Original Article

Proinflammatory cytokines profile in patients with Rheumatoid arthritis

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Summary:

Background:
Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder that causes the immune system to attack the joints. It is a disabling and painful inflammatory condition, which can lead to substantial loss of mobility due to pain and joint destruction. RA is a systemic disease, often affecting extra-articular tissues throughout the body including the skin, blood vessels, heart, lungs, and muscles.
Patients and Methods: Enzyme immunoassay for Determination of human TNF-α, IL-1β and GM-CSF in serum samples from 50 patients with a diagnosis of rheumatoid arthritis
Results: of cytokines showed a significant increase in TNF-alpha, IL-1 beta and GM-CSF in patients with rheumatoid arthritis (70.98±12.08) pg/ml,(238.6± 116.4)pg/ml and (96.1±12.08)pg/ml respectively. When compared with the control group (7.0±3.09)pg/ml, (15.4±3.8)pg/ml and (6.8±3.03)pg/ml respectively.
Conclusion: Increased serum levels of proinflammatory cytokine such as TNF-α, IL-1β and GM-CSF probably play important role in driving inflammatory process and promoting joint destruction in rheumatoid arthritis. Regulation of these cytokines is a crucial importance in the RA disease showing pleiotropic actions and many different targets.

Keywords: Rheumatoid arthritis, TNF-α, IL-1β and GM-CSF.

Introduction:

Rheumatoid arthritis (RA) is a chronic systemic disorder of unknown etiology (1). This disease effects about 1% of the population worldwide most commonly middle-aged women (2). It is characterized by chronic inflammation of the synovium, particularly of small joints, which often leads to destruction of articular cartilage and juxtaarticular bone (3). Cytokines regulate a broad range of inflammatory processes that are implicated in the pathogenesis of rheumatoid arthritis (4). In rheumatoid joints, it is well known that an imbalance between pro-and anti inflammatory cytokine activities favors the induction of autoimmunity (5). Chronic inflammation and thereby joint damage (6).The cytokine network in rheumatoid arthritis is a complex field, with a lot of cytokines. Pro-inflammatory cytokines in RA are IL-1 and TNF-α (6). To keep it simple, the network can be divided in two groups, the proinflammatory and anti-inflammatory cytokines (7) (8). Controlling the balance between these two groups is considered as an important therapeutic goal (9). TNF-α is a substance made by cells of the body that has an important role in promoting inflammation (10).TNF promotes the inflammation and its associates fever and signs (pain,tenderness, and swelling) in several inflammatory Conditions (11). IL-1β is a polypeptide with pro-inflammatory and immunopotentiating effects *in vivo* and *in vitro* (12). With relevance to rheumatoid arthritis IL-1 augments release of prostanoids , proteinases and oxygen metabolites and is a potent inducer of bone and cartilage resorption(13). The colony stimulating factors (CSFS) are glycoproteins believed to essential for the survival, proliferation, and differentiation of haematopoietic progenitor cells into monocyte/macrophage and granulocytes (14). They include granulocyte macrophage colony stimulating factor. GM-CSF can prime monocyte/ macrophages for the production of these and other proinflammatory mediators, led to the proposal of a CSF network loop in RA where GM-CSF has a central role in mainting joints inflammation and is a potent inducer of bone and cartilage resorption (15).

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Material and Methods:

Subjects: Patient study group: Serum samples from 50 patients with a diagnosis of rheumatoid arthritis (they had positive RF test) were collected during the period between January and May 2008. The range of age (35-56) years. In addition to patient group 20 individuals (blood donors) were further investigated, and were considered as a control group. Kits used in this study (TNF-α, IL-1β, and GM-CSF) by Immunotech A Beckman Coulter Company,France.
Methods: Enzyme immunoassay for Quantitative Determination of human TNF-α, IL-1β, and GM-CSF in serum.

Principle: The immunoenzyme assay of TNF-α, IL-1β, and GM-CSF is a sandwich type assay with two immunological steps.The first step leads to capture TNF-α, IL-1β, and GM-CSF by monoclonal anti TNF-α, IL-1β, and GM-CSF, antibody bound to the wells of microtiter plate. In the second step, a second monoclonal anti- TNF-α, IL-1β, and GM-CSF antibody, which is biotinylated is added together with streptavidin- peroxidase conjugate. The biotinylated antibody is bound to the solid phase antibody –antigen complex and in turn, binds the conjugate. After incubation period, the wells are washed and the binding of the streptavidin –peroxiase via biotin is followed by the addition of chromogenic substrate of the peroxidase. B- Immunoassay procedure: Procedure, according to the information supplied Immunotech A Beckman Coulter Company.

