Mans. Med. J. *Vol. 51. Issue 3*, 2022, pages 178-186

Mansoura Medical Journal

(Official Journal of Mansoura Faculty of Medicine)

pISSN: 1110-211X; **eISSN:** 2735-3990

The polyunsaturated fatty acid profile in erythrocyte cell membranes among group of Egyptian rheumatoid arthritis patients

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DOI: 10.21608/mjmu.2022.145254.1123

Submit Date: 18 June 2022
Accept Date: 15 August 2022
Available online: 30th Sept. 2022

Keywords

- polyunsaturated fatty acids
- rheumatoid arthritis
- erythrocytes cell membrane

Abstract

Feedback: Recently, it has been discovered that a disturbed polyunsaturated fatty acid ratio predisposes to inflammation and increases the risk of autoimmune disease. The assessment of polyunsaturated fatty acids in RBC cell membranes is an accurate method that reflects their actual level without being influenced by daily diet. Objectives: To determine the levels of various polyunsaturated fatty acids in erythrocyte membranes in the Rheumatoid arthritis (RA) cohort group versus the control group. In addition, to study the relation between their levels and activity as well as seropositivity. Results: When compared to a healthy control group, there was a significant rise in the erythrocytes cell membrane n-6 level and the n-6/n-3 ratio in RA patients. In RA patients, n-3 levels were significantly lower than in the control group. In individuals with rheumatoid arthritis, ESR levels were positively correlated with n-6 levels, while ESR levels were negatively correlated with n-3 levels. (docosahexaenoic acid and docosapentaenoic acid). The only polyunsaturated fatty acid parameter with a significant positive relationship to DAS28 –ESR and HAQ appeared to be the n-6/n-3 ratio. There was no correlation between both anti-CCP and RF antibodies and n-6, n-3 fatty acids, or n-6/n-3 ratio. Conclusion: RBC membranes in RA had low levels of n-3 and high levels of n-6, which significantly differ from the control group. N-3, n-6 and n-6/n-3 fatty acids had insignificant links with both RF and anti CCP antibodies.

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Introduction

Rheumatoid arthritis (RA) is chronic autoimmune illness defined by an inflammatory process that culminates in the breakdown of the synovial membrane of the small and large joints [1].

The body can synthesize most of fats it needs, however the Omega-3 (n-3) and omega-6 (n-6) are two essential fatty acids that the body cannot produce and must acquire from diet. [2]

Diets high in n-6 have been linked to an increased risk of chronic diseases linked to inflammation [3]. Increased n-3 intake, on the other hand, appears to lessen the inflammatory state [4].

These two types of poly unsaturated fatty acids (PUFAs) have been linked to the prevention and treatment of chronic inflammatory diseases.The optimal dietary intakes of the n-6: n-3 ratio should be around 1–4: 1. As a result of bad nutrition habits and consuming fast food, the ratio has now increased to be within the range of 10: 1 to 20: 1. This high ratio may play a role and contribute to the rise in the prevalence of inflammatory diseases like Rheumatoid arthritis [5].

A study, but on the other hand, discovered a significant inverse relationship between n-6 PUFA linoleic acid (LA) levels and pre-RA [6]. As a result, detecting the profile levels of different types of PUFA in rheumatoid arthritis and their association with disease activity is a major concern. PUFA are bound in cell membranes, and the fatty acid composition of red blood cell membranes reflects the 2–3 months dietary intake, as opposed to the profile of plasma fatty acids, which fluctuates based on daily dietary intake. As a result of the stability of RBCs over time within a person, the detection of these fatty acids in RBCs is more accurate than in plasma, and the evaluation is unaffected by fasting status [7,8,9]. Nonetheless, few studies have been conducted on this topic, particularly in Arab countries.

Consequently, it is an issue of interest to detect actual levels of different types of polyunsaturated fatty acids in RBCs cell membrane in cohort RA patients and their association with the disease activity and seropositivity.

Aim of work

The aim of this study is to determine the levels of various polyunsaturated fatty acids in erythrocyte membranes in the RA cohort group versus the control group. In addition, to study the relation between their levels and activity as well as seropositivity.

Subject and Methods

Study design and Subjects:

This is a case-control study.

