

ASSESSMENT OF SOME OXIDATIVE STRESS PARAMETERS IN METHOTREXATE TREATED PSORIASIS PATIENTS

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Abstract

Psoriasis is a chronic inflammatory skin disease. Reactive oxygen species produced in result of skin inflammation may cause disorders of the antioxidant defence systems and increased oxidative stress in psoriasis. The aim of the study was the assessment of certain redox stress parameters in methotrexate (MTX) treated psoriasis patients compared to a control group.

The study included 28 patients diagnosed with psoriasis according to clinical criteria and included into groups according to Psoriasis Area and Severity Index (PASI) score and treated with MTX. The control group included 32 healthy volunteers. We assessed the plasma levels of malondialdehyde (MDA) and total antioxidant status (TAS) (spectrophotometrically measured), as well as the PASI score of clinical progress.

MDA was significantly higher in psoriasis patients (640.28 ± 160.58 nmol/ μ L for MTX treated patients – on initiation, 540.20 ± 114.25 nmol/ μ L for MTX treated patients – at 24 weeks after initiation *versus* 490.20 ± 75.28 nmol/ μ L in the control group of patients $p=0.01$); TAS level was lower in psoriasis patients as compared to control patients (1.88 ± 0.28 mmol/mL – on MTX initiation, 2.54 ± 0.32 mmol/mL at 24 weeks after initiation *versus* 2.84 ± 0.22 mmol/mL – in the control group, $p=0.01$).

The pathogenic mechanism involved in the development of psoriasis seems to produce disorders of the endogenous antioxidative systems. MTX treated psoriasis patients show lower oxidative stress activity than untreated psoriasis patients.

Rezumat

Psoriazisul este o afecțiune dermatologică inflamatorie cronică. Speciile oxigen reactive produse ca urmare a inflamației de la nivel tegumentar pot determina tulburări la nivelul sistemului de apărare antioxidant și intensificarea stresului oxidativ în cazul psoriazisului. Scopul prezentului studiu a fost evaluarea unor parametri de stres oxidativ la pacienții cu psoriazis tratați cu metotrexat (MTX), comparativ cu un grup control.

Studiul a inclus 28 pacienți diagnosticați cu psoriazis conform criteriilor clinice, clasificați în funcție de scorul PASI (Psoriasis Area and Severity Index) și tratați cu MTX. Grupul control a inclus 32 de voluntari sănătoși. Au fost evaluate nivelurile plasmatiche de

MDA (malondialdehida), TAS (statusul total antioxidant) (printr-o metodă spectrofotometrică) și de asemenea, scorul PASI privind evoluția clinică.

Nivelul plasmatic de MDA a fost semnificativ mai mare la pacienții cu psoriazis (640.28 ± 160.58 nmol/ μ L la pacienții tratați cu MTX – la începutul studiului, 540.20 ± 114.25 nmol/ μ L la pacienții tratați cu MTX – la 24 săptămâni de la inițierea terapiei *versus* 490.20 ± 75.28 nmol/ μ L la pacienții din grupul control $p=0.01$); nivelul TAS a fost mai mic la pacienții cu psoriazis comparativ cu cei din grupul control (1.88 ± 0.28 mmol/mL – la inițierea terapiei cu MTX, 2.54 ± 0.32 mmol/mL la 24 săptămâni *versus* 2.84 ± 0.22 mmol/mL – grupul control, $p=0.01$).

Mecanismul patogenetic implicat în dezvoltarea psoriazisului determină tulburări la nivelul sistemelor antioxidante endogene. Pacienții tratați cu MTX prezintă un status al stresului oxidativ mai mic decât la pacienții cu psoriazis netratați.

Keywords: psoriasis, reactive oxygen species, methotrexate.

Introduction

The specialty literature indicates that oxidative changes determined by the imbalance between production of reactive oxygen species (ROS) in live organisms and the activity of endogenous antioxidative systems are associated with numerous pathologic conditions such as atherosclerosis, diabetes mellitus, neurodegenerative diseases, autoimmune rheumatic diseases (rheumatoid arthritis, systemic *lupus erythematosus*), as well as with skin inflammatory diseases such as psoriasis [7].

