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Original Article

THE INFLUENCE OF ADDING ANTIBIOTIC IN TREATMENT OF RHEUMATOID ARTHRITIS PATIENTS ON *STREPTOCOCCUS PYOGENES* CARRIER RATE AND ON THE LIPIDS PROFILE

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ABSTRACT

Objective: The main goal is to evaluate the clinical efficacy, safety, and tolerability of antibiotics and methotrexate (MTX) treatment on the disease severity, on *Streptococcus pyogenes* carrier rate and on the lipid profile of patients with rheumatoid arthritis (RA).

Methods: In a 6-months, double –blind trial, 130 patients with active RA were treated for four weeks with MTX therapy at a stable low dose of 12.5 mg/week instructed to receive either levofloxacin (500 mg) or placebo orally once daily while continuing to receive MTX. Before and after the treatment, disease activity parameters, rheumatoid factor (CF), C reactive protein (CRP), anti-streptolysin O (ASO) titer and lipid profile were measured. Throat swab cultures were done on suitable medium.

Results: Antibiotic adds to treatment causes a significant reduction in disease activity, lower the side effects and concomitant decrease in MTX treatment dose, most of the lipid levels had returned to baseline levels, decreased *S. pyogenes* carrier rate from 25-30% to 3.2-6% and lower ASO titers to undetectable.

Conclusion: RA patients who are treated with MTX, addition of antibiotics lower the signs, symptoms and risk factors of RA patient and *S. pyogenes* could be important in the etiopathogenesis of RA.

Keywords: Antirheumatic drug, Lipid profile, Antistreptolysin O, Rheumatoid arthritis.

INTRODUCTION

RA is a chronic, incurable disease characterized primarily by painful joint inflammation [1]. Patients with RA have an increased morbidity and mortality compared with the general population [2]. Cardiovascular disease (CVD) is the leading cause of high mortality in RA patients [3], this is predominantly due to accelerated atherosclerosis [4-5]. The etiology of RA remains elusive, although it appears that genetic, infectious, environmental and hormonal factors are all involved in complex, interrelated ways [6]. Several genetic markers have been identified; as human leukocyte antigen in DRβ1 region (HLA-DRβ1) [7], protein tyrosine phosphate (PTPN22) [8-9]. Smoking is one of the environmental factors suggested to increase the susceptibility for developing RA [10]. RA being more common among women than men in all age groups, gender and hormonal factors seem to play role since the difference become less obvious in post-menopausal women [11].

Moreover, infection, trauma, obesity and social deprivation among other factors, might increase the risk of developing RA [11]. RA is an autoimmune disease in which the immune system mistakes the body's own substances and cells for foreign invaders and releases friendly fire on them. However no specific auto-antigen has yet been identified [12]. Studies concerning the lipid profile in RA patients yielded contradictory data [13-21]. It is known that increased levels of total cholesterol (TC), low density lipoprotein- cholesterol (LDL-C) and low levels of high density lipoprotein- cholesterol (HDL-C), are associated with an increased incidence of CVD in general population [22-23]. In many previous studies, RA disease has been found at increased frequencies in individuals with periodontitis, and RA resembles periodontitis in many pathologic aspects [24-25]. Human leukocyte antigen in DR4 region (HLA-DR4) tissue antigens are found at high frequencies both in patients with periodontitis and in those with RA [26-27]. High levels of oral anaerobic bacterial antibodies have been found in serum and synovial fluid of RA patients [28-29]. A population- based study aim to investigate the frequency of *Streptococcus pyogenes* in a cohort of children with recent-onset of arthritis show that *S. pyogenes* was present in 18% of children with arthritis, rose steadily with age and peaked (35%) at ages 8-11 years [30]. RA has previously been linked with infection by pathogens such as mycoplasma, Epstein Barr virus, cytomegalovirus, parvovirus and rubella virus, but conclusive evidence is still lacking [31].

