

## Trends of inflammatory markers and cytokines after one month of phototherapy in patients with rheumatoid arthritis

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### Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by joint pain, swelling, stiffness, and progressive destruction of the small joints. Patients with RA should be treated appropriately in order to improve symptoms and inhibit structural joint damage. Treatment of RA has improved over the past decade. Of the treatment options available, Non-steroidal

**Objective.** to evaluate changes in the expression of tumor necrosis factor- $\alpha$  in patients with rheumatoid arthritis submitted to phototherapy. **Materials and methods.** This was an open label study, enrolling ten patients. The phototherapy scheme within a range of 425 to 650 nm, 11.33 Joules/cm<sup>2</sup>, 30 cm above the chest was as follows: a) 45-min daily sessions from Monday to Friday for 2 to 3 months; b) three, 45-min weekly sessions for 1 to 2 months; c) twice weekly 45-min sessions for 1 to 2 months, and d) one weekly session for 1 to 2 months until completion. Erythrocyte sedimentation rate, C-reactive protein and rheumatoid factor were measured in peripheral blood and tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and interleukin-10 in leukocytes by quantitative real-time Reverse transcriptase-Polymerase chain reaction. In all the patients the next indexes: Karnofsky scale, Rheumatoid Arthritis-specific quality of life instrument, Steinbrocker Functional Capacity Rating and the Visual Analog Scale were evaluated. **Results.** Erythrocyte sedimentation rate, C-reactive protein, and rheumatoid factor declined notoriously after the indicated sessions. In gene expression, there was a tendency in tumor necrosis factor- $\alpha$  to decrease after 1 month, from 24.5 $\pm$ 11.4 to 18 $\pm$ 9.2 relative units, without reaching a significant statistical difference. The four tested indexes showed improvement. **Conclusion.** Phototherapy appears to be a plausible complementary option to reduce the inflammatory component in rheumatoid arthritis.

anti-inflammatory drugs (NSAID) are the most widely used agents for symptomatic treatment. However, these drugs have several adverse effects (1). More options are low-dose glucocorticoids which have a modifying effect on structural damage in early RA (2) and disease-modifying antirheumatic drugs (DMARDs) such as hydroxychloroquine, methotrexate (MTX), leflunomide, cyclosporine, sulfasalazine, azathioprine, cyclophosphamide and biologics (3, 4). To

date, anti-Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) agents represent a milestone in RA treatment (5-7).

Although new therapies are increasingly available (8), a significant unmet medical need continues for patients with RA who have had inadequate response to prior treatments and require safe and effective therapy using a different mechanism of action. Alternative and complementary treatments for RA have been explored in many countries. One of these alternatives is phototherapy with diverse light spectra including Ultraviolet (UV), laser Photodynamic therapy (PDT), Light emitting diodes (LED) etc. (9, 10).

Our main objective was to evaluate the changes in the expression of TNF- $\alpha$ , Interleukin (IL)-1 $\beta$  and IL-10 in patients with RA while they are being submitted to a complementary treatment with phototherapy.

## Methods

### *Study population*

This was an open label study to evaluate the effect of phototherapy in patients with RA who met the criteria established by the American College of Rheumatology (ACR) (11). We included consecutive patients attending the Research Department, of the Maternal-Perinatal Hospital "Mónica Pretelini Sáenz" (HMPMPS), Health Institute of the State of Mexico (ISEM), Toluca, Mexico, in the period 2010-2012. Those with fewer than four criteria of the ACR, previous fractures, chronic diseases that limit functional capacity, other arthropathies, overlap syndromes, and no agreement of the patient to participate in the study were excluded. Patients who failed to comply with the phototherapy treatment program or severe disease progression were discarded. There was no any kind of restriction to the prescribed pharmacotherapy.

### *Phototherapy*

With the patient in supine position, after registering vital signs (blood pressure, heart rate, respiratory rate, and temperature), weight, height, and capillary glucose determination, we proceeded to place the phototherapy lamp (Federal Ministry of Health registration number: 1694E95) within a range between 425 and 650 nm, 11.33 Joules/cm<sup>2</sup>, 30 cm above the chest. The decision of placing the lamp above the chest was based on the location of the great vessels and the thymus as the aim of the treatment was to get a systemic effect rather than a local one.

