ORIGINAL ARTICLE

Clinical significance of soluble programmed death-1 (sPD-1) in rheumatoid arthritis patients: Relation to disease activity and functional status

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KEYWORDS
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Abstract Background: Programmed cell death-1 (PD-1) is an immunoreceptor that negatively regulates antigen receptor signaling and plays a critical role in the immunoregulation of autoimmune diseases.

Aim of the work: This study aimed to measure the plasma and synovial fluid levels of soluble programmed death-1 (sPD-1) in rheumatoid arthritis (RA) patients and to correlate them with the clinical and laboratory characteristics, disease activity, functional status and radiological severity.

Patients and methods: We measured sPD-1 in the plasma (n = 60) and synovial fluid (SF) samples (n = 24) from 60 RA patients and in the plasma from healthy control (n = 30). In the patients, disease activity score using 28 joint counts (DAS28) and the health assessment questionnaire (HAQ) score were assessed; immunoglobulin-M rheumatoid factor (IgM-RF) titer, anti-cyclic citrullinated peptide (anti-CCP) antibodies titer and C-reactive protein (CRP) levels were measured and total Sharp score calculated.

Results: In RA patients both plasma and SF sPD-1 levels (1416.9 ± 1037.9 pg/ml and 1503.9 ± 1129.48 pg/ml respectively) were highly significantly increased compared to its plasma level in the healthy control (165 ± 26.11 pg/mL) (p < 0.001). In RA patients, the plasma and SF levels of sPD-1 significantly correlated with DAS28 (r = 0.52 and 0.58 respectively, p < 0.05), HAQ scores (r = 0.48 and 0.51 respectively, p < 0.05) and anti-CCP titers (r = 0.55 and 0.58 respectively, p < 0.05).

Conclusions: Rheumatoid arthritis patients have significantly elevated plasma and synovial levels of sPD-1 that remarkably correlated with the DAS28 suggesting that it could be a useful marker to reflect RA disease activity. The considerable association of sPD-1 with autoantibodies production implies a possible role in the pathogenesis of RA.

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1. Introduction

Rheumatoid arthritis (RA), one of the most common systemic autoimmune diseases, is characterized by chronic joint inflammation and subsequent joint destruction [1]. The pathogenesis of RA is multifactorial with contributions from genetic polymorphisms, immune dysfunction, oxidative stress, apoptosis, infectious, environmental and hormonal factors [2–7]. Overexpression and dysfunction of clusters of differentiation may result in the development of autoimmune diseases [8].

The cluster of differentiation 4+ (CD4+) T-cells is responsible for the production of several pro-inflammatory cytokines, promotion of inflammation, activation of macrophages and osteoclasts, and subsequent joint destruction [9]. Costimulatory molecules are members of a growing family of receptors and ligands that play an important role in controlling and regulating the immune response, some of them are critical for the activation of T cells by providing the second signal to T-cell receptor (TCR) cross-linking whereas others, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death-1 (PD-1), and programmed death-ligand 1 (PD-L1), are thought to down-regulate T cell response [10].

PD-1 is a member of the CD28 family that plays a role in regulating T-cell activation and tolerance. PD-1 is inducibly expressed on T cells, B cells, macrophages, and natural killer cells after activation [11]. PD-1 has two ligands, PD-L1 and PD-L2, which have different expression patterns. PD-L1 is broadly expressed in both hematopoietic and non hematopoietic tissues and is upregulated on activated T cells, B cells, macrophages, and DCs upon stimulation, while PD-L2 expression is restricted to activated macrophages and dendritic cells (DCs) [12]. Cross-linking of PD-1 by its ligands, PD-L1 and PD-L2, leads to the down-regulation of T-cell responses by mediating programmed cell death and inhibiting cell proliferation [13]. Upregulation of PD-1 and PD-L1 is dependent on inflammatory cytokines such as interferon-gamma (IFN-γ) and members of the common γ-chain family [14]. In a similar manner to CTLA-4, PD-1 functions by inhibiting T-cell receptor (TCR)-mediated events such as cell proliferation and survival, and also interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), and IFN-γ production [15].

