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Study of the Effects of N-acetylcysteine on Inflammatory Biomarkers and Disease Activity Score in Patients with Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is considered as an autoimmune-related condition in which the overproduction of pro-inflammatory cytokines leads to an inflammatory cascade. N-acetylcysteine (NAC) is a potent anti-inflammatory and anti-oxidant agent. We aimed to explore the impact of oral NAC on cytokines activities and clinical indicators in RA patients.

In this placebo-controlled randomized double-blind clinical trial, 41 active RA patients were allocated in either NAC (600 mg, twice a day) or placebo group, as add-on therapy to the routine regimen, for 8 weeks. Disease activity score with an erythrocyte sedimentation rate (DAS28-ESR), and serum concentrations of interleukin (IL)-1β and IL-17 were assessed at baseline and end of the trial for all participants in the test and control groups.

The reduction of the DAS28-ESR was higher considerably in the NAC group compared to that of the control group. No statistically significant differences were seen in the reduction of IL-1β and IL-17 cytokines between the NAC and control groups. In addition, improvements in the patient global assessment, number of tender joints, number of swollen joints, and the ESR rates were in favor of the NAC group.

Our findings reveal that NAC may have a beneficial effect on all of the clinical features of RA. However, non-significant variations in the IL-1β and IL-17 levels suggest an alternative way of NAC effectiveness without influencing the measured cytokines. Nevertheless, these results need to be confirmed by further investigations.

Keywords: Acetylcysteine; Cytokines; Rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disorder whose exact etiology is unknown.[1] Prevalence of RA is

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estimated to be about 0.5 to 1.0% worldwide, varying between different geographic regions.[2]

It seems that environmental factors start the immune reaction in genetically susceptible persons which leads to the progression of synovitis, as well as joint and bone destruction. These eventually cause morning stiffness, swelling, pain, and inability with a broad spectrum of extra-articular manifestations. Besides, the social, psychosocial, and economic burden of RA on both patients and health systems is considerably high.[3,4]

Immune system stimulation results in the secretion of a great amount of pro-inflammatory cytokines that activate an inflammatory cascade. Interleukin (IL)-1β and IL-17 are the two critical cytokines in this cascade.[5] Overexpression of these two components has been shown in RA and the concentrations are correlated positively with an erythrocyte sedimentation rate (ESR), pain score, and disease severity.[6,7]

IL-1βand particularly IL-17 influence the activity of different cell types including tumor necrosis factor (TNF), IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), matrix metalloproteinase (MMPs), leukemia inhibitory factor (LIF), and nitric oxide (NO), that all lead to the development of inflammatory cascade and eventually destruction of the joints involved in RA patients. Additionally, these two cytokines can instantly stimulate chondrocytes to prevent the synthesis of matrix and induce enzymes that damage cartilage matrix, and thus have extra catabolic effects. In synergistic interplay, IL-17 reinforces the induced inflammation by other cytokines, mostly TNF.[8,9] Therefore, it is expected that inhibition of the activity of these cytokines and pro-inflammatory precursor can lead to relief in the inflammatory process and disease development.

Up to now, there isn't any certain cure for RA, however available guidelines strongly recommend disease-modifying anti-rheumatic drugs (DMARDs) to maximize functional ability through relieving symptoms and retardation or prevention of joint deformity. This may modify the quality of life and may reduce the burden of disease.[10] Although, there is a significant progression in introducing new drugs for the management of RA, nevertheless the existing therapies have limited efficacy and should be used with caution due to their relatively common and serious toxic adverse reactions.[11] Hence, targeting more effective, safer, and economically reasonable alternative treatments for RA has always been a goal for researchers.