One way to provide protection in case of wounds and lesions is inflammation, an essential response and the first step of the healing process, which may explain the importance of correct resolution of inflammation, whereby chronic overreaction can be avoided leading to increased tissue damage. Inflammatory injuries may be worsened by ROS prolonged excessive release at the skin level, promoting chronic swelling. Therefore, there is a strict mechanism operated by a number of prooxidants and enzymatic antioxidants, which closely regulates cellular redox balance. Occurrence of chronic inflammation is an instance of antioxidant system depletion, allowing the prolongation of oxidative stress [11, 14].

On the whole, it may be appreciated that psoriasis is a recurrent, chronic inflammatory skin disease, of unknown aetiology and pathogenic nature, characterised by circumscribed erythematous, dry, scaling plaques of various sizes, covered by silvery white lamellar layers. Many of the cellular disturbances underlying such pathologic conditions are known to be determined by formation of lipid, protein or DNA peroxidation products. Thus, lipid peroxidation products (among which 4-hidroxy-nonenal and

malondialdehyde) are very reactive towards proteins, with which they form a wide range of inter- and intra-molecular adducts [3, 4].

Notwithstanding, oxidative stress may emerge in case of not enough ROS detoxification or ROS excessive production, leading to cellular damage [9].

The two ROS related instances are associated with generalised oxidative processes as well as with emergence of lipid peroxidation products in the plasma. Lipid peroxidation results in depletion of polyunsaturated fatty acid molecules at cellular and subcellular membrane level, leading to loss of cell integrity, structural and functional impairment of membranes and membrane receptors [12, 13].

The aim of the study was the assessment of certain redox stress parameters in methotrexate (MTX) treated psoriasis patients compared to a control group.

Materials and Methods

Study design

A clinical trial was conducted in the Dermatology Department of “Prof. Dr. N. Paulescu” - National Institut of Diabetes Nutrition & Metabolic Diseases and designed including two groups:

- the first group was represented by 28 subjects diagnosed with psoriasis according to clinical criteria, and included into the group according to Psoriasis Area and Severity Index (PASI) score [3], treated with methotrexate (MTX) in increased doses (up to 15mg/week); patients (aged ≥ 18 years old) had stable, moderate to severe plaque psoriasis for more than 3 months, PASI score over 10 at baseline screening;
- the second group consisted of 32 healthy volunteers, none of whom received corticosteroids or tumour necrosis factor (TNF)- α antagonists.

Patients with active guttate, erythrodermic, pustular psoriasis, other skin condition, severe renal, hepatic or haematological diseases, overt cardiovascular disease or malignancy were excluded from the trial.

The patients included in the trial and the healthy volunteers were provided with detailed explanations about the aims and procedures involved in the trial, their informed consent was obtained and also was provided the approval of the ethic committee.

Equipment: UV-VIS Cary 100 BIO absorption spectrophotometer (Varian Inc.), provided with Peltier temperature controller, adapted for biochemical assays.

Reagents:

Total antioxidant status (TAS) kit: The principle of the antioxidant assay is formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, ABTS^{•+}, a green, soluble chromogen that can be determined spectrophotometrically at 405 nm.

Malondialdehyde (MDA) kit: lipid peroxidation is determined by MDA reaction with thiobarbituric acid (TBA) to form a colorimetric (532 nm)/fluorimetric ($\lambda_{\text{ex}} = 532 / \lambda_{\text{em}} = 553$ nm) product, proportionally with the MDA present.

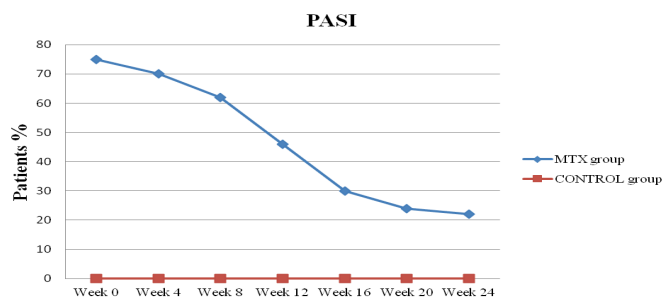
Sample preparation: Venous blood samples were collected à jeun, on anticoagulant (EDTANa₂), and allowed to rest for one hour. Next, plasma was separated by centrifugation at 2000rpm for 10 minutes and preserved at -20°C before analysis. Samples thus prepared were used for determination of plasmatic MDA and TAS. Results are presented as means±standard deviations and the statistical significance of determined parameters was assessed with the t Student test.