The critical role for PD-1 in immune regulation is highlighted by recent gene disruption studies demonstrating that strain-specific phenotypes, such as PD-1-deficient C57BL/6 mice, develop lupus-like autoimmune proliferative arthritis and glomerulonephritis with IgG3 deposition [16], whereas deficiency of PD-1 in BALB/c mice results in a severe autoimmune dilated cardiomyopathy followed by death due to congestive heart failure [17].

The mechanism of action, as to how co-stimulatory molecules are exactly involved in the activation and regulation of inflammatory autoimmune T-cells, remains elusive. Little is known about the role of co stimulatory molecules in many human autoimmune conditions [18]. For example, in RA, it is unclear as to how the peripheral immune system that integrates various regulatory mechanisms and networks fails to regulate persistent synovial T cell activation and inflammation. This may be reasoned to the overexpression of positive regulators, i.e., co stimulatory molecules capable of augmenting T cell activation (e.g., CD28) in synovial T cells and macrophages, and/or aberrant expression of those negative regulators (e.g., CTLA-4, PD-1) or functional antagonism of these molecules by soluble factors [19,20].

This study aimed to measure the plasma and synovial fluid (SF) levels of soluble programmed death-1(sPD-1) in rheumatoid arthritis (RA) patients and to correlate them with the clinical and laboratory characteristics, disease activity, functional status and radiological severity.

2. Patients and methods

2.1. Participants

Sixty patients, fulfilling the 2010 ACR-EULAR classification criteria for RA [21] were recruited from the in-patients and out-patients’ clinic of the Rheumatology and Rehabilitation department of Benha university hospitals between January and October 2014. Thirty age and sex matched apparently healthy individuals from the hospital personnel, undergraduates; medical and nursing staffs were also included as a control group. Patients’ evaluation included full history taking with recording of the disease duration, thorough physical examination, with particular focus on the pattern of joint involvement, the presence of nodules and other extra-articular features and ongoing medications. Disease activity using 28 joint counts (DAS28) [22] and Health Assessment Questionnaire (HAQ) [23] were assessed in all patients. X-rays (postero-anterior view) of the hands and feet were obtained for all patients and were scored by the total sharp score system [24]. The local ethics committee of our institution (Benha University, Faculty of Medicine) approved the study and all participants gave a written informed consent before being enrolled in this study.

2.2. Laboratory investigations

Blood specimens were collected after an overnight fasting analyzing for complete blood count (CBC), erythrocyte sedimentation rate (ESR) by Westergren’s method [25] in mm/hour, C-Reactive protein (CRP), Rheumatoid factor (RF) and anticyclic citrullinated peptide (anti-CCP) antibodies.

2.2.1. Measurement of plasma and synovial fluid levels of sPD-1

The sPD-1 levels were measured in the plasma collected from all RA patients and controls, in addition to the SF samples that were obtained from 24 of the RA patients who had knee effusion at the time of the study. All plasma samples were collected in heparinized tubes and stored at −80 °C until analysis. Synovial fluid samples were centrifuged and the supernatants carefully collected and stored at −80 °C until analysis. Assay of sPD-1 was made by the enzyme linked immunosorbent assay (ELISA) technique using the kit supplied from (My biosource, San Diego, California, USA) Human Soluble Programmed Death-1 (SPD-1) ELISA Kit Cat. No: MBS026745. The assay procedures were followed according to the manufacturer’s instructions. The detection range is 125 pg/ml–4000 pg/ml.
Significance of soluble programmed death-1 (sPD-1) in rheumatoid arthritis patients

parametric, and as median and range if non parametric. Chi square, Z-test, Mann Whitney U test, Kruskal–Wallis test and Spearman’s correlation coefficients were used as tests of significance. Two sided p-value < 0.05 was considered significant.