Alternative medicine such as supplements is a focus of interest and preferred by many patients since they might have the least harmful effects and beneficial additive effects when prescribed along with conventional treatments.[12] Anti-oxidants, as an adjuvant treatment, are amongst the most preferred therapeutic supplements used to reduce inflammation triggered by oxidative stress. There is growing proof for the positive properties of anti-oxidants on the clinical activity of RA.[13] They present a preventive role against the generation of free radicals.[14]

N-Acetylcysteine (NAC) is a safe, inexpensive, and available nutritional supplement. By oral administration, only 4-10% of the bioavailability of NAC is diminished.[15] NAC reacts directly with free radicals through its sulfhydryl groups.[16] The potent anti-oxidant effect of NAC is because of its role as a precursor of glutathione that is the most important occurring anti-oxidants. According to the literature, NAC is well tolerated in doses up to 2400 mg/day and has no major adverse reactions at the common doses of 600-1200 mg/day. Nausea, vomiting, skin rashes, and stomachache are the common probable adverse effects of oral NAC.[17]

Furthermore, NAC has anti-inflammatory properties by decreasing the activity of pro-inflammatory cytokines. Investigations revealed that NAC impedes the activity of nuclear factor kappa B (NF-Kb) and therefore, can stop the production of cytokines.[18] Some studies have shown that NAC can inhibit the IL-17-induced receptor activator of nuclear factor κ-B ligand (RANKL) in RA patients.[19,20]

Based on the role of inflammation and oxidative stress in the pathogenesis of RA and the anti-inflammatory and anti-oxidant features of NAC, possibly NAC might be an appropriate complementary therapeutic choice, that inhibits the inflammatory process and bone erosion in RA. This trial intended to explore the impact of oral effervescent NAC, as an adjunct treatment, on RA remission indicators and serum levels of IL-1β and IL-17.

MATERIALS AND METHODS

Study Population

A randomized double-blind placebo-controlled clinical trial was carried out in the rheumatology out-

patients clinic of the Loghman-e Hakim University Hospital, Tehran, Iran, from October 2019 to October 2020. The trial protocol was admitted by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.PHARMACY.REC.1398.078) and also recorded at the Iranian Registry of Clinical Trials with number IRCT20190626044030N1. Patients were asked to sign a written informed consent form before entering the trial.

Sample Size Estimation and Study Intervention

Individuals with certain active RA (DAS28-ESR>3.2), classified based on the American College of Rheumatology (ACR) criteria[21] were recruited in our trial. The eligible individuals were randomly allocated into two study groups receiving routine DMARDs regimen plus placebo oral effervescent tablet (Osvah, Iran) (control group), and routine DMARDs regimen plus oral effervescent tablet of NAC, 600mg (Osvah, Iran) twice daily (intervention group).

The sample size of 21 was calculated for each group based on α=0.05, power=80%, and standard deviation of 1.5 for DAS28-ESR. Patients were monitored for eight weeks and the intended outcomes and variables were gathered and recorded at the baseline (day 1) and the endpoint (8 weeks).

Study Outcomes

Patients' demographic data including sex, age, medical and medication history, disease duration, intra-articular *corticosteroid* injection, and history of drug or food allergy was documented. For all patients, clinical outcomes according to the components of the DAS28-ESR were recorded at baseline and the 8 weeks endpoint visits. Blood specimens also were collected at baseline and endpoint for measurement of IL-1β, IL-17, and ESR. Besides, the possible side effects were documented.

We used Disease Activity Score based on 28-joint counts and ESR (DAS28-ESR) as the main clinical outcome. This score includes four main components of "the number of tender joints", "the number of swollen joints, "ESR" and "patient global assessment (PtGA)". The following formula was used to calculate the DAS28-ESR for each patient:

DAS28-ESR = (0.56* sqr(TJC)) + (0.28* sqr (SJC)) + (0.7* ln (ESR)) + (0.014* PtGA)

DAS28-ESR is recommended by the ACR and is a useful criterion in clinical practice to evaluate the disease severity.[22]

Based on the DAS28-ESR, RA patients are assigned into four classes of disease activity: remission (DAS28-ESR<2.6), low (DAS28-ESR<2.6 to 3.2), moderate (DAS28-ESR> 3.2 to 5.1), and high (DAS28-ESR>5.1).[22]

Inclusion and Exclusion Criteria

Adult (age>18 yrs.) male and female RA patients with an activity of disease score of DAS28-ESR>3.2, not taking any complementary medicine, anti-oxidants and supplements interfering with IL-1β and IL-17 activity in the last two months, no recent infection, and body mass index <30 kg/m2 were entered into the trial.

Patients were excluded if they had any autoimmune condition other than RA, biologic agent therapy in the last six months and during the study, any adjuncts antioxidant therapy, any change in routine DMARDs regimen during the study, any addiction to psychotropic agents or opioids, and pregnancy or breastfeeding.