Many researcher recommended antibiotics treatment with the idea that perhaps there is an infectious bacterium that causes or worsens the inflammation in the joints [32], this was also approved by food and drug administration (FDA), national institutes of health (NIH), and the American College of Rheumatology (ACR) in 2004 recommended minocycline for patients with symptoms of mild RA [33]. It has been established that injection of cell wall from *S. pyogenes* produces acute joint lesions in mice and it has been used as a model of RA ([34], certain virulence factors such as superantigens and exotoxine may contribute to the pathogenesis of *S. pyogenes* in RA patients [35].

Since RA is chronic disease it leads to long-term patients suffering, with no know cure for it, early and effective treatment to reduce joint destruction, minimize pain and disability is important. The main goal is to evaluate the clinical efficacy, safety, and tolerability of antibiotics and antirheumatic treatment on the lipid profile of patients with RA, and to find any association of oral bacteria like *S. pyogenes* which causes pharyngitis, tonsillitis, bacteremia and necrotizing fasciitis in RA development.

MATERIALS AND METHODS

Subjects and clinical evaluation

130 RA patients, diagnosed according to the ACR criteria for classification of the disease [36], and 110 control subjects, matched for age, sex, smoking status (all were non smokers) and blood pressure were enrolled after giving informed consent. The patients had active disease, as evaluated by a Disease Activity Score (DAS 28=6.5 ± 0.2) according to European league against rheumatism (EULAR's) recommendations [37] and all were RF positive. No subject had any symptom or laboratory finding of kidney, liver or thyroid dysfunction, diabetes, malignancy, or was taking drugs affecting serum lipid or lipoprotein levels. None had been treated

with antihyperlipidemia drugs prior or during the study. All subjects had sedentary habits and were not participating in any regular physical exercises. The present investigation was a 6 month, double-blind, and placebo study and was approved by the hospital ethics committee and was in accordance with the principles outlined in the declaration of Helsinki. The selected patients had active joint symptoms (more than three swollen or five tender joints) and elevated inflammatory parameters.

Before receiving the study drugs, all patients had been taking MTX at stable dose, the median weekly dose was 12.5 (mean ± SD = 10.3 ± 3.9 mg/week) for the previous four weeks (RA patients group A). All patients were receiving folic acid to mitigate the toxic effects of MTX.

After informed consent, the patients were randomly assigned to one of two treatment groups: oral placebo with the stable dose of oral MTX (RA patients group B) or oral levofloxacin in 500 mg daily with the stable dose of oral MTX (RA patients group C). The use of analgesic for pain relief as acetaminophen was permitted. There was no significant difference between the groups in height, weight, body mass index, duration in rheumatic disease, seropositivity, the activity of RA. The basic data of the subjects are presented in table 1.

Table 1: Characteristics of RA patients and the control subjects

Variables	Females RA		Males RA	
	Control n=55	Test group n=66	Control n=55	Test group n=64
Age (years)	39.6±9.1	40.8±8.11	40.1±7.91	39.9±8.21
Height (cm)	163.6±6.3	161.2±5.9	162.9±5.3	161.8±6.1
Weight (kg)	69.9±3.1	68.9±4.8	68.8±4.1	67.3±5.0
Body mass index BMI (Kg/m^2)	26.7±6.8	27.4±9.1	27.3±10.1	26.8±7.9
ESR (mm/hr)	11.9±3.5	67.1±44.6	10.8±3.8	66.9±43.8
Rheumatoid factor (IU/ml)	8‹	›64	8‹	64‹
CRP (mg/L)	6‹	12	6‹	10
ASO (IU/ml)	200‹	›800	200‹	›600
Blood glucose (mg/dL)	91.8±10.3	98.6±11.6	95.9±9.9	94.810.8
Methotrexate dose (mg/wk)	0	12.5±2.5	0	12.5±2.5

