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Higher nitric oxide levels are associated with disease activity in Egyptian rheumatoid arthritis patients

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ABSTRACT

Background: Oxidative stress generated within inflammatory joints can produce autoimmune phenomena and joint destruction. Radical species with oxidative activity, including reactive nitrogen species, represent mediators of inflammation and cartilage damage.
Objectives: To assess serum nitric oxide as a marker of oxidative stress in Egyptian patients with rheumatoid arthritis and its relation to disease activity.
Methods: Eighty patients with rheumatoid arthritis were divided into 2 groups, according to the DAS-28 score: Group I: 42 patients with disease activity, and Group II: 38 patients with no disease activity. Forty age- and sex-matched individuals were included as control group (Group III). Routine laboratory investigations were done, and nitric oxide was measured using Elisa. Hand plain radiographies were done for radiological status scoring using the Sharp method.
Results: A comparison between nitric oxide in all three groups showed a highly significant difference ($p < 0.001$), significantly higher levels were obtained among rheumatoid arthritis patients in comparison to controls, and higher levels were obtained in patients with active disease (mean ± SD 82.38 ± 20.46) in comparison to patients without active disease (35.53 ± 7.15). Nitric oxide in Group I showed a significant positive correlation with morning stiffness (r = 0.45), arthritis (r = 0.43), platelet count (r = 0.46), erythrocyte sedimentation rate (r = 0.83), C-reactive protein (r = 0.76) and Disease Activity Score (r = 0.85). Nitric oxide showed a significant positive correlation (r = 0.43) with hand radiographies (Sharp score) in Group I.
Conclusion: There are increased levels of nitric oxide in the serum of patients with rheumatoid arthritis. Nitric oxide correlates significantly with disease activity, inflammatory markers and radiological joint status.

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Níveis de óxido nítrico mais elevados estão associados à atividade da doença em pacientes egípcios com artrite reumatoide

Palavras-chave:
Artrite reumatoide (AR)
Estresse oxidativo
Óxido nítrico (NO)

RESUMO

Introdução: O estresse oxidativo produzido no interior de articulações inflamadas pode produzir fenômenos autoimunes e destruição articular. As espécies radicais com atividade oxidativa, incluindo espécies reativas de nitrogênio, representam mediadores de inflamação e de lesão cartilaginosa.

Objetivos: Avaliar o óxido nítrico sérico como marcador de estresse oxidativo em pacientes egípcios com artrite reumatoide e sua relação com a atividade da doença.

Métodos: Oitenta pacientes com artrite reumatoide foram divididos em dois grupos, de acordo com a pontuação DAS28: Grupo I: 42 pacientes com doença ativa, e Grupo II: 38 pacientes com doença inativa. Quarenta indivíduos equiparados por idade e gênero foram incluídos como grupo controle (Grupo III). Foram realizados exames laboratoriais de rotina e o óxido nítrico foi medido usando Elisa. Radiografias simples das mãos foram feitas para a pontuação do estado radiológico utilizando o método de Sharpe.

Resultados: A comparação do nível sérico de óxido nítrico entre os três grupos mostrou uma diferença altamente significativa ($p < 0{,}001$). Obtiveram-se níveis significativamente mais elevados entre os pacientes com artrite reumatoide em comparação com os controles. Os níveis mais elevados foram obtidos em pacientes com a doença ativa (média ± DP 82,38 ± 20,46) em comparação com aqueles com a doença inativa (35,53 ± 7,15). O óxido nítrico no Grupo I exibiu uma correlação positiva significativa com a rigidez matinal (r = 0,45), artrite (r = 0,43), contagem de plaquetas (r = 0,46), velocidade de hemossedimentação (r = 0,83), proteína C-reativa (r = 0,76) e Índice de Atividade de Doença (r = 0,85). O óxido nítrico mostrou uma correlação positiva significativa (r = 0,43) com as radiografias das mãos (índice de Sharpe) no Grupo I.

Conclusão: Observa-se um aumento nos níveis séricos de óxido nítrico em pacientes com artrite reumatoide. O óxido nítrico se correlaciona significativamente com a atividade da doença, marcadores inflamatórios e estado radiológico das articulações.

