Serum TNF-Á Level in Medicated Rheumatoid Arthritis Patients

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Abstract: Rheumatoid arthritis (RA) is a chronic autoimmune, inflammatory disease characterized by inflammation and destruction of the synovial joints. It most commonly affects the peripheral joints of the hands and feet in a symmetric pattern leading to severe disability. Pro-inflammatory cytokines are an important part in the pathophysiology of RA, as tumor necrosis factor-alpha (TNF-Á), interleukin-1 (IL-1), IL-17, they stimulate inflammatory changes and degradation of bone and cartilage. The aim of the study was to assess the serum TNF-Á level in medicated RA patients and its correlation with disease activity and other disease factors. The study included 27 RA patients diagnosed according to 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria. Patients were further divided into 2 subgroups; 19 anti-cyclic-citrullinated peptide antibodies (ACPA) positive and 8 ACPA negative RA patients. The morning stiffness and disease duration were recorded, disease severity was assessed using disease activity score based on 28 joints- C-reactive protein (DAS 28-CRP). Serum ACPA and serum TNF-Á were measured by ELISA. Results showed that the median serum TNF-Á level was 90 pg/ml ± 112 for the ACPA positive RA patients and 40 pg/ml ± 28 for the ACPA negative RA patients. Eight patients (42%) of the ACPA positive RA patients had a serum TNF-Á level > 30 pg/ml, while only 1 patient in ACPA negative RA patients (12.5%) had a serum TNF-Á level > 30 pg/ml. The highest levels of serum TNF-Á level were found in ACPA positive RA patients, although the groups were of a small number to show statistical significance (P=0.145). Conclusion, serum TNF-Á levels are affected by several factors including disease duration and disease modifying anti-rheumatic drugs (DMARDs). So in patients with established RA or RA patients on DMARDs TNF-Á level doesn’t correlate well with disease activity or any other disease parameter.

Key words: Rheumatoid Arthritis • TNF-Á • ACPA

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation, hyperplasia and destruction of the synovial joints with autoantibody production including rheumatoid factor (RF) and anti-cyclic-citrullinated peptide antibodies (ACPA). It most commonly affects the peripheral joints of the hands and feet in a symmetric pattern leading to severe disability [1].

The exact cause of RA is unknown; it involves a complex interplay among genotype, environmental triggers and chance [2]. The micro-environmental changes associated with leukocytic infiltration and neoangiogenesis, followed by synovial reorganization and activation of the local fibroblasts, lead to the synovial hypertrophy and hyperplasia found in RA [3].

Pro-inflammatory cytokines that arise from the synovial cells are an important part in the pathophysiology of RA. They include tumor necrosis factor-alpha (TNF-Á), interleukin-1 (IL-1), IL-6 and IL-17. These cytokines stimulate inflammatory changes and degradation of bone and cartilage [4].

TNF-Á plays a fundamental role in the pathogenesis of RA through activation of cytokine, expression of endothelial- cell adhesion molecules and chemokine, protection of synovial fibroblasts, promotion of angiogenesis, suppression of regulatory T cells and induction of pain [5].
Results in regards to TNF-α levels reported in the literature are conflicting, as some studies in the past failed to detect circulating TNF-α in the serum of RA patients [6, 7] while others showed significant elevation of TNF-α levels in serum and other biological fluids in RA patients [8, 9].

The aim of the study was to assess the serum TNF-α level in medicated RA patients and its correlation with disease activity and other disease factors.

MATERIALS AND METHODS

The study included 27 RA patients, from those attending the Physical Medicine, Rheumatology and Rehabilitation outpatient clinic, at the Alexandria Main University Hospital over a period of 6 months, diagnosed according to 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria [10]. Patients were further divided into 2 subgroups; 19 ACPA positive and 8 ACPA negative RA patients.

Demographic data, disease and drug history was recorded for all patients. The morning stiffness and disease duration were recorded and disease activity was assessed using disease activity score based on 28 joints-C-reactive protein (DAS 28-CRP). Serum ACPA was measured by ELISA using the colorimetric method for detection and serum TNF-α was similarly measured by a coated human TNF-α platinum ELISA kit using the colorimetric method for detection provided by eBioscience.

Table 1: Comparison between the two studied groups according to TNF-A (pg./ml)

<table>
<thead>
<tr>
<th></th>
<th>ACPA +ve (No. = 19)</th>
<th>ACPA -ve (No. = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg./ml) Mean ± SD.</td>
<td>90 ± 112</td>
<td>40±28</td>
<td>0.145</td>
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<tr>
<td>No. patients &gt;30 pg./ml</td>
<td>8 (40%)</td>
<td>1(12.5%)</td>
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</tbody>
</table>

TNF-α: tumor necrosis factor-alpha, ACPA: anti-cyclic-citrullinated peptide antibodies, +ve positive, -ve negative, pg.: picogram, ml: milliliter, SD: standard deviation, No.: number, %: percentage.

Table 2: Correlation between TNF-α (pg./ml) and different parameters in each group.