Results and Discussion

This trial aimed the determination of the MDA, TAS levels and PASI 75 at baseline and after 24 weeks, for 28 psoriasis diagnosed patients and a control group, represented by 32 healthy volunteers. Patients included in the trial were treated with MTX (n=28), assessed at the treatment initiation and after 24 weeks of treatment.

All patients enrolled in the study completed the study. Most patients were men (64.29%) and the mean age was 41.2 ± 12.32 years old. At week 24, 82.8% of patients in the treated group were receiving methotrexate 15 mg/week.

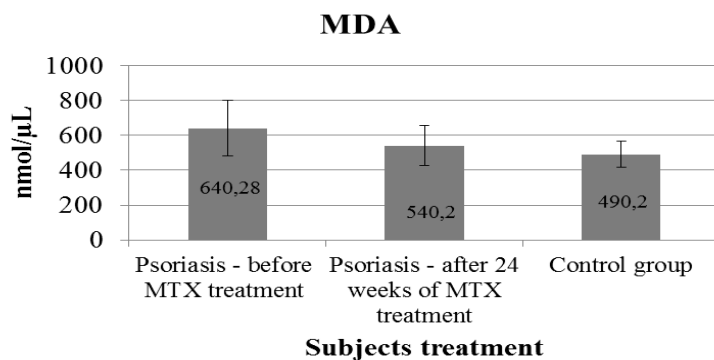
At week 24, PASI 75 response was higher for the treated group than for the control group and PASI 75 response at week 12 was also higher in the treated group than the control group (Figure 1).

**Figure 1**

PASI score in MTX treated psoriasis patients as compared to the control group

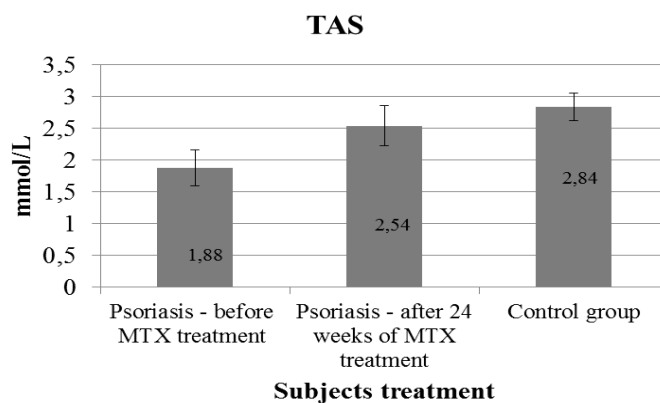
Statistical analysis of data revealed that MDA level (Figure 2) is high and TAS level (Figure 3) is lower as compared to subjects in the control group.

Outcomes related to lipid peroxidation susceptibility show MDA level values that are higher in psoriasis patients (640.28 ± 160.58 nmol/ μ L for MTX treated patients – on initiation, 540.20 ± 114.25 nmol/ μ L for MTX treated patients - after 24 weeks) as compared to the control group - 490.20 ± 75.28 nmol/ μ L, $p=0.01$) (Figure 2).

**Figure 2**

MDA level in MTX treated psoriasis patients as compared to the control group

Outcomes show that the TAS level was significantly lower in MTX treated psoriasis patients (1.88 ± 0.28 mmol/mL – on MTX initiation, 2.54 ± 0.32 mmol/mL – after 24 weeks) *versus* 2.84 ± 0.22 mmol/mL – in comparison to the control group, $p=0.01$ – but TAS level was higher in MTX treated patients after 24 weeks of treatment (Figure 3).

**Figure 3**

Total antioxidant status (TAS) in MTX treated psoriasis patients in comparison to the control group

Psoriasis is an inflammatory chronic disease of the skin and it consists of pathological cutaneous lesions induced by various factors of both exogenous and endogenous nature, accompanied by a range of immunological and biochemical disorders.

Tissues, cell membranes and biomolecules may suffer oxidative damage from higher production of free radicals [6, 10]. Oxidation of polyunsaturated fatty acids determined by free radicals leads to development of lipid peroxidation products, e.g. MDA [1, 5, 8].

Conclusions

Our study indicated that an increase in MDA level and a decrease in antioxidants levels are features of psoriasis. Clinical efficacy of MTX seems to correlate with the decrease of the oxidative status in the MTX treated group.

Although data are preliminary and based on a small number of subjects, MTX seems to have protective antioxidative properties during treatment of psoriasis.

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