Serum Protein Carbonyl Content, Total Thiol and Nitric Oxide in Patients with Rheumatoid Arthritis

Aida A .Mahmoud [1] and Mohamed A. Ismail [2]

[1]Biochemistry Department , Faculty of Medicine , Sohag University, Sohag, Egypt.
[2]Rheumatology Department, Faculty of Medicine , Sohag University, Sohag, Egypt.
aidamahmoud11@yahoo.com

Abstract: Serum protein carbonyl group, total thiol group and nitric oxide were studied in 30 patients with rheumatoid arthritis. Obtained results revealed that, Serum protein carbonyl content and nitric oxide (nitrite and nitrate) were significantly higher in rheumatoid arthritis patients than in the controls. However, total thiol group decreased significantly in rheumatoid arthritis patients than in the controls. In addition, protein carbonyl was negatively correlated with total thiol , while, nitric oxide (nitrite and nitrate) didn't correlate with protein carbonyl or total thiol .The impaired serum antioxidant defence mechanisms as evidenced by decreased total thiol group resulted in increased protein oxidation as indicated by increased protein carbonyl .
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1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder associated with synovial proliferation and excessive mononuclear infiltration, leading to the development of cartilage and subchondral bone erosions (Pap *et al*, 2000 and Gulden *et al* , 2005).

Reactive Oxygen Species (ROS) play an important role in the pathogenesis of RA exposing those patients to oxidative stress **(** Nourmohammadi *et al,* 2010 **).**

Oxidative stress is an important mechanism that underlies destructive and proliferative synovitis in RA (Mapp *et al,* 1995 , Hallwell ,1995, Tak *et al* , 2000 and Hitchoon and El-Gabalawy ., 2004)

Proteins are among the main targets of oxidation in the plasma (Dear *et al,* 1997). The action of ROS on proteins results in the formation of carbonyl groups with a relatively long half–life (Levine *et al,* 1990). The structure and activity of oxidized proteins change profoundly when compared with their native forms (Lemarechal *et al,* 2006).

Although the introduction of carbonyl groups in proteins is taken as a presumptive evidence of oxidative modification , as glycation of proteins may add carbonyl groups onto amino acid residues, assay of carbonyl groups in proteins provides a convenient technique for detecting and quantifying oxidative modification of proteins (Levine *et al,* 1990). Protein thiol groups may scavenge oxidants, thus sparing antioxidants and / or cellular constituents from attack. The measurement of thiol groups in serum provides an indirect reflection of the anti-oxidative defenses (Lemarechal *et al,* 2006).

Nitric oxide (NO) is a short-lived signaling molecule that plays an important role in a variety of physiologic functions, including the regulation of blood vessel tone, inflammation, mitochondrial functions and apoptosis (Brown ,1999 and Beltrán *et al,* 2000). NO also serves as a potent immunoregulatory factor, and influences the cytoplasmic redox balance through the generation of peroxynitrite ($ONOO^-$) following its reaction with superoxide (O_2^-) (Chung *et al* , 2001).

NO can also be involved in the production of angiogenic cytokines and the activation of matrix metalloproteases (Leibovich *et al,* 1994). Experimentally induced arthritis can be reversed by NOS inhibitors (Connor *et al,* 1995).

This investigation aimed to measure, serum protein carbonyl group as an evidence of oxidative modification of serum proteins , as well as , total thiol as an evidence of antioxidant defence mechanism and the free radical NO metabolites (nitrite and nitrate).

2. Materials and Methods

Subjects:

This study was conducted on 30 rheumatoid arthritis patients (24 ♀ and 6 ♂) fulfilling the American Collogue of Rheumatology criteria for the diagnosis of rheumatoid arthritis recruited from the Department of Rheumatology, Sohag University Hospital. Their age ranged from 29 to 50 years with mean ± SD (40.2 ± 6.6). A group of 20 healthy volunteers, recruited from the hospital staff , their age and sex distribution were similar to those of the rheumatoid arthritis group were included in the study as a control group. An informed consent was taken

from all the participants. Venous blood was collected from patients and controls and centrifuged immediately at 3000 r.p.m. for 15 minutes. Sera were stored in aliquots at -80 ċ till analysis.

N.B. The study was carried out in accordance with the guidelines of the ethical committee of Sohag University.

Materials:

Guanidine hydrochloride and 5,5'–Dithio-Bis (2 Nitrobenzoic acid) were purchased from Sigma (St. Louis, MO, USA), N-(1-Naphthyl) ethylenediamine dihydrochloride was from Sigma-Aldrich (Fluka) and other chemicals were form MERK (Darmstadt , Germany) .

All experiments were carried out using the spectrophotometer Biomeriux UV / Vis / Jr 60DT0249, Secoman C E France.

Determination of protein carbonyl levels;

Protein carbonyls are first derivatized with 2, 4 dinitrophenyl-hydrazine (DNPH). Proteins are then trichloroacetic acid (TCA) precipitated and free DNPH is removed by washing the protein pellet. The protein pellet is then dissolved in guanidine hydrochloride (GuHCL) and the absorbance of protein-hydrozone is measured at 370 nm. Protein carbonyls were expressed as nmol / mg protein (Levine *et al,* 1990).

Determination of thiol group:

It is based on thiol / disulfide reaction of thiol and Ellman ' reagent (5, 5'-dithiobisnitrobenzoic acid) (Hu, 1994).

