

CONCISE REPORT

Serum nitrate and nitrite levels in patients with rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis

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Objective: To assess and compare serum nitrate and nitrite levels in patients with ankylosing spondylitis (AS), rheumatoid arthritis (RA), and osteoarthritis (OA).

Methods: Thirty five patients with RA, 32 patients with AS, and 36 patients with OA were entered into this study. In addition, 30 healthy volunteers acted as a control group. Concentrations of nitrate and nitrite in serum were determined by direct and indirect Griess reactions. C reactive protein and erythrocyte sedimentation rate levels were determined as markers of systemic activity of disease (SAD) in RA and AS groups.

Results: Serum nitrate and nitrite levels were found to be higher in patients with AS and RA than in the OA group ($p < 0.01$). In addition, serum nitrate and nitrite levels were higher in all three groups than in the control group ($p < 0.01$). Moreover, serum nitrate and nitrite levels were higher in patients who had SAD than in those who had not in the RA and AS groups ($p < 0.01$ and $p < 0.05$, respectively), and there was a correlation between serum nitrate and nitrite concentrations and SAD variables in patients with RA (Spearman's $r_s = 0.414$, $p < 0.05$ and $r_s = 0.408$, $p < 0.05$, respectively) and AS ($r_s = 0.421$, $p < 0.05$ and $r_s = 0.412$, $p < 0.05$, respectively).

Conclusion: The findings suggest that nitrate and nitrite production is enhanced in patients with inflammatory arthritis compared with OA. In addition, serum nitrate and nitrite levels are enhanced in patients with RA, AS, and OA compared with healthy subjects. Furthermore, there is a correlation between the SAD variables and serum nitrate and nitrite levels in patients with RA and AS.

Previous studies on nitric oxide (NO), which is an inorganic, gaseous free radical, produced by the enzyme nitric oxide synthase (NOS), have suggested that it has several physiological roles, including the regulation of platelet function, neurotransmission, and the killing of intracellular pathogens.^{1–4}

NO itself is difficult to measure directly in serum, because of its very short half life in biological fluids (6–10 seconds). Under aqueous, aerobic conditions NO spontaneously oxidises to its inactive, stable end products nitrite and nitrate.^{1–6}

Little is known about the importance of the NO pathway in inflammatory joint disease.⁶ Several studies suggest that tissue injury in inflammation involves NO production.^{5,7} Although osteoarthritis (OA) is not generally considered to be an inflammatory disease, some studies have shown evidence of mild to moderate inflammatory changes in OA synovium, consistent with the fact that the proinflammatory cytokines have also been detected in OA synovial fluid.⁷

Growing evidence implicates NO in immune regulation, inflammation, autoimmunity, and arthritis.³ Raised levels of

NO in serum and synovial fluid have been reported in patients with rheumatoid arthritis (RA), OA,^{5,8} in animals with experimentally induced arthritis,⁹ and in autoimmune arthritis.¹⁰ Several cell types present within the joint, including synovial fibroblasts, endothelial cells and chondrocytes, can be induced by proinflammatory cytokines to produce NO in vitro.¹¹ In experimental arthritis, administration of NOS inhibitors profoundly reduces disease activity. In humans, the beneficial effects of NOS inhibition are inferred from indirect evidence: glucocorticoids, auranofin, salicylates, indometacin, and methotrexate inhibit induction of the inducible NOS and/or reduce enhanced NO synthesis and disease activity in different ways. Thus selective inhibition of the pathologically enhanced NO synthesis is a new experimental therapeutic approach in the treatment of inflammatory joint diseases.^{3,5}

As far as we know, no previously published study has compared the serum level of NO in patients with RA, ankylosing spondylitis (AS), and OA with controls. In this study we compare the levels of NO in these patients and correlate our findings with systemic activity variables in patients with RA and AS.

PATIENTS AND METHODS

Patients

Thirty two patients with AS (20 male, 12 female, mean (SD) age 45.6 (16.1) years), 35 patients with RA (nine male, 26 female, mean age 51.9 (15.7) years), and 36 patients with OA (17 male, 19 female, mean age 54.3 (12.1) years) who attended the outpatient clinic of the department of physical medicine and rehabilitation were studied. In addition, 30 healthy volunteers (13 male, 17 female, mean age 49.2 (10.3) years) served as a control group.

Study protocol

Informed consent was obtained from everyone who participated. For determination of serum nitrite and nitrate concentration, 5 ml of blood was required. At the same time blood samples were taken for determination of C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Blood samples for nitrate and nitrite were stored at -70°C until use.

Exclusion criteria were inflammatory diseases other than RA and AS, and treatment with prednisolone > 10 mg/day.