The ethical committee of Mansoura University's Faculty of Medicine approved the study, and the code number was Ms.18.04.119.

The study enlisted the participation of forty RA patients.. All selected patients fullfilled the criteria proposed by the EULAR/ACR in 2010 for the diagnosis of RA [9]. They were recruited from the rheumatology and rehabilitation department's outpatient clinics at Mansoura University Hospitals between August and October of 2018. The ages of the patients ranged from 18 to 50 years. Patients with other autoimmune disorders, chronic heart problems, metabolic

diseases, cancer, or infections were excluded from the study. As a control group, an additional gender and age matched 40 normal healthy population was included. The study's goal was explained to all participants, and they all agreed to take part and signed the consent form.

Patients' assessement:

A clinical evaluation was performed after taking each patient's history. The 28 joint count disease activity score (DAS28) - ESR was used to assess disease activity. This score was calculated using swollen joint count and tender joint count, as well as the patient's overall health rating as a 100 mm visual analogue score (VAS). A DAS 28 of 2.6 indicates remission, a DAS 28 of (2.6-3.2) indicates low disease activity, a DAS 28 of (3.2-5.1) denotes moderate disease activity, and a DAS 28 of >5.1 indicates high disease activity [10].

Health assessment questionnaire (HAQ) [11]. It was modelled after the functional classes of the American Rheumatism Association/American College of Rheumatology .There is a four-level difficulty scale for each item, with scores ranging from 0 to 3, denoting; (0) normal (no problem) (1) moderate difficulty, (2) major difficulty and (3) impossibility. There are 20 questions grouped into eight functional categories: clothing, rising, eating, walking, hygiene, reach, grip, and regular activities. In the absence of such aids or gadgets, the category score is determined by the component with the highest score in each of the many categories. A low score is bumped up to a level 2 when a person is dependent on equipment or physical assistance. The HAQ-DI scores range from 0 (no disability) to 3 (the highest possible score) (completely disabled).Rheumatoid factor (RF) and anti-citrullinated peptide (anti ccp) levels, ESR as well as a complete blood count, were measured.

The levels of omega-6 and omega-3 in RBC membranes were determined using GC – Mass Spectrometry (GCMS) [12]. All blood samples (5 ml) were collected by sterile venipuncture from each individual in the morning, these samples were collected in tubes containing Na Citrate. Then, samples were centrifuged to separate plasma above, leaving cells down. Later on, RBCs were separated from WBCs and platlets manually using n-hexan and methyl-propan. Samples were kept at 18^{o}C until they sent to cairo in nuclear energy authority for measuring these fatty acids by **(GCMS)**.

GCMS analysis

The chemical composition of the samples performed using Trace GC 1300-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness), the column oven temperature was initially held at 40°C and then increased by 6°C /min to 210°C withhold 2 min then increased to 310 with 12 C/min and hold for 3 min, the injector temperature were kept at 220°C. A 1 ml/min flow rate of helium was used as a carrier gas. Autosampler AS3000 in split mode was used to inject diluted samples of 1 l with a 2 minute delay in the solvent. T-SRM Scan EI mass spectra were acquired at 70 eV and the ion source and transfer line temperatures were set at 230°C and 280°C, respectively.

Statistical analysis:

SPSS for Windows version 20.0 was used for all statistical analyses (SPSS, Chicago, IL). All continuous data had a normal distribution and

were expressed as mean standard deviation (SD). Numbers and percentages were used to express categorical data. For two variables with continuous data of normal distribution, the comparisons were determined using the Student's t test. For comparing variables with categorical data, the Chi-square test was used. For evaluating the correlation between two variables with continuous data, the correlation coefficient test was used. The statistical significance level was set at p0.05.