Values are given as median (SD), BMI = Body Mass Index, ESR = Erythrocytes Sedimentation Rate, CRP = C-Reactive Protein, ASO = Antistreptolysin O titer

From all patients and the control group, blood samples and throat swabs were taken before and after ending the treatment and processed as follow; After overnight fast (12 h) venous blood samples from all participants (patients and controls) were collected into two set of tubes; in the first set anticoagulant were added and it is used for detection the Erythrocytes Sedimentation Rate (ESR) for the first hour. The other set a plain tubes were used. The samples after their complete coagulation were centrifuged at 4^{o}C (500 g; 15 minutes) to separate sera. All sera were assayed together to minimize inter-assay biases.

The sera were used for detection the total levels of ASO, RF, CRP and glucose using standard methods. Serum TC and triglyceride (TG) concentration were measured using enzymatic methods (Roche Diagnostic GmbH, Mannheim, Germany). Very low-density lipoprotein (VLDL) and LDL-C were calculated according to Friedewald formula [38]. Cholesterol was measured in HDL containing supernatant after phosphotungstic acid/magnesium chloride precipitation of Apolipoprotein B (Apo B) containing lipoproteins (Boehringer Mannheim, Mannheim, Germany). Apolipoproteins (Apo) AI, AII and B values were determined by immune-turbidimetric assay (Roche Diagnostic GmbH, Mannheim, Germany). Apo СП and СШ were determined by radial immunodiffusion technique (Daiichi pure chemicals Co. Ltd, Tokyo, Japan).

Rheumatoid factor detection (RF)

RF is detected by slide latex agglutination test using diagnostic reagent for measurement of RF from Chemej EX, S. A. Barcelona (Spain) and performed according to the manufacturer recommendations. Negative and positive sera were used as controls. After two minutes a clear agglutination is observed in the positive indicating the presence of RF. The presence of agglutination indicates a RF concentration equal or greater than 8 IU/ml.

C - reactive protein (CRP) concentration determination

CRP concentration was determined by slide latex agglutination test (semi-quantitative) using reagent purchase from Excel Scientific INC, USA; and performed as manufacturer recommendation; negative and positive control samples were included. Presence of agglutination indicates a content of CRP in the sample≥ 6 mg/L, normal levels is < 6 mg/L.

The erythrocyte sedimentation rate (ESR)

Was determined using modified Westergren method [39].

Anti-Streptolysin O level (ASO)

ASO was determined by slide latex agglutination test (semi-quantitative) using reagent purchased from Chemelex, S. A. Barcelona, Spain, and performed according to the manufacturer recommendations. Negative and positive sera were used as controls. After two minutes a clear agglutination is observed in the positive indicating the presence of ASO. The presence of agglutination indicates ASO concentration equal or greater than 200 IU/ml.

Throat swab culture

Throat swabs of all (patients and controls) were taken and inoculated onto two plates containing 5-10% horse blood agar for aerobic and anaerobic cultures, and on MacConkey's agar plate (Oxoid limited, UK). All plates were incubated for 18-24 hr at 37^{o}C. *S. pyogenes* were identified macroscopically, microscopically and by sensitivity to bacitracin and resistance to trimethoprim/ sulfa methoxazole (SXT) disk testing. Antibiotics susceptibility testing was done according to Kirby- Bauer disk diffusion method [40], accordingly levofloxacin was used.

All patients underwent evaluations initially and after 6-months treatment. The assessments consisted of a tender and swollen joint count, the Health Assessment Questionnaire (HAQ), and patient's assessment of pain in addition to the laboratory data.

Statistical analysis

Statistical analysis was carried out using Student's t test by statistical packages for social science software (SPSS). Values are expressed as mean ± SD and values of $p < 0.05$ were considered statistically significant. The relationships between variables were calculated using Pearson Correlation Coefficients.