The phototherapy scheme was the following: a) 45-min daily sessions from Monday to Friday for 2 to 3 months; b) three 45-min sessions per week for 1 to 2 months; c) twice weekly 45-min sessions for 1 to 2 months, and d) one weekly session for 1 to 2 months until completion. Weekly frequency and progressive reduction of phototherapy sessions were determined according to the patients' own improvement.

### *Clinical follow-up*

The patients' data was obtained from their medical history. Clinical evaluation was performed by the Research Team Leader once weekly, including the next indexes: Karnofsky scale, Rheumatoid Arthritis-specific quality of life (RAQoL) instrument, Steinbrocker Functional Capacity Rating and the Visual Analog Scale (VAS). An essential aspect of the study was absolute respect for the management, evaluation, and subsequent appointments instituted by the treating Rheumatologist. Phototherapy alone was considered for naïve patients with contraindications for the antirheumatic drugs.

### *Biochemical assessment*

Laboratory tests were done in the first clinical visit and 4 weeks after the initiation of

treatment. Fasting blood samples (10 ml) were taken at the HMPMPS Laboratory at an early-morning after an overnight fast. Serum samples were analyzed for globulin (Dimension Rx L Max, Dade Behring, USA), hemogram (Advia 120, Bayer Health, USA), Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and Rheumatoid factor (RF). All these tests were measured according to standardized procedures recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

#### *Leukocyte collection and storage*

Blood samples were taken by venipuncture in tubes (Vacutainer) containing Ethylenediaminetetraacetic acid (EDTA) as anticoagulant for subsequent centrifugation at 2,500 rpm for 10 min. Using a 1-ml micropipette, leukocytes were separated and deposited in sterile Eppendorf® 1.5-ml tubes. Once separated, the leukocytes were purified in 1 ml Red blood cell (RBC) ACK Lysing buffer (ACK), stirring by gentle inversion and allowing these to sit for 1 min at 30°C. Following this, they were centrifuged at 2,000 rpm for 5 min, the supernatant was discarded, and 1 ml of ACK was again added, repeating the process until visualizing a pellet without RBC. The final step was the addition of 100 µl of phosphate buffer and resuspension for further storage at -80°C (Forma -86°C ULT Freezer, Thermo Electron Corporation, USA) until analysis, which was carried out in the Laboratory of Molecular Biology, Medical Sciences Research Center (CIC-MED), Autonomous University of the State of Mexico (UAEMex).

#### *Gene expression*

Messenger RNA (mRNA) was isolated using the Magna Pure LC RNA Isolation Kit III

and retrotranscribed with the Transcriptor High Fidelity cDNA Synthesis Kit (Roche) to obtain complementary DNA (cDNA). Samples were then quantified using a nanophotometer set (Nano Photometer, Implen) at two wavelengths (260 nm and 280 nm), with an acceptable degree of purity between 1.8 and 2.

Quantitative real-time Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR) was performed in a 7500 Fast Real Time PCR System (Applied Biosystems, Applied Biosystems, Cheshire, UK), using the TaqMan® Gene Expression Assays (Life Technologies, USA) for TNF-α (Catalog #4331182), IL-1β (Catalog #4331182), and IL-10 (Catalog #4331182), following the manufacturer's instructions. The relative expression of these genes was calculated through the  $2^{-\Delta\Delta CT}$  method against 18S (NCBI: NC\_000012.11) as follows: fw: 5'-ctttggtatcgtggaaggactc-3', and rv: 5'-gtagaggcagggatgatgttct-3' (Catalog #Hs99999901-s1; Life Technologies).

#### *Ethics statement*

The protocol was approved by the Research Committee of the HMPMPS (November 2010) and followed the Declaration of Helsinki indications. All patients were asked to sign written informed consent.

#### *Statistical analysis*

Descriptive analysis, Wilcoxon test to compare whether the group presented differences through time and Spearman correlation were performed with the SPSS v. 17 program. A difference was considered significant at  $p \leq 0.05$ . Results are expressed as absolute numbers, means  $\pm$  standard deviation (SD) for all variables except TNF-α, IL-1β and IL-10 that are expressed as absolute numbers, means  $\pm$  standard error (SE).

## Results

### Anthropometric data

A total of 10 women, mean age of 41.2±8.8 years, with RA were enrolled for this study. Mean time within the protocol was 140±7.7 days. The average number of antirheumatic drugs that patients were taking at the time of the initiation of the protocol was of 3.7 (Table 1).

### Clinical evolution

Table 2 shows the results of the evaluated scales. While the Karnofsky increased 1.3 times, the RaQol questionnaire was reduced by almost half. Additionally, the Steinbrocker Functional Capacity Rating improved from Class III to Class II. Finally, the VAS showed a reduction in pain from “dreadful” to “annoying”.