Introduction

Nitric oxide (NO) is an endogenously produced small molecule that has critical roles in cellular signaling and is involved in a variety of physiological processes. NO can have opposite biological effects, depending upon various environmental and pathophysiological conditions.[1]

Oxidant stress generated within an inflammatory joint can produce autoimmune phenomena and connective tissue destruction within the synovium. Radical species with oxidative activity, which include reactive nitrogen species and reactive oxygen species, represent the mediators and effectors of cartilage damage.[2]

NO mediates many different cell functions at the site of synovial inflammation, including signal transduction, mitochondrial function and apoptosis.[3]

NO has emerged as an important mediator in rheumatoid arthritis (RA) synovium. Increased levels of NO in serum and synovial fluid have been reported in patients with RA, ankylosing spondylitis and osteoarthritis.[4]

The aim of this work is to assess NO level as a marker of oxidative stress in Egyptian patients with RA and to correlate it with various disease parameters and disease activity.

Methods

In a cross sectional study, 80 patients with RA, diagnosed according to the American College of Rheumatology/European League against Rheumatism (ACR/EULAR) criteria,[5] were divided into 2 groups according to disease activity: Group I: 42 patients with disease activity, and Group II: 38 patients with no disease activity. A third group was included: Group III: 40 age and sex matched healthy individuals as control. All patients were recruited from the Rheumatology Outpatient Clinic and Internal Medicine Ward at Ain Shams University Hospital. Informed consents were obtained from all participants, and the study was approved by the Ain Shams Medical ethics committee. The nature of the present study was explained, and laboratory and radiological procedures represent standard care posing no ethical conflicts. For all the patients, the following were done:

I. Detailed medical history and thorough clinical and musculoskeletal examination with assessment of disease activity by using DAS28-ESR:[6]

Detailed history of disease duration and progression and constitutional symptoms and signs of fever, weight loss and fatigue were assessed, and a thorough musculoskeletal examination was performed.

Disease activity was assessed using DAS28-ESR, and a DAS28-ESR≤2.6 was considered indicative of no disease activity.[6]

II. Laboratory investigations:

a) Routine laboratory investigations:

Complete blood count;

ESR in the first hour, estimated by Western method;

CRP (mg/dL with titer).

b) Immunological investigations:

Rheumatoid factor: measured by using biotic RA factor latex agglutination slide for the qualitative determination of RF in serum.

c) Serum nitric oxide

NO was performed using ELISA (R&D Systems, Inc., Minneapolis, USA). This assay determines NO concentrations based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. A preliminary deproteinization step has been done using ultrafiltration method, the filter used was centristart 1, supplied by vivascience sartorius group (www.sartorius.com). The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction.[7]

III. Radiological investigations:

Plain X-ray hands and wrists for scoring the radiological damage using Sharp method.[8]

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 17.0. Description of qualitative variables as number and percentage was done. Chi-square test was used to compare qualitative variables. Fisher exact test was used instead of chi-square test when one expected cell or more ≤ 5. Unpaired t-test was used to compare two independent groups as regards quantitative variables. Spearman & Person Correlation coefficient rank test was used to rank different variables against each other's positively or inversely. P-value = level of significance, where $p > 0.05$ = not significant (NS), $p < 0.05$ = significant (S), and $p < 0.001$ = highly significant (HS).

Results

In Group I, 23 were females, and 19 were males. Their age ranged from 27 to 63 years with mean ± SD 48.78 ± 12.41, and disease duration 5.48 ± 6.63 years. In Group I, 21 were females, and 17 were males. Their age ranged from 28 to 65 years with mean ± SD 44.10 ± 10.54, and disease duration 6.59 ± 5.81 years. There was no significant difference between both groups as regards gender, age or disease duration.

Fatigue and morning stiffness were significantly more frequent in Group I than in Group II patients ($p < 0.001$). Patients from Group I also had significantly higher ESR and CRP values than Group II patients ($p < 0.001$) (Table 1).

There was a significant difference between Group I and Group II as regards X-ray Sharp score (Table 2).

On comparing NO level among the three groups, significantly higher levels were detected among RA patients in comparison to controls, with highest levels obtained among RA patients with disease activity (Group I) (Table 3).

Correlation between serum NO level and various disease parameters in Group I showed a significant positive correlation ($p < 0.05$) with morning stiffness, arthritis and platelet count, and a highly significant positive correlation ($p < 0.001$) with disease activity, ESR and CRP. (Table 4); furthermore, NO showed

Table 1 – Comparison between Group I and Group II as regards demographic, clinical and laboratory data.