RESULTS

The characteristics of the patients and the control group before starting the study are summarized in table 1. Sixty four male and sixty six female were enrolled in the study. The mean age was 40 years. There were no significant differences in age, body mass index,

height, white blood cell or platelet counts, glucose blood levels, disease duration, or disease activity indicating that the physical conditions were comparable in the groups before starting drug treatment. All patients were started treatment with MTX for 4 weeks (RA patients group A). The patients were then divided into two groups; to the first group one oral placebo tablet was added to their MTX treatment (RA patients group B), to the second group oral tablet contain 500 mg levofloxacin were added once daily to their stable MTX treatment (RA patients group C), the treatment was continue to six months. As shown in table 2 the serum lipid and lipoprotein lipid levels in RA female patients group A and group B had significant higher levels of TC, LDL-C, TTG, VLDL-, LDL-, and HDL-TG when compared to the RA female patients group C and to the controls.

Table 2: Serum lipids and lipoprotein concentrations (mmol/L) in RA female patients (untreated and treated) compared to the control subjects

Parameters	Controln=55	RA female patients		
		An= 66	Bn=33	Cn=33
TC	4.64 ± 0.43	5.78 ± 0.56[a]	5.10 ± 0.41[ac]	4.66 ± 0.36[c]
VLDL-C	0.41 ± 0.06	0.49 ± 0.09[a]	0.41 ± 0.03[d]	0.38 ± 0.08
LDL-C	2.71 ± 0.40	4.18 ± 0.52[a]	3.43 ± 0.38[ac]	2.80 ± 0.31[cd]
HDL-C	1.51 ± 0.11	± 0.08[a] 1.08	1.18 ± 0.09[c]	1.58 ± 0.16[ac]
TTG	0.96 ± 0.10	2.41 ± 0.30[a]	1.61 ± 0.11[ac]	1.01 ± 0.06[cd]
VLDL-TG	0.61 ± 0.16	1.70 ± 0.27[a]	1.13 ± 0.21[ac]	0.72 ± 0.10[a]
LDL-TG	0.24 ± 0.08	0.48 ± 0.11[a]	0.33 ± 0.051[ad]	0.16 ± 0.02[a]
HDL-TG	0.13 ± 0.03	0.24 ± 0.06[a]	0.18 ± 0.04[ad]	0.11 ± 0.04

Values are means ± SD,

TC= total cholesterol, VLDL-C= very low density lipoprotein – cholesterol, LDL-C low density lipoprotein – cholesterol, HDL-C= high density lipoprotein – cholesterol, TTG= total triglyceride, VLDL-TG= very low density lipoprotein – triglyceride, LDL-TG= low density lipoprotein – triglyceride, HDL-TG= high density lipoprotein – triglyceride.

a p <0.001; bp < 0.05 compared to control subjects; c p < 0.001; dp < 0.05 between RA groups; A = RA patient treated with stable dose of MTX for 4 weeks; B =A group + placebo treated for 6 months ; C = A group+ Levofloxacin treated for 6 months

The level of HDL-C was significantly lower in RA female patients group A and B when compared to group C and the controls. There were no significant differences in RA female patients group C and the control groups regarding TC, VLDL- and LDL-C TTG, VLDL-TG, whereas RA female patients group C had significant higher HDL-C and significant lower LDL-, HDL-TG values compared to the control group. Table 3 shows serum lipid and lipoprotein lipid levels in RA male patients (groups A, B, and C) compared to the levels in the control group.

Table 3: Serum lipids and lipoprotein concentrations (mmol/L) in RA male patients (untreated and treated) compared to the control subjects