In addition, patients with non-adherence to therapy (to take NAC or placebo in doses less than 80% of the prescribed dose), intolerance to NAC or placebo, uncontrolled hypertension, active gastric ulcer, receiving nitroglycerin, and those with a history of severe skin disorders were excluded.

Laboratory Measurements

Five mL of venous blood was obtained from each participant at baseline and after 8 weeks of intervention. Two mL of blood specimens were used to measure ESR and the residue was centrifuged to separate serum. Samples were kept at –70 °C for measurement of IL-1β and IL-17 concentrations applying high-sensitivity sandwich enzyme-linked immunosorbent (ELISA) test (Human IL-1β and IL-17 ELISA kit; Zellbio (GmbH, Germany)), according to the company's instruction.

Statistical Analysis

For statistical analysis, the Chi-square and the Fisher's exact tests were used for qualitative data; and the Student's t-test and the Mann-Whitney U-test were applied for quantitative data with normal and non-normal distributions, respectively. For within-group

analyses, the paired t-test (for data with normal distribution) and the Related-Samples Sign test (for data with non-normal distribution) were applied. p value<0.05 was considered as a significance level.

RESULTS

As shown in Figure 1, 41 patients (22 and 19 patients in the intervention and control groups, respectively) finished the study. Table 1 represents that there were not any statistically significant differences in demographic features, baseline medical and medication history, as well as biochemical parameters among the two groups. Also, no significant differences were found in DAS28-ESR and its components including tender joint counts, PtGA, and ESR except for swollen joint counts which were higher in the NAC group compared to that of the placebo group (p=0.008) (Table 1).

Routine anti-RA regimens of DMARDs received by patients during the study are presented in Table 2. The main components of the routine DMARDs were methotrexate and hydroxychloroquine, however, sulfasalazine, leflunomide, azathioprine, and NSAIDs were also received occasionally by patients in both groups. In respect of the DMARDs used by patients, no significant differences were detected among the study groups (Table 2).

Participants in both groups had comparable disease activity distribution at baseline, without any statistically significant difference based on Fisher's Exact test (p=0.65). In the intervention group, 20 (90.90%) and 2 (9.10%) patients had moderate and high disease severity, respectively. Similarly, in the control group, 16 (84.21%) and 3 (15.79%) patients were in the moderate and high disease severity categories, respectively. As it is seen, most of the patients are categorized as moderate disease severity group. Individuals with a high disease severity score have poly-articular or severe involvement of the joints, and consequently, they may need to be hospitalized or receive intra-articular/systemic corticosteroid pulses, as well as biological agents. These treatments would include patients in the category of cases who were excluded from our study.