3. Results

Sixty RA patients (ages ranged from 20 to 65 years) with a mean of 41.3 ± 13.3 years and thirty age and sex matched apparently healthy control (ages ranged from 19 to 63 years) with a mean of 40 ± 13.1 years were included in the study. Patients’ clinical and laboratory features are shown in Table 1. The mean plasma and synovial sPD-1 levels in the RA patients (1416.9 ± 1037.9 and 1503.9 ± 1129.48 pg/ml respectively) showed a highly statistically significant (p < 0.001) increase as compared to the mean plasma levels in the control (165 ± 26.11 pg/ml). (Fig. 1). Although the synovial fluid sPD-1 level was higher than in the plasma of the RA patients, the difference showed no statistical significance (p > 0.05).

Regarding RF positivity, the mean sPD-1 plasma level in the seropositive RA patients (1581 ± 1069 pg/ml) showed a highly statistically significant increase (p < 0.001) compared to the seronegative patients (760.4 ± 551.7 pg/ml) but there was no statistically significant difference (p > 0.05) between the mean sPD-1 synovial fluid levels of the seropositive and seronegative RA patients (1503.9 ± 1129.48 and 1416.9 ± 1037.9 pg/ml respectively) (Table 2).

Plasma sPD-1 levels showed a statistically significant correlation with the ESR (r = 0.45, p < 0.05), CRP level (r = 0.57, p < 0.05), RF titers (r = 0.55, p < 0.05), anti-CCP titers (r = 0.55, p < 0.05), DAS28 scores (r = 0.52, p < 0.05) and HAQ (r = 0.48, p < 0.05). Synovial sPD-1 levels showed a statistically significant correlation with the ESR (r = 0.61, p < 0.05), CRP (r = 0.55, p < 0.05), anti-CCP titers (r = 0.58, p < 0.05), DAS28 (r = 0.58, p < 0.05) and HAQ (r = 0.51, p < 0.05) (Table 3). There was a highly statistically significant correlation between both plasma and SF sPD-1 levels in the RA patients (r = 0.73, p < 0.001).

4. Discussion

It has been suggested that PD-1 possibly contributes to the pathogenesis of RA especially as the PD-1 gene is significantly associated with RA susceptibility [26]. Contrarily, in another study it has been demonstrated that PD-1 ligand gene polymorphisms were not associated with susceptibility to RA [27]. T cell activation and function are critically regulated by positive and negative co-stimulatory molecules. Aberrant expression and function of co-stimulatory molecules have been associated with persistent activation of self-reactive T cells in autoimmune diseases such as RA [3]. PD-1 and its ligand, are known to play an important immunoregulatory role by controlling the development of immune effector cells [28,29]. The precise function of the soluble form of PD-1 is not well described, and sPD-1 has been reported to exhibit both ‘functional antagonism’ [30] as it functionally blocks and inhibits the immune regulatory effect of membrane bound PD1 on T cell activation and attenuates the PD-1 pathway leading to worsening the disease; and ‘agonism’ as others claimed that sPD-1 functions like the membrane form, exhibiting immune regulation by limiting TCR induced events [31].

In our study both plasma and SF sPD-1 levels were statistically significantly elevated in the RA patients than in the plasma level in the healthy controls, and these levels significantly correlated with the clinical and laboratory parameters of disease activity, DAS 28 and HAQ. Our results confirmed that results of others which found an elevated plasma and synovial sPD-1 level in RA patients than in the controls [10,32].

We found that both plasma and SF sPD-1 are significantly correlated to each other and this increased SF sPD-1 level indicates that sPD-1 in the given compartment reflects cellular expression. PD-1 expression is upregulated by low-affinity ligation of the TCR, and these low-affinity peptides are overexpressed in the RA synovium confirming the ideal conditions in the joint for PD-1 up-regulation [33].