Results:

Table (1) the comparison of patients with RA and a control group:

			Student's t test	
	RA patients (40)	Control group (40)	T	P
Age (years)				
Range	26 – 49	28 – 48		
Mean ±SD	41.4 ±6.8	39.8 ±5.9	1.089	0.279
Sex (n, %)				
Females	34, 85.0%	30, 75.0%		
Males	6, 15.0%	10, 15.0%	1.250	0.264
Body mass index (BMI) (kg/m^2)				
Range	22.5 – 33.8	22.2 – 33.7		
Mean ±SD	28.1 ±3.4	28.2 ±3.7	0.174	0.863

A comparison of the patient and control groups' ages, genders, and BMIs revealed no significant differences (Table 1). (P: 0.279, 0.264 and 0.863 respectively)

Table (2) Comparison between levels of n-6, n-3 and n-6/n-3 ratio between RA patients and healthy controls:

			Student's t test	
	RA patients	Controls	T	P
Omega-6 FAs (ng/dl)				
Arachidonic acid				
Range	17.5 – 23.7	8.6 – 24.6		
Mean ±SD	18.3 ±1.5	14.1 ±3.6	6.811	<0.001
Linoleic acid				
Range	111.6 – 185.9	55.3 – 128.7		
Mean ±SD	133.1 ±20.2	92.3 ±18.2	9.490	<0.001
Dihomo-gamma-linolenic acid				
Range	25.2 – 62.1	11.5 – 37.9		
Mean ±SD	44.8 ±7.3	23.4 ±6.4	13.941	<0.001
Gamma-linolenic acid				
Range	7 – 21	4.5 – 14.5		
Mean ±SD	13.7 ±4.1	9.7±3.1	4.922	<0.001
Docosadienoic acid				
Range	15.5 – 22.9	6.1 – 15.8		
Mean ±SD	19.0 ±2.4	11.6 ±2.6	13.227	<0.001
Omega-3 FAs (ng/dl)				
Docosahexaenoic acid				
Range	11.1 – 15.4	19.7 – 25.2		
Mean ±SD	13.4 ±1.3	23.3 ±1.1	36.768	<0.001
Docosapentaenoic acid				
Range	18 – 20	28 – 30		
Mean ±SD	18.8 ±0.7	28.8 ±0.6	66.964	<0.001
Omega-6/omega-3				
Range	5.8 – 9.3	1.7 – 4.0		
Mean ±SD	7.1 ±2.2	2.9 ±0.9	11.175	<0.001

There was a substantial rise in the level of n-6 and the n-6: n-3 ratio in RA patients compared to healthy controls (P< 0.001), whereas there was a significant drop in the level of n-3 in RA patients compared to healthy controls (P< 0.001).

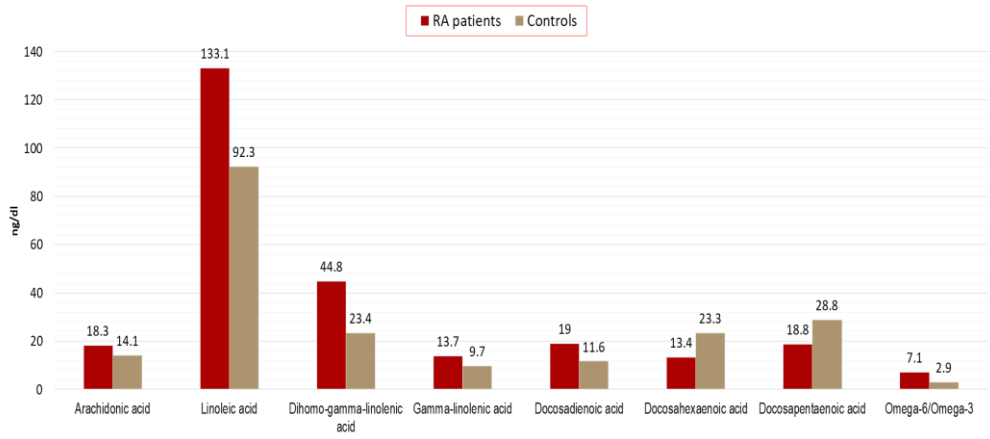

Figure 1. Level of omega-6, omega-3 and omega-6/omega-3 ratio in RA patients and control group.

Correlation of n-6, n-3 as well as n-6/n-3 ratio and disease activity of RA patients:

I Correlation of n-6, n-3 and n-6/n-3 ratio in RA patients with ESR.

There was a positive significant correlation between ESR level and levels of n-6, n-3 and n-6/n-3 ratio while there was a negative significant correlation between ESR and *levels of n-3* (docosahexaenoic acid and docosapentaenoic acid) as showm in figure (2).