<table>
<thead>
<tr>
<th></th>
<th>ACPA +ve (No. = 19)</th>
<th>ACPA -ve (No. = 8)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.083</td>
<td>0.735</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>-0.119</td>
<td>0.628</td>
</tr>
<tr>
<td>Morning Stiffness (min)</td>
<td>-0.134</td>
<td>0.585</td>
</tr>
<tr>
<td>ACPA (U/ml)</td>
<td>-0.172</td>
<td>0.481</td>
</tr>
<tr>
<td>DAS 28-CR</td>
<td>0.175</td>
<td>0.488</td>
</tr>
</tbody>
</table>

TNF-α: tumor necrosis factor-alpha, pg.: picogram, ml: milliliter, ACPA: anti-cyclic-citrullinated peptides antibody, +ve positive, -ve negative, No.: number, r: Spearman coefficient, min: minutes, U: unit, DAS 28-CR: disease activity score based on 28 joints- C-reactive protein

RESULTS

The mean age was 42.11 years of age ± 6.72 for the ACPA positive and 50.88 years of age ± 8.25 for the ACPA negative RA patients. The median disease duration was 7.84 years ± 5.81 for the ACPA positive and 6.75 years ± 6.09 for the ACPA negative RA patients. All patients in both groups were on triple disease modifying anti-rheumatic drugs (DMARDs) therapy; methotrexate (MTX), hydroxychloroquine (HCQ) and sulfasalazine (SSZ) in different doses. All patients with high disease activity were on low dose corticosteroid treatment (<5mg/day). The median disease activity by DAS-CRP was 5.56 ± 1.19 for the ACPA positive and 5.67 ± 0.79 for the ACPA negative RA patients.

The median ACPA level was 233.82 u/ml ± 306.67 for the ACPA positive and 7.01 u/ml ± 5.08 for the ACPA negative RA patients. The median serum TNF-α level was 90 pg/ml ± 112 for the ACPA positive and 40 pg/ml ± 28 for the ACPA negative patients. Eight patients (42%) of the ACPA positive RA patients had a serum TNF-α level > 30 pg/ml, while only 1 patient in ACPA negative RA patients (12.5%) had a serum TNF-A level > 30 pg/ml. The highest levels of serum TNF-α level were found in ACPA positive RA patients, although the groups were of a small number to show statistical significance (P=0.145) (Table 1). There was no statistically significant correlation between serum TNF-α level and any other disease parameter in ACPA positive and negative groups; age, disease duration, disease activity and ACPA level (Table 2).
DISCUSSION

Genetic factors are involved in disease initiation, course and severity. TNF-α gene expression and TNF-α itself are important regulators for inflammation in RA. TNF-α production was shown to vary between individuals and the variation was associated with certain HLA-DR alleles [11, 12]. In a study done on Egyptian patients it was demonstrated that a correlation between TNF-α production and the TNF-α genotype in these patients was found, where most (62%) RA patients had a GG TNF-α (-308 G/A) gene promoter polymorphism, these patients showed a lower level of TNF-α than the other genotypes [8]. This could explain why most patients in this study had low serum TNF-α level (=30pg/ml) in both the ACPA positive (58%) and ACPA negative groups (87.5%).

Although the role of TNF-α as a pro-inflammatory cytokine is well documented, the biological functions of its soluble receptors are not well understood. They are thought to block TNF-α function, but they could also prevent its inactivation, thus increasing the half-life of TNF-α in the circulation [13, 14]. TNF-α was shown to have a relatively short half life 3-4 hrs, while its soluble receptors persist longer. By utilizing immunoassays that discriminate between total serum TNF receptor-I (sTNFR-I) complex and sTNFR-I not bound to TNF-α, it has shown that sTNFR-I/TNF-A complexes may circulate even in the absence of detectable free TNF-α [15, 16]. It was also found that the presence of sTNFR in biological fluids interferes with different degrees in TNF-α bioassays and immunoassays [17]. This also supports the low results of serum TNF-α (≤ 30 pg/ml) found in most patients, as only the free unbound form of serum TNF-α was measured.

A study showed that low TNF-α secretion after 3 months also correlated significantly with a more chronic course of disease. Although this was seen more in patients with reactive arthritis, it was also seen in RA patients. The diminished TNF-α production was thought to reflect a state of relative immunodeficiency in patients with chronic disease [18]. This is consistent with the findings of this study as all patients had established RA and the median disease duration was 7.84 years ± 5.81 for the ACPA positive and 6.75 years ± 6.09 for the ACPA negative RA patients, where 1 year was the shortest disease duration.

A couple of studies have shown the different effects of DMARDs on serum TNF-α levels, where a study showed that neither MTX nor azathioprine (AZA) alter serum TNF-α levels [19]. While another study proved that MTX has an inhibitory effect on the production of inflammatory mediators including TNF-α on a co-culture of RA T-lymphocytes and synovial macrophages [20]. A significant decrease in circulating TNF-α in circulating TNF-α levels has also been seen during therapy with corticosteroids [21] and SSZ [22]. This is also consistent with the low level of serum TNF-α levels (≤ 30pg/ml) detected in most patients in this study, as all patients were on triple DMARDs therapy and those with high disease activity were also on low dose corticosteroids.

CONCLUSIONS

Serum TNF-α levels are affected by several factors including disease duration and DMARDs. So patients with established RA or patients on DMARDs TNF-α level doesn’t correlate well with disease activity or any other disease parameter. This article is the original work, neither published nor under publication process and I agree to publish in this journal.

REFERENCES