### Laboratory analysis

There were no differences in the hematological evaluation. The acute inflammation variables declined notoriously after the indicated sessions. In gene expression, there was a tendency in TNF-α to be decreased after 1 month, from 24.5±11.4 to 18±9.2 relative units without reaching significant statistical difference. Neither IL-1β nor IL-10 showed significant statistical differences (Table 2).

IL-1 and IL-10 showed a significant negative correlation in their trends (-0.829, p=0.042). On the contrary, there was a positive correlation in the reduction values of TNF and the VAS (0.894, p=0.041).

The Karnofsky scale showed a negative correlation with the RaQol (-0.851, p=0.002) and VAS (-0.757, p=0.011) scales. The last scale showed a positive correlation with RaQol (0.665, p=0.036), Steinbrocker Functional Capacity Rating (0.774, p=0.009) and with the TNF-α reduction (0.894, p=0.041).

Table 1 General characteristics of the population

Case	Age*	Disease duration†	Concomitant treatment
1	41	4	Metamizol 500 mg/day PO‡; Paracetamol 650 mg/day PO; Ketorolac 30 mg/day IM‡
2	47	60	Methotrexate 10 mg/week PO; Hydroxychloroquine 200 mg/day PO; Acemetacin 60 mg/12 h PO; Methylprednisolone 40 mg bimonthly IM; Diclofenac 75-150 mg/day PO
3	32	58	Methotrexate 15 mg/week PO; Sulfasalazine 500 mg/12 h PO; Diclofenac 100 mg/12 h PO
4	41	9	Prednisone 10 mg/day PO; Diclofenac 100 mg/12 h PO; Paracetamol 500 mg/12 h PO; Meloxicam 15 mg IM only 2 doses
5	44	59	Methotrexate 5 mg/week PO; Deflazacort 2 mg/day PO; Diclofenac 100 mg/12 h PO
6	44	45	Methotrexate 15 mg/week PO; Sulfasalazine 500 mg/8 h PO; Prednisone 20 mg/day, 10 days/month PO; Acemetacin 60 mg/12 h PO; Fluoxetine 20 mg/day PO
7	48	34	Leflunomide 20 mg/day PO; Sulfasalazine 500 mg/12 h PO; Diclofenac 100 mg/12 h PO
8	42	2	Leflunomide 20 mg/day PO; Hydroxychloroquine 200 mg/day PO; Deflazacort 3 mg/day PO; Naproxen 275 mg/12 h PO; Paracetamol 300 mg/12 h PO
9	21	17	Prednisone 75 mg/day P.O. §Dexamethasone 8 mg IM only 4 doses; §Ibuprofen 400 mg/12 h PO
10	52	120	Chloroquine 150 mg/12 h PO; Sulfasalazine 2.5 g/day PO; Diclofenac 100 mg/day PO

\*Years; †Months; PO=oral administration; IM=intramuscular; ‡Self-medication, §This treatment was suspended 7 months previous to the study due to pregnancy.

Table 2 Clinical and laboratorial characteristics

Characteristics	Values of laboratory tests		p value
	Basal	After <sup>†</sup>	
Hb (g/dl)*	12.4±1.6	12.7±1.6	0.134
Ht (volume %)*	38.6±4	39.35±4.9	0.386
Leukocytes (cells/mm <sup>3</sup> )*	6390±1757	6060±1509	0.327
Granulocytes (cells/mm <sup>3</sup> )*	4554±1561	4302±1414	0.285
Lymphocytes (cells/mm <sup>3</sup> )*	1360±536	1270±309	0.386
Monocytes (cells/mm <sup>3</sup> )*	284±80	296±88	0.203
CRP (titers)*	1:204 ±169	1:122 (±186)	0.008
ESR (mm/h)*	45±14	38.6±13	0.017
Rheumatoid factor (titers)*	1:808 ±1554	1:178 (±387)	0.026
IL-1 $\beta$ (RU) <sup>†</sup>	0.23±0.18	0.66±0.32	0.917
IL-10 (RU) <sup>†</sup>	1.6±1.2	0.58±0.32	0.374
TNF- $\alpha$ (RU) <sup>†</sup>	24.5±27.9	18±22.6	0.249
Karnofsky scale (points)*	46±17.1	64±12.6	0.005
RAQoL (points)*	25.7±8.5	13.9±6.1	0.001
VAS (points)*	6.9±2.4	3.2±2.2	0.001

\*Mean±SD; <sup>†</sup>Mean±SE; <sup>‡</sup>One month of phototherapy; CRP=C reactive protein; ESR=Erythrocyte sedimentation rate; Hb=Haemoglobin; Ht=Haematocrit; IL=Interleukin; RAQoL=Rheumatoid Arthritis-specific Quality of Life; RU=Relative units; TNF- $\alpha$ =Tumor necrosis factor- $\alpha$ ; VAS=Visual Analogue Scale.