Assay of NO:

Because NO has a very short half life (1–10 seconds) as it reacts with oxygen to form nitrite and nitrate. Nitrite an nitrate (NO_x) was measured using the Griess ' reagent after addition of Vanadium III Chloride to reduce nitrate into nitrite (Mirinda *et al* , 2001)..

Statistical analysis:

Statistical analysis was performed using prism 3 version 5 www.Graphpad.com.

3. Results:

The clinical and laboratory characters of the patients are represented in table (1).

Table 1 . Clinical and laboratory characters of RA patients.

Clinical and laboratory characters of R A patients (n=30)	Mean ± SD
Age (years)	40.2 ± 6.6
Duration of disease (years)	5.3 ± 3.6
ESR (erythrocyte sedimentation rate , mm / h)	55.7 ± 17.1
CRP (C-reactive protein , mg / dl)	5.1 ± 2.3
Rheumatoid factor (number , %)	24 (80 %)

Serum protein carbonyl content. It was significantly higher in rheumatoid arthritis patients than in the controls ($P < 0.001$, fig. 1.)

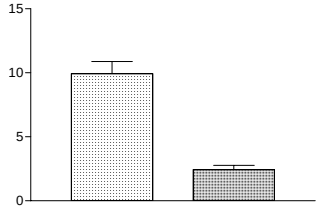

Fig.1. Mean ± SD levels of protein carbonyl in controls and RA patients (nmol / mg protein) .

Total thiol group. It decreased significantly in rheumatoid arthritis patients than in the controls ($P < 0.001$, fig. 2.).

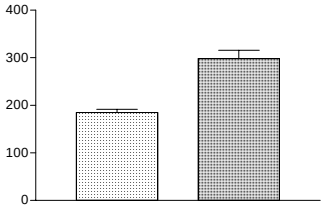

Fig . 2. Mean ± SD levels of total thiol in controls and RA patients (µmol / ml).

Nitric oxide levels ($N0_X$). It increased significantly in rheumatoid arthritis patients than in the controls ($P < 0.05$, fig. 3.).

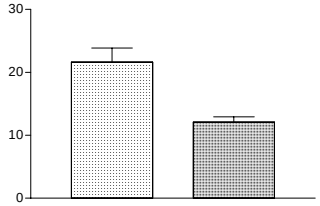

Fig . 3. Mean ± SD levels of NO_x in controls and RA patients (µmol / ml).

4. Discussion

This investigation revealed that protein carbonyl group content and NO_x (nitrite and nitrate) significantly increased in RA patients than in controls. while total thiol were significantly decreased in RA patients than in controls. Our data demonstrated an excessive degree of oxidative stress in patients with RA as evidenced by increased protein carbonyl , increased NO_x and decreased total thiol . Lemarechal *et al,* 2006 and Tutik *et al,* 2010 found elevated levels of protein carbonyls and decreased levels of total thiol group in patients with rheumatoid

arthritis. Decreased thiol concentration showed that RA patients are at considerable risk of tissue oxidant injury because of impaired serum antioxidant defence mechanisms. Thiols participate in several processes ranging from simple electron donation (GSH) and antioxidant catalysis to complex biochemical sensing and antioxidant responses (Winyard *et a* , 2005).

Carbonylation is an irreversible, non enzymatic modification of proteins. Carbonyl groups are introduced into proteins by a variety of oxidative pathways (Dall-Donne *et al,* 2003). The oxidation of protein can modulate biochemical properties such as enzyme activities, the DNA binding of transcription factors and susceptibility to proteolytic degradation (Garibadli *et al,* 1994 and Renke *et al,* 2000).The usage of protein carbonyl group as biomarker of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated protein (Dall-Donne *et al,* 2003).

NO is an important mediator of diverse physiologic and pathologic processes, including arthritis (Wallace , 2005). NO is synthesized from L-arginine by NO synthases (NOSs) which are; neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (Bredt, 1999). NO have relatively short half-life (for example 1 to 10 s), which has make it technically difficult to quantify in solution. Instead of directly measuring NO, investigators have estimated NO production by measuring levels of nitrate (NO_3^-) and nitrite (NO_2^-), stable anions derived from the reaction of NO with superoxide. In general, serum levels and urinary excretion of nitrite and nitrate (NO_x) reflect the total production of NO by the body(Green *et al,* 1981and Granger *et al,* 1999).

In the present study NO was found to increase significantly in RA patients , but NO didn't correlate with ESR or CRP. Also NO didn't correlate with total thiol or protein carbonyl group. Farrell et al ,1992, found that patients with RA had higher serum nitrite (not nitrite + nitrate) levels than normal controls. Similarly, Ueki *et al,* 1996 , found that serum nitrite was higher in patients with RA than in normal controls , and that nitrite levels were correlated with C-reactive protein. Also, Choi *et al,* 2003, found increased NO production in RA patients , however , this increase not correlated to parameters of disease activity. NO has been shown to regulate T cell functions under physiological conditions, but overproduction of NO may contribute to T lymphocyte dysfunction. NO-dependent tissue injury has been implicated in a variety of rheumatic diseases, including RA (Nagy *et al,* 2010). The increase in NO production in RA patients may be due to the increase in NO synthase activity (Mäki-Petäjä *et al,* 2008).

NOS polymorphism has been observed in RA (Gonzalez-Gay *et al,* 2004). iNOS is regulated at the transcriptional level, while eNOS and nNOS are regulated by intracellular Ca2+. Several different cell types are capable of generating NO in the inflamed synovium, including osteoblasts, osteoclasts, macrophages, fibroblasts, neutrophils and endothelial cells (Nagy *et al,* 2007).

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