Parameters	Control n=55	RA male patients		
		A n=64	B n=33	C n=31
TC	5.41 ± 0.81	6.20 ± 1.7[a]	5.91 ± 0.41[ad]	5.39 ± 0.61[c]
VLDL-C	0.68 ± 0.07	1.10 ± 0.04[a]	0.89 ± 0.03[c]	0.60 ± 0.11
LDL-C	3.20 ± 0.61	4.31 ± 0.90[a]	3.70 ± 0.85[bd]	3.11 ± 0.52
HDL-C	1.61 ± 0.20	± 0.06[a] 1.01	1.31 ± 0.30[bc]	1.71 ± 0.13
TTG	1.31 ± 0.50	2.33 ± 0.60[a]	2.13 ± 0.60[ad]	1.81 ± 0.18[bc]
VLDL-TG	0.80 ± 0.07	1.66 ± 0.64[a]	1.52 ± 0.09[ad]	1.29 ± 0.26[bd]
LDL-TG	0.32 ± 0.08	0.44 ± 0.08[a]	0.41 ± 0.08[ad]	0.31 ± 0.072
HDL-TG	0.20 ± 0.08	0.24 ± 0.09[a]	0.23 ± 0.07[a]	0.23 ± 0.051

Values are means ± SD

a p <0.001; b p < 0.05 compared to control subjects; c p < 0.001; d p < 0.05 between RA groups; A = RA patient treated with stable dose of MTX for 4 weeks; B =A group + placebo treated for 6 months; C = A group+ Levofloxacin treated for 6 months

TC= total cholesterol, VLDL-C= very low density lipoprotein – cholesterol, LDL-C low density lipoprotein – cholesterol, HDL-C= high density lipoprotein – cholesterol, TTG= total triglyceride, VLDL-TG= very low density lipoprotein – triglyceride, LDL-TG= low density lipoprotein – triglyceride, HDL-TG= high density lipoprotein – triglyceride.

Apo AI was significantly lower in RA female patients group A and B compared to the levels in RA female patients group C and to the control group, while Apo B, Apo CII and Apo CIII were significantly higher in the same groups when compared to RA female patients group C and to the control. On the other hand the levels of Apo AI, Apo B, and Apo CII did not differ in RA female patients group C and the control.

Their were no differences in the levels of Apo AII in all RA female groups when compared to the controls, whereas Apo CIII was significantly increased in RA female patients group C compared to the control group. While in RA male patients, Apo AI and Apo AII levels were significantly lower in RA male patients group A and B when compared to group C and to the control.

In RA male patients group A and B there were significant higher levels of TC, VLDL- and LDL-C, TTG, VLDL-and LDL-TG when compared to group C who were treated with MTX and levofloxacin and to the control group, Whereas no significant differences were found in TC, VLDL-, and LDL-C, LDL-, HDL-TG between the control group and RA male patients group C. On the other hand, the levels of HDL-C was significantly lower in RA male patients group A and B when compared to RA male patients group C and to the control group, whereas HDL-C, TTG, and VLDL-TG were significantly increased in RA male patients group C when compared to the control group.

Table 4 shows apolipo protein levels in the serum of RA female, male patients and the control group.

Table 4: Serum apolipoprotein concentration (mg/dL) in untreated and treated RA patients compared to the control subjects

RA female patients	Apolipoproteins (mg/dL)	Controls n=55	A n=66	B n=33	C n=33
	A-I	141.8± 20.2	110.0±18.3[a]	125.6±21.3[ac]	143 ± 18.8
	A-II	54.1±8.3	41.6±6.3[b]	45.1±10.6[c]	54.3 ± 8.6
	B	80.6±10.1	116.3±11.3[b]	101.1±8.2[c]	79.1± 6.8
	C-II	4.20±0.03	5.31±0.04[b]	4.81±0.03[ad]	3.99 ± 0.088
	C-III	6.30±0.021	9.31±0.03[a]	8.90±0.02[ad]	7.21 ± 0.071[bc]
RA male patients	**Apolipoproteins (mg/dL)**	**Controls n=64**	**A n=64**	**B n=33**	**C n=31**
	A-I	149.0±18.1	109.0±17.3[a]	130.3±16.6[ac]	148.20±19.3
	A-II	51.3±5.3	39.3±8.31[a]	41.31±6.9[c]	52.1±7.2
	B	86.3±9.3	110.6±13.6[a]	100.9±10.6[ad]	82.6±8.1
	C-II	4.3±0.04	5.11±0.04[ab]	4.90±0.03[abd]	5.31±0.09[a]
	C-III	6.9±0.04	10.8±0.1[a]	9.1±0.91[adc]	7.21±0.01[ac]