	Group I	Group II	p
Demography			
Age, mean (SD)	48.78 (12.41)	44.10 (10.54)	> 0.05
Gender, n (%)			
Male	19(45)	17(45)	> 0.05
Female	23(55)	21(55)	> 0.05
Disease duration (years), mean (SD)	5.48 (6.63)	6.59 (5.81)	> 0.05
Clinical manifestations			
Weight loss, n (%)	16 (38)	15 (39)	> 0.05
Fatigue, n (%)	21(50)	10 (26)	< 0.001
Fever, n (%)	4 (10)	0 (0)	> 0.05
Morning stiffness, n (%)	36 (85)	13 (34)	< 0.001
Laboratory data			
Hemoglobin, mean (SD)	11.06 (1.64)	11.87 ± 1.61	> 0.05
Total leucocytic count, mean (SD)	7.68 (2.61)	7.12 ± 2.38	> 0.05
Platelet, mean (SD)	382.81 (102.24)	261.14 ± 74.37	< 0.05
Erythrocyte sedimentation rate (ESR), mean (SD)	60.69 (24.32)	19.76 ± 5.29	< 0.001
C-reactive protein (CRP), mean (SD)	24.48 (12.64)	8.52 ± 3.01	< 0.001
Rheumatoid factor (RF), n (%)	36 (86)	30 (79)	> 0.05

There was a highly significant difference ($p < 0.001$) between Group I and II as regards fatigue (50%, 26% respectively) and morning stiffness (85%, 34% respectively). There was a highly significant difference ($p < 0.001$) between Group I and II as regards ESR and CRP protein with higher levels for both detected in Group I.

Table 2 – Comparison between group I and II as regards X-ray Sharp score.

Sharp score	Group I		Group II		p
	n	%	n	%	
0	2	5	3	8	<0.05
1	4	10	16	42	<0.001
2	6	14	11	29	<0.01
3	18	43	5	13	<0.001
4	10	23	2	5	<0.001
5	2	5	0	0	<0.01

Table 3 – Comparison between Groups I, II, and III as regards serum NO.

Serum NO	Group I	Group II	Group III	p-value
Mean ± SD	82.38 ± 20.46	35.53 ± 7.15	14.25 ± 4.09	<0.001

Significantly higher levels of NO were detected among RA patients in comparison to controls with highest levels obtained among RA patients with disease activity (Group I).

Table 4 – Correlation between serum NO level and various disease parameters in Group I.

Variable	r	p-value
Age	0.231	>0.05
Disease duration	-0.252	>0.05
Morning stiffness	0.453	<0.05
Arthritis[a]	0.432	<0.05
Platelet	0.458	<0.05
ESR	0.832	<0.001
CRP	0.763	<0.001
Disease activity	0.846	<0.001

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
NO showed a significant positive correlation ($p < 0.05$) with morning stiffness, arthritis and platelet count, and a highly significant positive correlation ($p < 0.001$) with disease activity, ESR and CRP.
[a] 28-tender and/or swollen joint count.

a significant positive correlation with X-ray hand Sharp score ($p < 0.05$) in Group I (Fig. 1). No significant correlation between NO and X-ray hand Sharp score in Group II could be detected.

Discussion

Inflammation and tissue injury related to oxidative stress has been implicated in the pathogenesis of RA. Oxidative stress and decreased antioxidant status are the hallmarks in patients of RA as observed in recent years.[9] NO-dependent tissue injury has been implicated in a variety of rheumatic diseases, including RA.[10]

The present study assesses NO level as a marker of oxidative stress in patients with RA and its relation to disease activity. The study included 80 patients with RA, 42 patients with disease activity (Group I) and 38 without activity (Group II).

Patients with disease activity complained significantly more ($p < 0.001$) of morning stiffness and fatigue as compared to those without disease activity. This is most likely due to the fact that clinical manifestations, such as morning stiffness, have long been associated with disease activity in RA, and so it is expected that significantly higher values are to be obtained in active RA patients.[11]

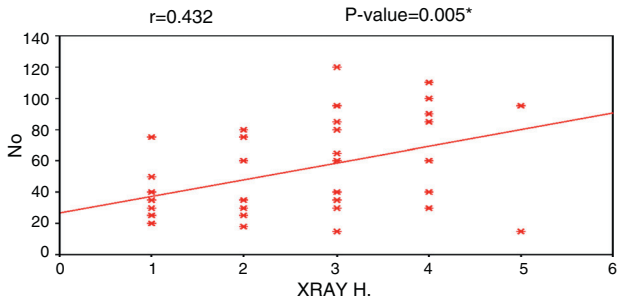

Figure 1 – Correlation between serum NO level and hand X-ray Sharp score in Group I. NO showed a significant positive correlation with X-ray hand Sharp score ($p < 0.05$).