Laboratory analyses

Nitrite levels were measured by direct Griess reaction, which is the simplest and most commonly used assay method.³ Nitrate levels were measured by subtraction of the nitrite levels from

Abbreviations: AS, ankylosing spondylitis; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; NO, nitric oxide; NOS, nitric oxide synthase; OA, osteoarthritis; RA, rheumatoid arthritis; SAD, systemic activity of disease

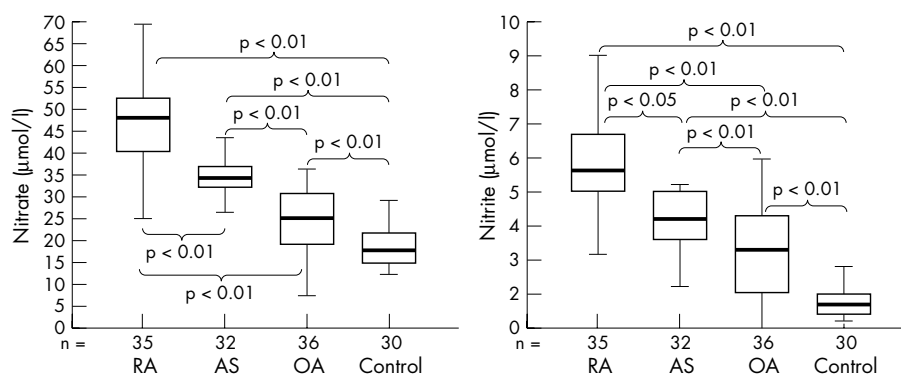


Figure 1 Nitrate and nitrite levels in each group.

the total NO measured by indirect Griess reaction. Excess dietary nitrite/nitrate intake was excluded, although a standardised diet was not used. Blood sampling time was standardised two to four hours after intake of a low nitrite/nitrate breakfast. Under these conditions, the effects of dietary nitrite/nitrate intake should be minimised and the different nitrite and/or nitrate serum levels between patients should indicate increased NO synthesis.

The ESR was determined according to the method of Westergren and serum CRP was measured by a nephelometric method in RA and AS patient groups. For men, an ESR $>$ age/2 and/or CRP $>$ 6 mg/l, and for women, an ESR $>$ (age+10)/2 and/or CRP $>$ 6 mg/l have been accepted as criteria for the systemic activity of disease (SAD).

Statistical analyses

Statistical analysis was undertaken using the statistical package for social sciences (SPSS version 10.0). Data in tables are expressed as the mean (SD). Independent sample *t* test, Mann-Whitney U test, and Spearman's non-parametric correlation tests were used as appropriate statistical methods for analyses. Differences were considered to be significant if *p* values were ≤ 0.05 .

RESULTS

There were no significant differences in age and body weight between the groups ($p > 0.05$). As expected, there was a significant difference in the proportions of men and women between the RA and AS groups of patients and healthy subjects ($p < 0.05$).

Serum nitrate and nitrite levels were higher in patients with AS (33.8 (5.1) $\mu\text{mol/l}$ and 4.9 (2.2) $\mu\text{mol/l}$, respectively) and RA (51.7 (21.3) $\mu\text{mol/l}$ and 6.1 (2.1) $\mu\text{mol/l}$, respectively) than OA (24.2 (7.4) $\mu\text{mol/l}$ and 3.4 (1.2) $\mu\text{mol/l}$, respectively) group ($p < 0.01$). In addition, serum nitrate and nitrite levels in healthy volunteers (18.6 (5.0) $\mu\text{mol/l}$ and 1.7 (0.5) $\mu\text{mol/l}$, respectively) were lower than those in all patient groups ($p < 0.01$) (fig 1).

Table 1 and fig 2 show the SAD variables in patients with RA and AS with or without systemic activity. Serum nitrate and nitrite levels of patients with active disease were increased at least twofold in comparison with patients with inactive disease in the RA group ($p < 0.01$). Similar higher levels of serum nitrate and nitrite concentrations were detected in patients with active disease in the AS group in comparison with patients with inactive disease ($p < 0.05$). Furthermore, we found a correlation in both serum nitrate and nitrite concentrations with SAD variables in RA ($r_s = 0.414$, $p < 0.05$ and $r_s = 0.408$, $p < 0.05$, respectively) and AS ($r_s = 0.421$, $p < 0.05$ and $r_s = 0.412$, $p < 0.05$, respectively) groups.