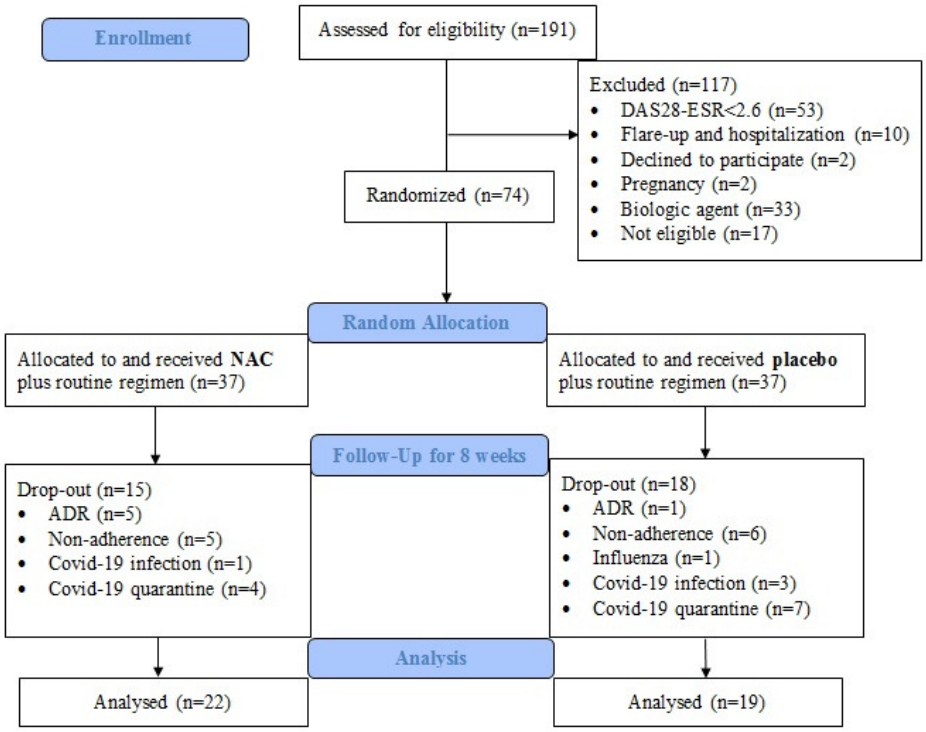

Figure 1. Study flowchart

Table 1. Demographic characteristics, baseline medical and medication history, DAS28-ESR and its components as well as serum levels of cytokines in the study groups

Variable	Subgroup	Intervention group	Control group	*p*
Age(year)	N/A+	54.14±9.11*	54 ± 9.12	0.96
Sex	Male	3 (13.63%)	2 (10.52%)	1.00
	Female	19 (86.36%)	17 (89.47%)	
RA history(years)	N/A	10.50±6.52	9.63±5.26	0.65
Concurrent Disease	Yes	11 (50%)	10 (52.63%)	0.87
	No	11 (50%)	9 (47.36%)	
Concurrent Drugs	Yes	11 (50%)	10 (52.63%)	0.87
	No	11 (50%)	9 (47.36%)	
Confounder Drugs	Yes	3 (13.63%)	7 (36.84%)	0.14
	No	19 (86.36%)	12 (63.15%)	
RA Regimen Changes	Yes	9 (40.90%)	8 (42.10%)	0.94
	No	13 (59.09%)	11 (57.89%)	
IA CS	Yes	5 (22.72%)	4 (21.05%)	1.00
	No	17 (78.94%)	15 (78.94%)	
Smoking	Non-smoker	20 (90.90%)	15 (78.94%)	0.39
	Ex-smoker	2 (9.09%)	4 (21.05%)	
No of tender joints	N/A	4.41±3.46	4.95±5.65	0.77
		[4.00, 2.75-5.00] **	[4.00, 2.00-5.00]	
No of Swollen joints	N/A	2.60±2.40	1.11±0.81	**0.008**[#]
		[2.00, 1.00-3.00]	[1.00, 1.00-2.00]	
PtGA	N/A	59.54±11.74	58.94±14.86	0.88
		[60.00, 50.00-70.00]	[60.00, 50.00-70.00]	
ESR	N/A	23.45±11.92	23.26±17.25	0.39
		[19.50, 13.75-33.00]	[15.00, 10.00-39.00]	
DAS28-ESR	N/A	4.45±0.65	4.26±0.74	0.38
IL-1β pg/L	N/A	5561.95±2289.37	5094.53±3051.59	0.81
		[6324.00, 2852.75-7077.75]	[4243.00, 2204.00-8044.00]	
IL-17 ng/L	N/A	374.20±223.61	351.72±268.71	0.60
		[260.34, 210.85-531.67]	[260.61, 148.30-542.92]	

+Not Applicable; *Continuous data are provided as mean ± standard deviation; ** [Median, Interquartile range] for discrete or non-normally distributed variables; #Significant difference; RA: Rheumatoid Arthritis; IA CS: Intra-articular *corticosteroid*; PtGA: Patient global assessment; ESR: Erythrocyte Sedimentation Rate; DAS28: Disease Activity Score with 28-joint counts; IL: Interleukin, $p<0.05$ was considered as a significance level.