In the present study, both ESR and CRP were significantly higher in the active group ($p < 0.001$). Although CRP appears to be the better test regarding measurement of the acute phase, yet because ESR is sensitive to immunoglobulins and RF, it may measure general severity better than CRP, even though it is a poorer measure of inflammation.[12] In a study to assess clinical utility of measurements of CRP and ESR in evaluating patients with RA, it was found that both ESR and CRP were significant predictors of swollen joint count ($p < 0.001$ for each).[13]

In this study, comparison between serum NO in all three groups showed a highly significant difference ($p < 0.001$), where significantly higher levels were obtained among RA patients than controls and even higher levels were obtained in active RA patients (mean ± SD 82.38 ± 20.46), than the inactive group (mean ± SD 35.53 ± 7.15). Serum NO level in Group I showed a significant positive correlation with morning stiffness ($r = 0.45$), arthritis ($r = 0.43$) and platelet count ($r = 0.46$), and a highly significant positive correlation was found between NO and each of ESR ($r = 0.83$) and CRP ($r = 0.76$), as well as DAS ($r = 0.84$).

On comparison between both groups as regards radiological damage using Sharp score, there was a highly significant difference between both groups ($p < 0.001$). NO levels showed a significant positive correlation ($r = 0.43$) with X-ray hand Sharp score only in Group I, yet no correlation between NO and X-ray hand Sharp score in Group II could be detected. Studies have demonstrated a longitudinal relationship between disease activity and radiographic status.[14] A study concluded that the level of disease activity, as well as the duration of remission, affect subsequent progression of radiographic damage in RA.[15] Consequently, since disease activity may be one of the drivers of joint damage and radiographic progression in RA, it would be expected that since NO correlates with disease activity, it would also correlate with radiographic damage in patients with active disease, as has been detected in the present study. In contrast, however other studies have concluded that, despite clinical improvement and remission in patients with active RA, the radiographic progression continues over time because of the underlying inflammatory process.[16]

In line with this study, several studies have proved increased serum NO level in RA patients compared to

control groups,[17,18] One study suggested that nitrate and nitrite production is enhanced in patients with RA compared with healthy subjects.[4]

NO has been shown to regulate T-cell functions under physiological conditions, but overproduction of NO may contribute to T-lymphocyte dysfunction. The increase in NO production in RA patients may be due to the increase in NO synthase activity.[19]

Similarly, a significant correlation between serum nitrate concentrations and number of tender joints, number of swollen joints, DAS score and CRP level has been detected.[20] The results suggest that these findings can serve as a reliable parameter of disease activity in patients with RA.

Although it has been suggested that reactive nitrogen species are produced within the inflamed joints of RA patients and that the levels correlate directly with disease activity,[21] several studies concluded that NO level did not correlate with ESR neither with CRP, and that there was a non-significant correlation between the serum levels of each of nitrogen species and the duration of disease or ESR as a marker of disease activity.[18,22]

Oxidative stress generated in an inflamed joint can contribute to autoimmune phenomenon and connective tissue destruction in RA, and the production of NO and prostaglandin E2 from articular chondrocytes probably contribute to the cartilage destruction seen in arthritis.[23] In the present study, a significant positive correlation was detected between serum NO level and radiological status in both RA groups, suggesting a role for NO not only in disease activity, but also in joint damage.

In the present cross sectional study conducted on a cohort of Egyptian RA patients, NO was not only detected with significantly higher levels in active RA patients, but correlated significantly with various disease parameters and activity. The study also included radiological assessment of X-ray hands using Sharp score, which also showed significant correlation with NO levels. However, mainly due to the limitation imposed by its cross sectional design, the results of the study could either demonstrate a causative effect of NO or simply reflect the inflammatory status of disease activity.

Finally, it can be concluded from the present study that there is an increased level of NO in the serum of RA patients and that NO level correlates significantly with disease activity as well as radiological joint status. This could suggest the involvement of free radicals not only in the inflammatory process of RA, but also in the joint destruction. New therapeutic protocols based on correcting oxidative stress levels may prove effective in restricting disease progression and limiting deformities.

Conflicts of interest

The authors declare no conflicts of interest.

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