DISCUSSION

In this study the most intriguing finding was that serum nitrate and nitrite concentrations were increased in patients with RA, AS, and OA compared with healthy subjects. This accords with nitrite levels in synovial tissue and serum in a previous study.⁸ The origin of these increased levels of NO is not clear; widespread synovial inflammation might increase serum levels of NO when synovial fluid cleared by the lymphatic system enters the systemic circulation and equilibrates with the vascular compartment within the synovium. However, this may not entirely account for the higher serum NO concentrations in RA and AS compared with controls and does not seem to be a likely explanation in patients with OA. A possible source of increased NO is the systemic vasculature and other cells in which the induction of NO has been shown.⁸

Increased serum and synovial nitrite concentrations have been found in patients with RA and OA⁸ and in the spondyloarthropathies.^{3,6} In the spondyloarthropathies a correlation between nitrite, CRP, and ESR was found. The ESR decreased with six weeks' treatment with non-steroidal anti-inflammatory drugs which reduced clinical disease activity, although serum nitrate and CRP concentrations remained unchanged.³ Positive correlations between serum nitrate levels and parameters of clinical presentation and severity of disease have been shown in patients with RA.¹²

Table 1 Systemic activity variables and serum nitrite and nitrate concentrations in patients with RA and AS with or without systemic activity. Results are shown as mean (SD)

Parameters	RA		<i>r</i>	<i>p</i>	AS		<i>r</i>	<i>p</i>
	Active (n=25)	Inactive (n=10)			Active (n=24)	Inactive (n=8)		
ESR (mm/1st h)	55.08 (26.18)	24.20 (6.42)			38.86 (9.56)	23.00 (2.83)		
CRP (mg/l)	13.62 (3.62)	3.60 (3.29)			11.14 (3.98)	3.06 (0.55)		
Nitrate ($\mu\text{mol/l}$)	54.64 (21.77)	36.31 (8.53)	0.414	< 0.05	35.14 (3.89)	24.70 (2.40)	0.421	< 0.05
Nitrite ($\mu\text{mol/l}$)	6.46 (2.12)	4.40 (0.76)	0.408	< 0.05	5.22 (2.14)	2.40 (0.28)	0.412	< 0.05

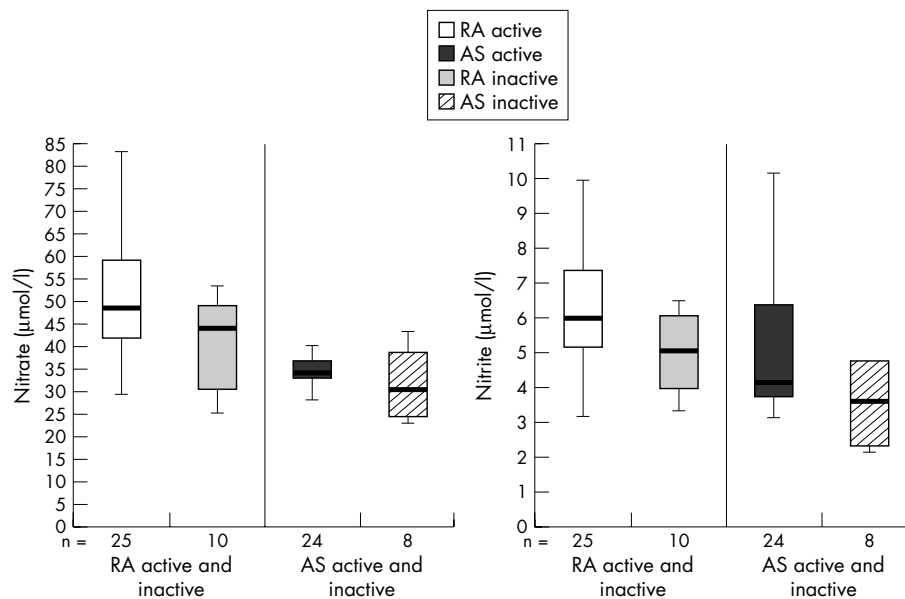


Figure 2 Comparison of nitrate and nitrite levels in patients with active and inactive RA and AS.

Our data confirm that serum nitrate/nitrite levels are raised in patients with arthritis in comparison with controls.^{8, 13, 14} In addition, we found a correlation between SAD variables and serum nitrate/nitrite concentrations in patients with RA as previously detected.¹⁵ Moreover, we determined a correlation between serum nitrate and nitrite concentrations with SAD variables in patients with AS and also found increased serum NO concentrations in patients with AS in comparison with patients with OA and healthy volunteers.

Our findings suggest that nitrate and nitrite provide a measure of endogenous NO synthesis, and this study shows that nitrite and nitrate concentrations in serum samples may be measured in humans without complex preparatory steps.

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