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ORIGINAL ARTICLE

Inflammatory markers in patients with rheumatoid arthritis

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Abstract
Background: Autoimmune diseases such as rheumatoid arthritis (RA) are the consequence of a persistent imbalance between pro- and anti-inflammatory immune mechanisms, leading to chronic inflammation. The objective of this study was to determine whether the high sensitive C-reactive protein (hs-CRP) and cytokines are elevated in RA patients and to investigate the relationship between these markers and disease activity in RA, measured by disease activity score 28 (DAS28).
Methods: We studied 110 RA patients according to American College of Rheumatology revised criteria for RA, and 55 controls matched by age and sex. Serum levels of hs-CRP and cytokines interleukin (IL)-6, IL-10 and tumour necrosis factor-α (TNF-α) were estimated and correlated with the DAS28. Serum hs-CRP was assayed immunoturbidimetrically and cytokines were analysed by commercially available ELISA kit.
Results: We found that RA patients had significantly higher levels of serum hs-CRP ($p < 0.001$), IL-6 ($p < 0.001$), TNF-α ($p < 0.001$), and IL-10 ($p < 0.01$) as compared to healthy controls. hs-CRP, IL-6 and TNF-α correlated positively ($p < 0.001$) and IL-10 correlated negatively ($p < 0.01$) with DAS28.
Conclusions: These results demonstrate that RA patients have high levels of inflammatory markers, and these levels are correlated with the DAS28. These findings suggest a possible role of these markers in the pathogenesis of RA. Moreover, these biomarkers can be used as markers of disease activity in the diagnosis and treatment of RA.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that is characterised by polyarthritis with often progressive joint damage and disability, immunological abnormalities, systemic inflammation, increased co-morbidity, and premature mortality. It affects 1% of the adult population worldwide and also occurs among one in a thousand children as juvenile RA. RA is much more common in women and affects women 2–3 times more frequently than men, and during pregnancy 70% of women suffering from RA experience remission, with flare-ups after birth.[1] The aetiology of RA is not known, but it is classified as one of the autoimmune diseases.[2] It is associated with reduced life expectancy and a major cause of chronic disability and handicap, and conditions become more dangerous with time. Many studies have shown that advance therapy including the use of early, aggressive therapy, and the introduction of anti-cytokines agent have improved patient's quality of life, eased clinical symptoms, retarded the progression of joint destruction, and delayed disability.[3]

Inflammatory processes play a pivotal role in the pathogenesis of RA. Markers of inflammation such as C-reactive protein (CRP), interleukin (IL)-6, tumour necrosis factor (TNF)-α and anti-inflammatory marker IL-10 are highly expressed in synovium fluid and serum of arthritic patients and play an important role in the pathophysiology of RA. CRP is an acute-phase protein produced by hepatocytes, upon stimulation by the cytokines IL-1, IL-6 and TNF-α, during an acute-phase response.[4,5] CRP is a general marker of systemic inflammation and is elevated in patients with RA. Some studies reported a higher frequency of increased CRP concentrations in serum samples of RA patients before the onset of RA.[6]

In RA, several cytokines are involved in almost all aspects of articular inflammation and destruction.[7] Increased levels of pro-inflammatory cytokines lead to the proliferation of synovial tissue, and thereby cause damage in the articular cartilage and bone destruction in the adjacent area. Anti-inflammatory cytokines can also be found in the affected joints, and it has been postulated that chronic synovitis may reflect an imbalance in pro- and anti-inflammatory cytokines production in RA. IL-6 is the most abundantly expressed cytokine in RA patients with biological activities that include regulation of immune response, inflammation, and haematopoiesis. IL-6 stimulates the secretion of immunoglobulin by plasmacytes, activates and promotes the proliferation of T and B cells (thus it is involved in the production of the rheumatoid factor), induces synthesis of acute-phase proteins such as CRP, fibrinogen, haptoglobin and serum amyloid-A, regulates the proliferation and differentiation of osteoclasts, and induces bone resorption.[8]

TNF-α is one of the pivotal pro-inflammatory cytokines responsible for inflammation and joint destruction in RA. TNF-α and its two receptors (p55 and p75 TNFR) are readily detected in both synovial fluid and serum of patients with RA. The severity of this disease is correlated with the concentration of TNF-α in RA patients.[9] TNF-α is a potent stimulator of mesenchymal cells, such as synovial fibroblasts, osteoclasts, and chondrocytes that release tissue-destroying matrix metalloproteinases. TNF-α also inhibits the production of tissue inhibitors of metalloproteinases by synovial fibroblasts. These dual actions are thought to lead to joint damage. Although, TNF-α and IL-6 have overlapping and synergic actions, some of the effects of these two cytokines are regulated by distinct mechanisms.[10] IL-10 is a potent immunosuppressive and anti-inflammatory cytokine, produced as a part of the homeostatic response to infection and inflammation, and plays a critical role in limiting the duration and intensity of immune and inflammatory reactions. As an anti-inflammatory cytokine, IL-10 has been shown to inhibit the synthesis of pro-inflammatory cytokines.

In the present study, we have screened 110 RA cases attending the Rheumatology OPD of a tertiary care hospital in Delhi, India. Acute phase protein hs-CRP, pro-inflammatory markers IL-6 and TNF-α and anti-inflammatory marker IL-10 were estimated in the serum of RA patients to rule out the levels of these biomarkers during active RA and compared them with healthy controls, and then investigated the correlation between serum levels of these inflammatory markers with the disease activity score 28 (DAS28) in the patient group.

Methods

The present study was carried out on 110 RA patients, fulfilling the 1987 revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association).[11] All cases were selected from the Rheumatology Department of a tertiary care hospital in Delhi, India, under the guidance of a specialist rheumatologist. All patients had active RA (>3 swollen and >3 tender joints). Some of them had evidence of erosive disease on X-rays of hands or feet. Disease activity in RA patients was measured using the DAS28, which includes the 28 tender and swollen joint counts, the erythrocyte sedimentation rate (ESR) and the patients' assessment of disease activity measured with a visual analogue scale. The body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height (m^2). Fifty-five controls, matched by age and sex were selected from blood donors and hospital staff. The controls were healthy persons who had no personal or family history of a rheumatic disease. These healthy controls were screened for diabetes, hypertension and dyslipidaemia. The study had local Research Ethics Committee and Research and Development approval, and all participants gave their written informed consent.

Overnight fasting blood samples were collected for measurement of all parameters. Blood was allowed to clot at room temperature, and serum was obtained immediately by centrifugation at 3500 rpm for 10 min. Serum was aliquoted into plastic tubes and stored at $-27\,^{\circ}C$ until assayed. Serum levels of hs-CRP were determined by immunoturbidimetric assay with the use of reagents and calibrators from Roche diagnostics. The levels of IL-6, IL-10 and TNF-α were estimated by means of commercially available quantitative ''sandwich'' enzyme-linked immunosorbent assay (ELISA) kits obtained from R&D Systems, according to the instructions of the manufacturer. Rheumatoid factor (RF) and ESR were measured by routine hospital procedure. Statistical Package for the Social Sciences 16 (SPSS 16.0) was used for all statistical analyses. All the descriptive variables were