Results are means ± SD in milligrams per deciliter

a $p < 0.001$, b $p < 0.05$ compared to control subjects; c $p < 0.001$, d $p < 0.05$ between RA patients; A = RA patient treated with stable dose of MTX for 4 weeks; B =A group + placebo treated for 6 months; C = A group+ Levofloxacin treated for 6 months

Apo B, Apo CII and Apo CIII were significantly elevated in RA male patients group A and B when compared with group C and to the control group. Apo CII and Apo CIII were significantly increased in RA male patients group C compared to the control group.

As shown in table 5 the ratios of lipids and apolipoproteins levels in untreated and treated RA female and male patients were comparable to the levels in control subjects.

Table 5: Ratios of lipids and apolipoproteins levels in treated and untreated RA patients compared to the levels in the control subjects

RA female patients	Ratios	Controls n=55	A n=66	B n=33	C n=33
	LDL-C/HDL-C	1.71±0.031	3.9±0.31[a]	1.80±0.21[c]	1.77±0.61
	TC/HDL-C	3.1±0.26	5.4±0.31[a]	3.09±0.33[c]	2.91±0.61
	Apo B/Apo AI	0.57±0.42	1.06±0.08[a]	0.65±0.051[b]	0.56±0.13
	Apo CII/Apo CII	1.5±0.31	1.75±0.23[b]	1.91±0.34[ad]	1.69±0.31[b]
	TTG/Apo CIII	9.7±1.81	22.9±7.3	16.0±0.81[ac]	16.12±3.9[a]
RA male patients	**Ratios**	**Controls n=55**	**A n=64**	**B n=33**	**C n=31**
	LDL-C/HDL-C	1.98±0.31	4.3±0.38[a]	2.85±0.30[ac]	1.81±0.26
	TC/HDL-C	3.4±0.51	6.1±0.71	4.5±0.53	3.2±0.81
	Apo B/Apo AI	0.23±0.03	1.01±0.013[a]	0.77±0.05[bc]	0.56±0.03
	Apo CII/Apo CII	1.61±0.14	2.11±0.21[a]	1.90±0.31[bc]	1.58±0.31[c]
	TG/Apo CIII	16.80±3.4	19.1±2.6[a]	20.71±3.51[a]	23.5±9.80[ac]

Results are mean ± SD in milligrams per deciliter

a $p < 0.001$, b $p < 0.05$ compared to control subjects; c $p < 0.001$, d $p < 0.05$ between RA patients; A = RA patient treated with stable dose of MTX for 4 weeks; B =A group + placebo treated for 6 months; C = A group+ Levofloxacin treated for 6 months

TC= total cholesterol, LDL-C low density lipoprotein – cholesterol, HDL-C= high density lipoprotein – cholesterol, apo B= apolipoprotein B, apo AI= apolipoprotein AI, apo CII= apolipoprotein CII, apo CIII= apolipoprotein CIII, TTG= total triglyceride.

The rations of LDL-C/HDL-C, TC/HDL-C, Apo B/Apo AI, Apo CII/Apo CII and TG/ Apo CIII in RA female patients group A and B had significantly higher levels when compared to group C patients and to the control group. There were no significant differences in RA female patients group C and the control group regarding LDL-C/HDL-C, TC/HDL-C, and Apo B/ Apo AI where as RA female patients group C had significant higher TTG/Apo CIII and Apo CII/Apo CII values compared to the control group.

