A (SAA) variants and their concentration-dependent functions during host insults. Current Medicinal Chemistry, 2016; 23(17): 1725-1755.
9. Zhang Y, Zhang J, Sheng H, Li H, Wang R. Acute phase reactant serum amyloid A in inflammation and other diseases. Advances in Clinical Chemistry, 2019; 90: 25-80.
10. Brijesh R. Rheumatoid arthritis: An understanding in the light of herbal medicines. Era's Journal of Medical Research, 2018; 5(1): 45-58.
11. Rodríguez DA, Pluchino N, Canny G, Gabay C, Finckh A. The role of female hormonal factors in the development of rheumatoid arthritis. Rheumatology, 2017; 56(8): 1254-1263.
12. Favalli EG, Biggioggero M, Crotti C, Becciolini A, Raimondo MG, Meroni PL. Sex and management of rheumatoid arthritis. Clinical Reviews in Allergy & Immunology, 2019; 56: 333-345.
13. Valverde JML, Salazar F, León L, Toledano E, Jover JA, *et al*. Development and validation of a multivariate predictive model for rheumatoid arthritis mortality using a machine learning approach. Scientific Reports, 2017; 7(1): 1-10.

14. Castro VA, Sampedro MA, Temprano NV, Tünes C, Rey D, *et al*. Factors influencing erythrocyte sedimentation rate in adults: New evidence for an old test. Medicine, 2019; 98(34): E16816.
15. Ito H, Ogura T, Hirata A, Takenaka S, Mizushina K, *et al.* Global assessments of disease activity are age-dependent determinant factors of clinical remission in rheumatoid arthritis. Seminars in Arthritis and Rheumatism, 2017; 47(3): 310-314.
16. Bray C, Bell LN, Liang H, Haykal R, Kaiksow F, *et al*. Erythrocyte sedimentation rate and C-reactive protein measurements and their relevance in clinical medicine. WMJ, 2016; 115(6): 317-321.
17. Chao R, Das M, Purat N, Efthimiou P. 14-3-3eta positivity is associated with higher rheumatoid arthritis disease activity measured by multi-biomarker disease activity assay. BMJ, 2018; 77(2): 1326-1327
18. Liu Q, Li Y, Yang F, Xu T, Yao L, Sun J, Liang W. Distribution of serum amyloid A and establishment of reference intervals in healthy adults. Journal of Clinical Laboratory Analysis, 2020; 34(4): e23120.
19. Shen C, Sun XG, Liu N, Mu Y, Hong CC, Wei W, Zheng F. Increased serum amyloid A and its association with autoantibodies, acute phase reactants and disease activity in patients with rheumatoid arthritis. Molecular Medicine Reports, 2015; 11(2): 1528-1534.
20. Schoeman CC, Gay MAG, Kaplan I, Boy M, Geier J, *et al*. Effects of tofacitinib and other DMARDs on lipid profiles in rheumatoid arthritis: Implications for the rheumatologist. Seminars in Arthritis and Rheumatism, 2016; 46(1): 71-80.
21. Fard MT, Arulselvan P, Karthivashan G, Adam SK, Fakurazi S. Bioactive extract from *Moringa oleifera* inhibits the pro-inflammatory mediators in lipopolysaccharide stimulated macrophages. Pharmacognosy Magazine, 2015; 11(4): 556-563.
22. Pandey P, Bhatt PC, Kumar V. *Moringa oleifera* lam ameliorates adjuvant induced arthritis via inhibition of inflammatory mediators and down-regulation of mmp3 and mmp-9 proteins. BMJ, 2017; 75: 32.
23. Mahdi, HJ, Khan NAK, Asmawi MZB, Mahmud R, Vikneswaran A, Murugaiyah L. In-vivo anti-arthritic and anti-nociceptive effects of ethanol extract of Moringa oleifera leaves on complete Freund's adjuvant (CFA)-induced arthritis in rats. Integrative Medicine Research, 2018; 7(1): 85-94.