C- Calculation of results: The standards curve was drawn by plotting on horizontal axis the TNF-α, IL-1β, and GM-CSF concentrations of the standards and on the vertical axis the corresponding average absorbance. To locate the concentration of TNF-α, IL-1β, and GM-CSF in the sample, the average absorbance for each sample on the vertical axis was located and the corresponding TNF-α, IL-1β, and GM-CSF concentrations were located on the horizontal axis. Statistical analysis: Data have been analyzed statistically using UPSS program version 10. Results were expressed using simple statistical parameters such as mean and standard deviation. Analysis of quntitation data was done using t-test and ANOVA. Acceptable level of significance was considered to be less than o.o5

Results:

Serum levels of TNF-α, IL-1 β, and GM-CSF were significantly higher in patients with RA (70.98±12.08) pg/ml, (238.6± 116.4) pg/ml and (96.1±12.08) pg/ml respectively when compared with control group which were significantly lower (7.0±3.09) pg/ml,(15.4±3. pg/ml 8) and (6.8±3.03) pg/ml respectively($p< 0.001$). See figures (1, 2, and 3).

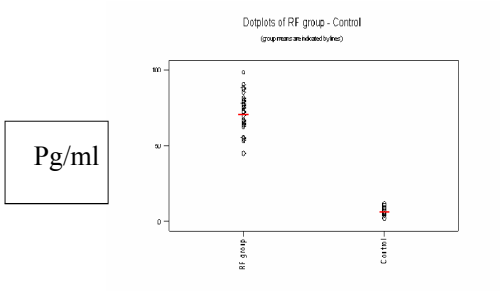

Figure1: Serum levels of TNF-α measured by ELISA in patients with RA and control group.

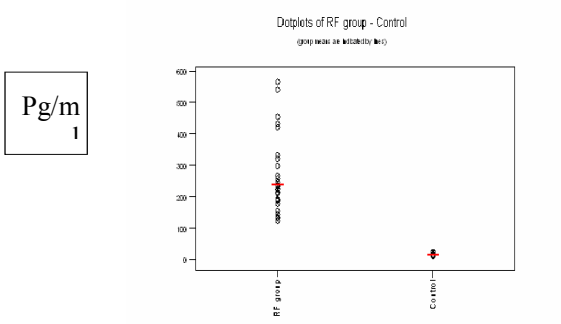

Figure2: Serum levels of IL-1 β measured by ELISA in patients with RA and control group.

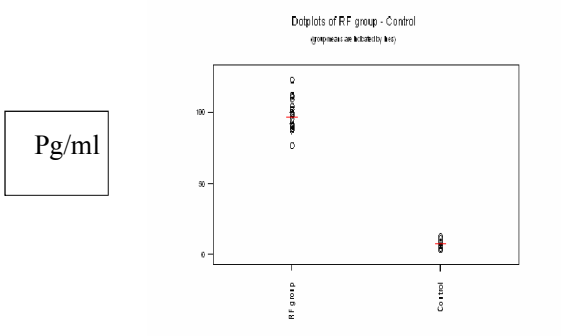

Figure3: Serum levels of GM-CSF measured by ELISA in patients with RA and control group.

Discussion:

The current concept is that inflammation and tissue destruction results from complex cell-cell interactions between antigen presenting cells (APC) and CD4+ T cells; APC display complexes of class II major histocompatibility complex (MHC) molecules and peptide antigens that bind to specific receptors on the T cells. Macrophage activation occurs, with abundant secretion of proinflammatory cytokines such as IL-1 and TNFα. These cytokines stimulate synovial fibroblasts and chondrocytes in the nearby articular cartilage to secrete enzymes that degrade proteoglycans and collagen, leading to tissue destruction (16). In this study the level of serum proinflammatory cytokines (TNF-alpha, IL-1 beta and GM-CSF) was significantly higher compared with healthy control. This result agrees with previous study which concluded that the development of arthritis in TNF transgenic mice could be prevented with

antibodies to TNFα, more interestingly, pathology could also be fully blocked with antibodies against the IL-1 receptor (17) (18) This strongly indicates that IL-1 is the secondary mediator responsible for the arthritic changes, and TNFα alone is neither arthritogenic nor destructive towards joints (19). Another study showed that the concept that TNF alpha is pathogenic in inflammatory arthritis has been validated by showing that neutralizing monoclonal anti-TNF antibodies significantly attenuate collagen-induced arthritis in mice (20) In preliminary trials in rheumatoid patients anti-TNF appears to have an impressive effect on indices of disease activity including C-reactive production and serum amyloid-A production. TNF alpha appears to be a relevant therapeutic target in rheumatoid disease (21). IL-1 and TNF-α play a pivotal role in the RA. TNF-α is responsible for the inflammatory and proliferative aspects, and IL-1 is responsible for the destructive aspects of RA (22). Other proinflammatory cytokines also play very important role in RA, because they are responsible for activation of enzymes in synovial fluid, which induce degradation of bone (23).

Conclusion:

Increased serum levels of proinflammatory cytokine such as TNF-α, IL-1β and GM-CSF probably play important role in driving inflammatory process and promoting joint destruction in rheumatoid arthritis. Regulation of these cytokines is a crucial importance in the RA disease showing pleiotropic actions and many different targets.

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