## Discussion

DMARDs, TNF inhibitor treatments, or their combination are considered first-line treatment (12) in RA, but collateral effects such as hepatotoxicity and nephrotoxicity are commonly associated with the former option. In addition, immunosuppression predisposes to increased risk of infection, the potential for development of certain types of malignancy, as well as the significant increased cost of therapy.

It has been demonstrated that UV radiation poses an immunosuppression effect due in part to changes in the expression of IL-10, transformation of urocanic acid from its trans to cis isomer, and induction of CD4+ CD25+ T regulatory cells (9). Evidence indicates that phototherapy exerts a significant impact on neutrophils, the effect of which varies according to the specific type of phototherapy (13).

A previous study by Goats et al. tested the therapeutic effects of combined low-intensity laser and phototherapy upon the articular, systemic, and functional sequelae of RA affecting weight-bearing joints without finding significant differences between the active or placebo cohorts (14). To date, the majority of trials have unsuccessfully tested laser therapy with a wide range of parameters in Joules and wavelength (15). Contrariwise, the present study noted a tendency of TNF decrement.

In mice models, LED irradiation has been effective for inhibition of the inflammatory reactions caused by RA within a period of four weeks (16); but to the best of our knowledge, the present study is the first specifically designed to investigate the short-term effect of visible light and changes in peripheral TNF- $\alpha$ , IL-1 $\beta$ , and IL-10 expression. Despite the lack of significant differences after the month of follow up, we

did find significant correlation between the clinical improvement and the reduction in TNF- $\alpha$ .

The primary cellular effects of phototherapy are related with the interaction of photons and the intracellular molecules that absorb them, i.e., the cytochromes. Visible light is absorbed by cytochromes, many of which are located in the mitochondria. It has also been postulated that light can act as a catalyst, influencing molecules, organelles, and cells without being absorbed (17). Whether or not the primary effects of light induce the changes in inflammatory variables is a matter that has yet to be discerned. In an initial attempt, this study shows a possible reduction in TNF- $\alpha$  secondary to the phototherapy sessions, but more studies are needed to clarify this issue.

A limitation of this study is the low number of participants. Notwithstanding, the quantification of gene expression of inflammatory markers along with the clinical scale evaluation, gives a good support for a possible recommendation of phototherapy in RA. Another limitation of this study in terms of the interest for the clinician is that we didn't collect any activity index of the disease such as DAS28 or SDAI which are the topic of a new project.

## Conclusion

Phototherapy could be useful to treat patients with RA, which is clearly evident with the reduction in the following inflammatory markers: ESR, CRP, and RF. Due to scarce knowledge on the mechanism of action of phototherapy, the results should be treated with caution.

### What is already known on this subject

*Rheumatoid arthritis (RA) is a chronic and painful inflammatory disease. To date, anti-Tumor necrosis factor- $\alpha$  agents represent a milestone of RA treatment in addition to non-steroidal anti-inflammatory drugs (NSAID), low-dose glucocorticoids, and Methotrexate. Despite the development of new pharma-*

*cological therapies, alternative and complementary treatments for RA have been explored extensively.*

### What this study adds

*As a complementary option, phototherapy with diverse light spectrums has shown potential clinical utility in treating RA. In this line, we evaluated the effect on inflammatory markers in RA after 1 month of phototherapy within a range of 425 to 650 nm, 11.33 Joules/cm<sup>2</sup>, yielding a reduction in the levels of Erythrocyte sedimentation rate, C-reactive protein, and rheumatoid factor.*

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**Authors' contributions:** Conception and design: JMC, HMZ; Phototherapy lamp design: GAH; Molecular studies: ASAB, MCCF; Acquisition, analysis and interpretation of data: JMC, HMZ, IGS; Drafting the article: JMC, HMZ; Revising it critically for important intellectual content: JMC, HMZ, MCF.

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