RA male patients group A and B had significantly higher levels of LDL-C/HDL-C, TC/HDL-C, Apo B/Apo AI, and TTG/Apo CIII when compared to RA male patients group C and to the controls. There were no significant differences in RA male patients group C and the control groups regarding LDL-C/HDL-C, TC/HDL-C, and Apo B/Apo AI, where as RA male patients group C had significantly higher TTC/Apo CIII, and lower Apo CIII/Apo CII values compared to the control group.

Table 6: The number and percentage of isolation of *Streptococcus pyogeenes* from throat swab of RA patients.

Groups	female		Male	
	Total No.	No. with positive *S. pyogenes* No. %	Total No.	No. with positive *S. pyogenes* NO. %
RA group A	66	20 (30.3%)	64	16 (25%)
RA group B	33	10 (30.3%)	33	8 (24.2%)
RA group C	33	2 (6%)	31	1 (3.2%)
Control group	55	5 (9.09%)	55	3 (5.4%)

Group A = RA patient treated with stable dose of MTX for 4 weeks
Group B = group A + placebo treated for 6 months
Group C = group A + Levofloxacin treated for 6 months
Control = healthy no treatment

Table 6 shows the results of throat swab cultures; as shown in the table *Streptococcus pyogenes* was isolated from 20 (30.3%), 16 (25%) RA female and male patients group A, and it was isolated from10 (30.3%), 8 (24.2%) RA female and male patients group B, while significant lower percentage results were detected in RA female and male patients group C as 2 (6%), 1(3.2%) respectively.

The percentage of isolation in RA patients groups A and B were significantly higher than the percentage of isolation in the control group which was 5 (9.09%) in female and 3 (5.4%) in male, treatment with levofloxacine as in RA patients group C decrease the levels of ASO titer to undetectable and lower the percentage of isolation *Streptococcus pyogenes* from 25-30.3% to 3, 2-6% which was even lower than the level detected in the control group.

Table 7 shows the effect of treatment on measures of disease activity at 6 months, as shown in the table addition of Levofloxacin to MTX treatment was associated with significant reductions in pain, swollen joints and duration of morning stiffness, and significant reductions in disease activity as assessed by objective laboratory measures (as shown in table 7).

Table 7: Effect of treatment on measures of disease activity at 6 months

Characteristic	AN=130 (male + female)	BN=66 (male +female)	CN=64(male +female)
Tender joint count	24±2.8	20±2.1†	12±1.7*
Swollen joint count	18±6.3	16±5.3†	9±2.8*
Morning stiffness that may last for hours	5.6±1.2	3.5±0.85†	1.8±0.61*
Patient's assessment of pain (0-10 VAS)[a]	7.8±2.3	7.1±1.8†	3.4±0.90*
HAQ	2.4±1.11	2.1±0.91†	1.2±0.33*
ESR titer	60.2±36.4	30±26.2†	15.8±5.6*
DAS 28 score	6.82±1.8	6.03±1.3	4.69±0.98*
CRP	12	10	‹6*

*P‹0.0001 between C compared to A and B, †P‹0.01 between B compared to A, A = RA patient treated with stable dose of MTX for 4 weeks, B =A group + placebo treated for 6 months ,C = A group+ Levofloxacin treated for 6 months, VAS= Visual Analogue Scale, HAQ =Health Assessment Questionnaire, a =0 is best and 10 worst

DISCUSSION

Our aim was to determine the lipid profile in patients with RA and to investigate whether the profile will change after starting early treatment with low dose MTX and to detect any association of RA development with oral bacteria that found in the carriers which may cause repeated tonsillitis, and if the lipid profile could be influenced by adding antibiotic to the treatment protocol. The lipid profile of RA patients has been evaluated in several studies with controversial results [41-42]. We tried to exclude patients with classic risk factors for atherosclerosis. According to our results, patients with RA exhibited an atherogenic lipid profile characterized by an increase of TC, LDL-C, TTG, Apo AI, Apo CII and Apo CIII serum levels and a reduction in serum HDL-C and Apo AI levels before starting therapy, thus increase in the atherogenic ratio of LDL-C/HDL-C, TC/HDL-C, Apo B/Apo AI and Apo CIII/Apo CII were observed suggesting that RA patients may exposed to a higher risk of atherosclerosis, this is in accordance with a previous reports [41, 43-45].