Table 2. Routine disease-modifying anti-rheumatic drugs (DMARDs) regimen received by patients at the beginning of the study

Drug	Subgroup	Intervention group	Control group	*p*
Methotrexate	Yes	20 (90.90%)	19 (100%)	0.49[a]
	No	2 (9.09%)	0 (0%)	
Hydroxychloroquine	Yes	13 (59.09%)	15 (78.94%)	0.17[b]
	No	9 (40.90%)	4 (21.05%)	
Sulfasalazine	Yes	5 (22.72%)	5 (26.31%)	1.00[a]
	No	17 (77.27%)	14 (73.68%)	
Leflunomide	Yes	6 (27.27%)	6 (31.57%)	0.76[b]
	No	16 (72.72%)	13 (68.42%)	
Azathioprine	Yes	2 (9.09%)	2 (10.52%)	1.00[a]
	No	20 (90.90%)	17 (89.47%)	
Nonsteroidal Anti-inflammatory Drug	Yes	1 (4.54%)	3 (15.78%)	0.32[a]
	No	21 (95.45%)	16 (84.21%)	

[a] Based on the Fisher's exact test; [b] Based on the Chi-square test, $p<0.05$ was considered as a significance level.

In Table 3, results of the within-group comparisons of differences between the baseline and endpoint (after 8 weeks) values of the IL-1β and IL-17, DAS28-ESR, and its components are presented. All of the study outcomes were significantly ameliorated in the NAC group, while in the placebo group only there was a significant reduction in the PtGA and IL-1β.

Table 3. Within-group comparisons of differences between the baseline and endpoint (after 8 weeks) values of the IL-1β and IL-17, DAS28-ESR and its components

Variable	Group	Baseline	8 weeks after intervention	*p*
No of tender joints	Intervention group	4.41±3.46* [4.00, 2.75-5.00] **	0.95±1.22 [1.00, 0.00-1.00]	**<0.0001**
	Control group	4.95±5.65 [4.00, 2.00-5.00]	3.47±3.70 [2.00, 1.00-4.00]	0.09
No of swollen joints	Intervention group	2.59±2.40 [2.00, 1.00-3.00]	0.55±0.74 [0.00, 0.00-1.00]	**0.001**
	Control group	1.11±0.81 [1.00, 1.00-2.00]	0.89±0.85 [1.00, 0.00-1.00]	0.23
PtGA	Intervention group	59.54±11.74 [60.00, 50.00-70.00]	40.45±13.30 [40.00, 30.00-42.50]	**<0.0001**
	Control group	58.94±14.87 [60.00, 50.00-70.00]	49.47±18.10 [50.00, 40.00-60.00]	**0.04**
ESR	Intervention group	23.45±11.92 [19.50, 13.75-33.00]	14.18±9.19 [12.50, 6.50-20.00]	**<0.0001**
	Control group	23.26±17.25 [15.00, 10.00-39.00]	22.47±11.88 [20.00, 12.00-33.00]	0.82
DAS28-ESR	Intervention group	4.45±0.65	2.79±0.78	**<0.0001**[b]
	Control group	4.26±0.74	3.85±0.75	0.07[b]
IL-1β pg/L	Intervention group	5561.95±2289.37 [6324.00, 2852.75-7077.75]	3706.04±2287.93 [3899.00, 1710.25-4964.00]	**<0.0001**
	Control group	5094.53±3051.59 [4243.00, 2204.00-8044.00]	3904.26±2111.17 [4089.00, 1619.00-5179.00]	**0.02**
IL-17 ng/L	Intervention group	374.20±223.61 [260.34, 210.85-531.67]	234.53±162.39 [161.57, 102.73-354.27]	**<0.0001**
	Control group	351.72±268.71 [260.61, 148.30-542.92]	294.66±217.99 [206.00,140.76-381.38]	0.06

* Mean ± Standard Deviation; ** [Median, Interquartile range]; [a]Based on the Related-Samples Sign test; [b]Based on the Paired t-test; PtGA: Patient global assessment; ESR: Erythrocyte Sedimentation Rate; DAS28: Disease Activity Score with 28-joint counts; IL: Interleukin, $p < 0.05$ was considered significant.

Table 4 depicts the results of the between-groups comparisons of the changes in the values of the serum concentrations of the inflammatory biomarkers (IL-1β and IL-17) and the DAS28-ESR along with its components. The student t-test revealed that reduction in the DAS28-ESR was significantly higher ($p<0.0001$, 95% CI: -1.86 - -0.64) in the NAC group (1.66±1.03) compared to that of the control (0.41±0.90) group. Though, according to the Mann-Whitney U-test, no statistically significant differences were seen in the reduction of IL-1β and IL-17 between the intervention and control groups ($p=0.32$ and $p=0.12$, respectively). Moreover, improvements in the PtGA ($p=0.03$), number of tender joints ($p=0.02$), number of swollen joints ($p=0.007$), and ESR ($p=0.008$ without outlier data) were in favor of the NAC group.