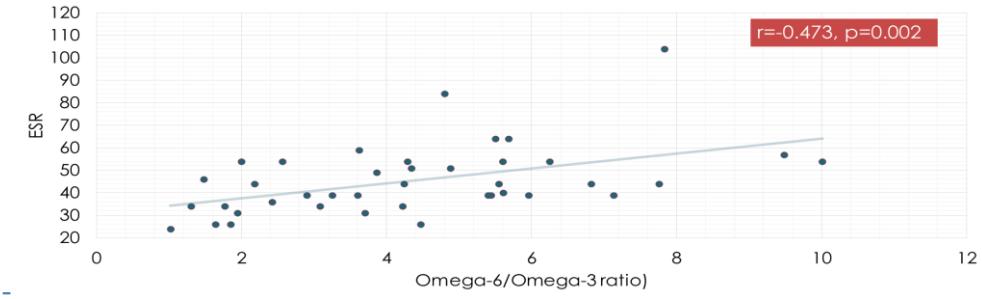

Figure2. Correlation of omega-6/omega-3 ratio in RA patients with ESR.

Table (3) Correlation of n-6, n-3 levels and n-6/n-3 ratio with DAS 28 and HAQ score in RA patients

	DAS28-ESR		HAQ score	
Variable	R	P	R	P
n-6 Fatty acids				
Arachidonic acid	-0.167	0.303	-0.123	0.448
Linoleic acid	-0.163	0.314	0.080	0.623
Dihomo-γ-linolenic acid	0.114	0.483	-0.111	0.494
Gamma-linolenic acid	0.169	0.298	-0.109	0.504
Docosadienoic acid	0.043	0.792	0.087	0.593
n-3 Fatty acids				
Docosahexaenoic acid	0.242	0.132	0.138	0.395
Docosapentaenoic acid	0.303	0.057	0.224	0.166
n-6/n-3	0.421	0.007	0.343	0.030

The omega-6/omega-3 ratio appeared to be the only polyunsaturated fatty acid parameter with a significant positive relationship to DAS28 –ESR and HAQ.

Table (4) Correlation between n- 3 and n- 6 levels and both rheumatoid factor (RF) and anti citrullinated (anti-CCP) antibodies:

PUFA		RF	Anti-CCP
Arachidonic acid (omega 6)	R	.050	-.006
	P	.767	.972
Linoleinic acid (omega 6)	R	.057	.296
	P	.734	.071
Di homo -γ-linolinic acid (n- 6)	R	.005	-.073
	P	.977	.663
gamma-Linolenic acid (n-6)	R	-.112	-.193
	P	.639	.415
Docosadienoic acid (n-6)	R	.146	-.023
	P	.380	.890
Docosahexaenoic acid (n-3)	R	.295	.106
	P	.096	.557
Docosapentaenoic acid (n-3)	R	.300	.197
	P	.096	.280

Table (5) Correlation between n- 3 and n- 6 levels and n6/n3 ratio:

		RF	ANTICCP	RATIO
RF	r	1.000		
	p	.		
	N	42		
ANTICCP	r	.184	1.000	
	p	.244	.	
	N	42	42	
RATIO	r	-.069	.103	1.000
	p	.665	.515	.
	N	42	42	42

The n-6, n3 levels and n-6/ n-3 ratio had an insignificant correlation with both anti CCP and RF antibodies after further correlation analysis was done.

Discussion:

Different forms of polyunsaturated fatty acids can influence the inflammatory response. Omega-3 fatty acids have been demonstrated to decrease or regulate the inflammatory response. While eicosapentaenoic acid can be synthesised from n-6 by desaturating linoleic acid and is primarily converted into arachidonic acid which stimulate inflammatory response [13].

The role of n-3 fatty acids as suspected RA-protective factors has been examined in numerous research. The risk of RA was shown to be reduced in individuals who consumed more fatty fish (>8 g fat/100 g fish) than in those who consumed less fatty fish (between 3 and 7 g fat/100 g fish) in a Danish prospective cohort study. Women who consumed more than 0.21 g/day of long-chain n-3 polyunsaturated fatty acids had a 35% decreased chance of having RA[14].