Clinical use of low dose MTX for treatment RA for 6 months is associated with clinical and laboratory improvement was noted specially in those treated with MTX and antibiotics. This was correlated with changes in lipid profile, mainly increase of HDL-C, Apo AI and slightly deceases in the levels of LDL-C, Apo B and the ratio of Apo B/Apo AI with slight increase in TTG and VLDL-TG. Chen and his group [46] in their work they reported that there were no differences detected in plasma TC, TG, HDL-C, LDL-C in non-MTX users and subjects taking MTX which may be possibly attributed to low prevalence of dsylipidemia in their study subjects. In respect of this difference, low-dose MTX treatment has the potential to protect against dyslipidemia through facilitation of cholesterol outflow in patients by elevated expressions of athero protective proteins (27-hydroxylase and ATP-binding cassette transporter A1) [46]. Systemic inflammation may play role as a risk factor in the RA development, in fact increase of acute phase proteins [42] as CRP. An important observation in this study is that RA patients show low HDL-C and high CRP serum levels with high ESR value which are significantly changed after starting treatment with increased in HDL-C and reduction of CRP serum levels and ESR values.

To identified if there is any correlation between pathogenic bacteria as *Streptococcus pyogenes* which is associated with pharyngitis, tonsillitis, bacteremia and necrotizing fasciitis in RA development. Throat swab culture was done and ASO level was determined in the patients sera and compared with healthy control. In this study, the percentage of positive streptococcal tests was found to be 25-30.3% with ASO levels ›600-800 IU/ml in patients with RA while in the control group it was 5.4-9.09% with AS0 levels ‹200 IU/ml which is not in accordance with Riise[30] study that reported 35% positive streptococcal tests for arthritis patients aged 8-11 years, this variation may be due to our tested patients were older and had a longer disease duration. Starting treatment with antibiotic significantly lower those percentage levels to 3.2-6%, with ASO levels undetectable (‹200 IU/ml), it also improved the lipid profile of RA patient and lower the disease activity which may be due to it anti-inflammatory characters, and the improvement was better in RA patients positive for *S. pyogenes*.

The results of this study show the clinical efficacy of levofloxacin in patients with active RA receiving MTX. In this six-month study addition of levofloxacin provided additional benefit without potentiating the toxic effects of MTX or inducing any toxic effects of its own.

Levofloxacin is used in the treatment of infections of upper respiratory tract specially periodontopathic bacteria, facultative anaerobic bacteria and anaerobic bacteria which found normally in the gastrointestinal tract, were they cause chronic sinusitis, chronic recurrent tonsillitis and others in immune-suppressed individuals [47].

According to the ACR, antibiotics is prescribed for patient with symptoms of mild RA, it is sometimes combined with other medications to treat patients with persistent symptoms of this form of arthritis. Not only did levofloxacin significantly reduce symptoms (as shown in table 7), but side effects were minimal and less severe than observed for MTX and placebo treatments. After 6 months of treatment with MTX and levofloxacin a significant clinical and laboratory improvement was noted. This was correlated with improvement in lipid profile, mainly the increase of HDL-C. In conclusion Levofloxacin treatment in RA in conjugation with MTX is effective treatment for RA. Not only did the antibiotic reduce the symptoms, may reduce the risk of atherosclerosis and cardiovascular events, but the side effects were minimal and less severe than observed for most other common rheumatoid treatments. This work may indicate that oral and gut bacteria could be important in the etiopathogenesis of

RA. However, further studies are needed to confirm that *S. pyogenes* may have significant role in RA.

CONFLICT OF INTEREST

All authors indicate absence of any conflict of interest: declared none

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