Furthermore, a comparison of the frequency of the individuals in the study groups using the Fisher's Exact

test and based on the disease severity score (DAS28-ESR), revealed that there was a statistically significant difference between study groups (p=0.004) at the end of the 8th week, so that in the NAC group, the frequency of the individuals in the remission [n=9, (40.91%)] and low severity [n=6, (27.27%)] categories were considerably higher than those of the control group [n=1, (5.26%) and 2 (10.53%), respectively]. Interestingly, 9 (40.91%) patients in the NAC group versus 1 (5.26%) patient in the placebo group reported "no pain".

Reported adverse drug reactions (ADR), that led to drug discontinuation in our study, included nausea (n=3), heartburn (n=1), and hypertension (n=1) in the NAC group; and itching and dermal irritation on hands and face (n=1) in the placebo group. No serious ADR was reported, and between-groups comparison of the total incidence rate of the ADRs was not significant.

Table 4. Between groups comparisons regarding changes in the DAS28-ESR and its components, the plasma levels of IL-1β and IL-17

Variable	Intervention group	Control group	*p*
Reduction in number of tender joints	3.45 ± 3.10* [3.00, 1.75-4.00] **	1.47 ± 2.61 [1.00, 0.00-3.00]	**0.02**
Reduction in number of Swollen joints	2.05 ± 2.50 [1.50, 0.00-3.00]	0.21 ± 1.08 [0.00, 0.00-1.00]	**0.007**
Reduction in PtGA	19.09 ± 15.71 [20.00, 10.00-30.00]	9.47 ± 17.47 [10.00, 0.00-20.00]	**0.03**
Reduction in ESR	9.27 ± 10.43	0.79 ± 18.22	0.07
Reduction in ESR(without outliers)[b]	8.95 ± 7.48	0.12 ± 11.50	**0.008**
Reduction in DAS28-ESR	1.66 ± 1.03	0.41 ± 0.90	**<0.0001**
Reduction in IL-1β pg/L	1855.91±1987.10	1190.26±2214.10	0.32
Reduction in IL-17 ng/L	139.66±153.32	57.06±176.65	0.12

[a] Difference between baseline and endpoint (8 weeks) measurements.

[b] Two outlier values of 37 and -12 in the intervention group and two outlier values of 50 and -37 in the control group were excluded from the analysis.

* Mean ± Standard Deviation, ** [Median, Interquartile range]; PtGA: Patient global assessment; ESR: Erythrocyte Sedimentation Rate; DAS28: Disease Activity Score with 28-joint counts; IL: Interleukin.

p value <0.05 was considered significant.

DISCUSSION

This investigation was the first randomized double-blind placebo-controlled clinical trial weighing the effect of oral effervescent NAC on serum concentrations of IL-1β and IL-17 in RA patients.

Our trial indicated that clinical features of RA could considerably be improved after 8 weeks of add-on therapy with oral NAC 1200 mg/day. The severity of disease determined by DAS28-ESR and its components of PtGA, numbers of tender and swollen joints, as well as ESR were considerably modified in patients using NAC. In addition, although it was not statistically significant, however, NAC could also decrease serum concentrations of IL-1β and IL-17.

RA is an immune-mediated disease in which joints are primarily and progressively involved. Inflammation related to RA can cause systemic disorders, including pulmonary, cardiovascular, gastric, and musculoskeletal complications.[2,23]

The role of the inflammatory pathway is confirmed in the progression of RA. Serum concentrations of IL-1β, IL-6, IL-17, IL-23, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) are elevated in RA.[5,7,24] Imbalance between these pro-inflammatory and anti-inflammatory cytokines like IL-4, IL-10 and IL-13 lead to immune dysfunction and synovium destruction.[24,25] Eastgate et al, reported that plasma concentration of IL-1β was correlated positively with pain score, ESR, and disease severity in RA patients.[6] IL-1β stimulates the growth and activation of synovial cells to produce MMPs and collagenases, which cause bone and joint cartilage erosion. It also expresses other pro-inflammatory genes and mediators, too.[26]