Based on this analysis, GC mass spectrometry in RBC membranes is required to accurately detect the plasma levels of various types of polyunsaturated fatty acids in a group of Egyptian RA patients. To discover if their levels in patients' plasma have a role in rheumatoid arthritis development. As well as which types are most common and whether their levels are linked to disease activity.

In this study, high levels of n- 6 were detected in RBCs membrane in RA patients; (Arachidonic acid, Linoleic acid, Dihomo-gamma-linolenic acid, Gamma-linolenic acid and Docosadienoic acid):18.3 ±1.5, 133.1 ±20.2, 44.8 ±7.3, 13.7 ±4.1, 19.0 ±2.4 respectively. While lower levels of n- 6 were observed in healthy control group: 14.1 ±3.6, 92.3 ±18.2, 23.4 ±6.4, 9.7±3.1 and 11.6 ±2.6 respectively.

Low levels of n-3 were observed in RBCs membrane in RA patients: Docosahexaenoic acid and Docosapentaenoic acid 13.4 ±1.3 and 18.8 ±0.7. Higher levels of n- 3 were detected in healthy control group: 23.3 ±1.1 and 23.3 ±1.1.

These findings were similar to those of Lee and Park (2013) [15], who discovered that erythrocyte levels of eicosapentaenoic, -linolenic acids, and n-3 index (DHA + EPA) were significantly lower in RA patients than in healthy controls.

In this study we discovered significant differences in RBC membrane n-6, n-3, and n-6/n-3 ratios between RA patients and normal controls.

This work detected a positive significant correlation between ESR level and n -6 levels as well as n-6/n-3 ratio. On the otherhand, a negative significant correlation was detected between ESR and omega -3 levels (docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)). In addition, Docosapentaenoic acid and DAS28-ESR showed a positive correlation. Furthermore, the omega-6/omega-3 ratio was found to have a positive correlation with DAS28 –ESR and HAQ.

These results indicated correlation between these fatty acids and inflammation of the disease. This is consistent with the results of experimental study performed by **Olson et al., 2013** [16] who demonstrated that DHA administration, when started 4 weeks before arthritis induction, reduced the incidence and severity of arthritis, whereas DHA/Eicosapentaenoic acid (EPA) combination did not. DHA also reduced pannus formation, cartilage destruction, bone damage, pro-inflammatory cytokine levels, and anti-collagen antibodies, whereas the DHA/EPA combination had no beneficial effect on synovial histopathology or anti-collagen antibody production.

On the other hand , **Bärebring et al., (2018)** studied the relation between diet quality and inflammation among RA patients . Their study involved 66 subjects, each completed a FFQ at screening .The activity of the disease was evaluated as DAS28 based on ESR , CRP , patients underwent VAS of perceived global health , the number of swollen and tender joints out of 28 evaluated .They found that higher dietary quality rich in n - 3 than n- 6 in patients with RA was associated with lower ESR and CRP only but not with the disease activity including DAS-ESR or DAS-CRP [17].

In the present study , there were no significant associations between levels of n-3 in RA patients and RF or anti-CCP positivity. This was in agreement with **Rodríguez-Carrio et al., (2016) [18]** as they found that both types of FAs had no effect on either RF or anti-CCP positivity.

On the other hand , a nested case control study with 40 anti-CCP positive patients, 69 controls negative for RF and anti-CCP, and 27 participants positive just for high titre of RF, which was demonstrated to be a powerful predictor of future RA, was carried out. They discovered an inverse relationship between anti-CCP, RF positivity, and n-3 FAs in RBC membranes. This could imply that omega-3 fatty acids protect against RA-related autoimmunity **[19]**.

Conclusion:

It was shown that the n-6, n-3, and n-6/n-3 ratios of the RBC membranes of RA patients and healthy controls differed significantly. In RA, RBC membranes contained low levels of n-3 and high levels of n-6. The omega-6/omega-3 ratio appeared to be the only polyunsaturated fatty acid parameter with a significant positive relationship to DAS28 – ESR and HAQ. Both n-3 and n-6 showed insignificant link with both RF and anti CCP antibodies.

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