It is approved that IL-17, which is a production of the Th17 cells, induces TNF-α, IL-1β, IL-6, and IL-8 activates the generation and function of MMPs in synovial fibroblasts and also increases the expression of RANKL in osteoblasts that result in an increased RANKL signaling in osteoclasts and finally bone erosion in RA patients.[27] In 2014, Pavlovic et al, found that there is a positive association between serum IL-17A concentrations with ESR, CRP, and DAS28 in early RA patients.[28] Therefore, IL-1β and IL-17 have a pivotal role in the progression of RA not only by inducing inflammatory reactions but also through activating the neuronal, endocrine and immune systems.

Several clinical trials have confirmed the positive impact of anti-oxidant and anti-inflammatory supplements on the pathophysiology of RA. Supplements like omega-3 fatty acids, vitamins B6, and coenzyme Q10 (CoQ10) probably modify pathways connected to inflammation.[29-32]

In a study, consumption of fish oil (3.6 g/day for 12 weeks) by RA patients, could decrease the plasma level of IL-1β.[29] In a single-blind co-intervention study on patients with RA, vitamin B6, 100 mg/day, suppressed IL-6 and TNF-α activities.[30] A double-blind, randomized controlled clinical trial by Abdollahzad et al, showed beneficial properties of CoQ10 supplementation on serum levels of TNF-α, IL-6, malondialdehyde (MDA), and total antioxidant capacity (TAC) in RA patients.[31] In 2016, Veselinovic et al, in a study on 60 patients with active RA examined the impact of omega-3 fatty acid consumption along with standard therapy in a 3-month randomized trial. Patients were allocated in 3 groups of omega-3 fatty acids, omega-3 fatty acids plus primrose evening oil, and no supplement. They reported a notable decline in visual analog scales core (for pain), DAS28, and the number of tender joints in both of the study groups.[32]

Furthermore, *in-vitro* and *ex-vivo* investigations have also confirmed the effects of supplements on reducing IL-1β and IL-17 levels. Jhun et al studied the anti-inflammatory effects of CoQ10 on a mice model with RA. The most interesting finding was that CoQ10 could down-regulated IL-17 expression and Th17 cell population that induce an inflammatory response in autoimmune arthritis. Also, a significant decline in IL-1β, IL-17, IL-21, and TNF-α was detected in the joints of mice.[33] Another *ex-vivo* investigation evaluated the immune-regulatory effect of quercetin, in IL-17-produced osteoclastogenesis. They found that quercetin could inhibit IL-17-stimulated RANKL level in RA fibroblast-like synoviocytes and IL-17-stimulated osteoclast formation as well as Th17 cell differentiation.[34] Currently, it is accepted that Th17 activity and expression of IL-17 can lead to inflammation and exacerbation of RA.[33,34]

Anti-oxidant and anti-inflammatory properties of NAC, both in-vitro and in-vivo, were shown in previous studies.[15,18,20] In recent decades, various clinical indications *e.g.* ulcerative colitis, prophylaxis of contrast media-induced nephrotoxicity, polycystic ovary syndrome, cardiovascular and psychiatric disorders have been introduced for NAC, owing to its anti-oxidant and anti-inflammatory effects.[15] An *ex-vivo* study by Lee et al showed that NAC can prevent the expression of IL-17-induced RANKL and differentiation of IL-17-induced osteoclast from precursor cells in RA synovial fibroblasts. This study suggests that NAC also represses Th17 induced osteoclastogenesis and cytokine production. Consequently, the researchers propose that NAC could be an adjunct therapeutic choice for inflammation and bone erosion in RA.[19]

Nakagawa et al, accomplished an investigation to determine the impact of NAC on a rat model of osteoarthritis (OA). They reported that NAC could substantially prevent cartilage damage in-vivo.[35] Another in-vivo study conducted by Roman-Blas et al, on the synovial fluid of patients with OA, indicated that NAC inhibits prostaglandin E2 synthesis, Cyclooxygenase 2 expression, and activity of the metalloproteinase-13 protein and nuclear factor-kappaB (NF-κB). All of these could be induced by IL-1β.[36]

Few trials are weighing the effectiveness of NAC on clinical improvement and inflammatory cytokines in RA. A study in 1997 by Remans et al, assessed the efficacy of high dose intravenous NAC in active RA patients in 24 hours. They claimed a significant decline in swollen and tender joint counts which were similar to our findings.[37] On the contrary; they reported no significant improvement in DAS28-ESR.[37] Jonsson et al, administered oral NAC at doses of 600-1200 mg/day up to 12 months for treatment of seven patients with refractory RA. They observed no significant effects. This observation is under question due to the small sample size or the insufficient dose of NAC.[38]

In 2018, in a randomized double-blind clinical trial, Batooei et al, evaluated the impact of NAC1200

mg/day for 12 weeks, as add-on therapy of RA. They reported significant positive outcomes in PtGA, patients' physical performance, and severity of pain, while no satisfying impact on DAS28-ESR and ESR were seen. They concluded that oral NAC might improve the health status and clinical activity of RA patients possibly via a reduction in inflammation and oxidative stress.[39]

Some studies have also suggested that NAC may present analgesic properties.[40,41] Ozcamdalli et al, carried out a study to compare the intra-articular hyaluronic acid and NAC injection efficacy on pain severity and cartilage destruction in OA patients. They demonstrated that the efficacy of NAC on pain scores and function of patients were comparable to those of hyaluronic acid.[42] Satisfying improvement in PtGA observed in our trial might be correlated to the analgesic properties of NAC.

Hashemi et al evaluated the oral NAC effects on TNF-α, IL-6, and few oxidative stress biomarkers as well as ESR. Based on the within-group analysis, they found that the serum levels of TNF-α, IL-6, and ESR have reduced in the NAC group, while between groups analysis revealed no statistically significant differences.[43] Similarly, our findings revealed that serum levels of IL-1βand IL-17 have not significantly reduced, based on the between-groups analyses.

The impacts of NAC on inflammatory cytokines and oxidative stress markers have also been reported in numerous studies on diseases rather than RA. Purwanto and Prasetyo performed a placebo-controlled trial on the impacts of oral NAC on plasma levels of inflammatory markers in patients on regular continuous ambulatory peritoneal dialysis (CAPD). Their trial included 2 groups of 16 patients on regular CAPD. They showed that 8 weeks consumption of oral NAC 1200 mg/day could significantly diminish plasma levels of IL-1, IL-6, TNF-α, C3, and CRP.[44] Conversely, according to the result published by Nascimento et al, NAC (effervescent tablets, 600 mg, twice daily) did not significantly affect the inflammatory and oxidative stress markers except IL-6.[45]

Another study assessed the NAC efficacy for cardiac protection in individuals undergoing elective abdominal aortic aneurysm repair. Fifty patients randomly received NAC infusion (0.3 mg/kg/min through the surgery then reduced to 0.2 mg/kg/min for 24 hours after operation) or placebo infusion. The results showed that NAC infusion could significantly decrease postoperative levels of myocardial-specific protein, IL-1β, and TNF-α. Moreover, MDA concentration was less and TAC was higher in the NAC group.[46]

Although, most of the previous reports are in agreement with the results obtained in our study, confirming a satisfactory role for NAC in improving clinical indicators of the disease severity i.e. DAS28-ESR, however, dissimilar results in particular about the inflammatory biomarkers of IL-1β and IL-17 may be explained by differences in the duration and dose of NAC, route of administration, or unexplained mechanism of the action exerted by NAC in patients with RA. Furthermore, the study population, type of the disease, and its severity, as well as comorbidities can be a source of variation in outcomes obtained by different studies.

Improvements in the DAS28-ESR and its constituents reveal that NAC supplementation could have a beneficial impact on all of the clinical features of RA. This is reinforced with the safe ADR profile of NAC. On the other hand, non-significant variations in the IL-1β and IL-17 concentration suggest that the positive effects of NAC may be intermediated by mechanisms other than those involving these two cytokines. However, these results need to be confirmed by further investigations. Probably, conducting randomized controlled trials with larger sample sizes and higher doses of NAC used for a longer period will lead to more precise and convincing findings.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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