The significant correlation between sPD1 levels and the disease activity that had been found in our study is against the original hypothesis because of an obvious contradiction between over-expression of co stimulatory molecules capable of down-regulating T cell response and persistent activation of self-reactive T cells known to exist in rheumatoid synovium. It had been suggested by Wan et al. [10] to revise the precise role of co-stimulatory molecules in persistent T cell activation.

No, number; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; DAS, disease activity score; HAQ, health assessment questionnaire.

Table 1 Clinical and laboratory characteristics, disease activity, radiological severity and functional disability of the rheumatoid arthritis (RA) patients (n = 60).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (years)</td>
<td>7.7 ± 4.6</td>
</tr>
<tr>
<td>Tender joints (No)</td>
<td>10.5 ± 3.1</td>
</tr>
<tr>
<td>Swollen joints (No)</td>
<td>8.3 ± 2.4</td>
</tr>
<tr>
<td>Morning stiffness (min)</td>
<td>84.2 ± 26.3</td>
</tr>
<tr>
<td>ESR (mm/1st h)</td>
<td>53.5 ± 17.6</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>28.1 ± 14.5</td>
</tr>
<tr>
<td>DAS28</td>
<td>4.8 ± 0.8</td>
</tr>
<tr>
<td>Total sharp score</td>
<td>26.2 ± 24.9</td>
</tr>
<tr>
<td>HAQ</td>
<td>1.08 ± 0.9</td>
</tr>
</tbody>
</table>
confirmed in our study as we found that plasma sPD-1 is of autoantibodies and the shedding of PD-1, this was tenot et al. [34] suggested that it is conceivable that persistent T in RA and how to explain the findings contradictory to the known inhibitory properties of these negative regulators. Fon- four inflammatory cytokines are seen as a danger signal by the immune system and disease activity score; HAQ, health assessment questionnaire. * Significant p < 0.05.

Table 3 Correlations between plasma and synovial fluid sPD-1 levels and different variables in rheumatoid arthritis (RA) patients.

<table>
<thead>
<tr>
<th>Variable in RA patients</th>
<th>sPD-1 (pg/ml)</th>
<th>r test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.1</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>0.01</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Morning stiffness (min)</td>
<td>0.24</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>ESR (mm/1st h)</td>
<td>0.45*</td>
<td>0.61*</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>0.57*</td>
<td>0.55*</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>0.55*</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>0.55*</td>
<td>0.58*</td>
<td></td>
</tr>
<tr>
<td>DAS-28</td>
<td>0.52*</td>
<td>0.38*</td>
<td></td>
</tr>
<tr>
<td>HAQ</td>
<td>0.48*</td>
<td>0.51*</td>
<td></td>
</tr>
<tr>
<td>Total sharp score</td>
<td>0.29</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable in RA patients</th>
<th>sPD-1 (pg/ml)</th>
<th>r test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1581 ± 1069</td>
<td>760.4 ± 551.7</td>
<td>1.21</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>1507 ± 1166.7</td>
<td>1494 ± 1100</td>
<td>0.19</td>
</tr>
</tbody>
</table>

sPD-1, soluble programmed death-1; RF, rheumatoid factor.

in RA and how to explain the findings contradictory to the known inhibitory properties of these negative regulators. Fon- tenot et al. [34] suggested that it is conceivable that persistent T cell activation and overly produced pro-inflammatory cytokines are seen as a danger signal by the immune system that reacts by selectively up-regulating the expression of negative costimulatory molecules. Apart from T-cell-associated cytokines, low-affinity ligation of the TCR will also result in increased expression of PD-1, and the inhibitory effect of PD-1 is therefore dependent on the degree of T-cell activation [13]. This possible mechanism may have a broader spectrum to include negative regulators of other family or network, such as Foxp3 and Fas/Fas ligand. It was further evident that both soluble PD-1 and PD-1, in a form of recombinant fusion protein, are able to effectively block the function of membrane-bound PD-1 and PD-L1 at a concentration range of 20 ng/ml in the in vitro experimental system. It appears that higher concentrations of recombinant fusion proteins are required to affect synovial T cell function in the system than the concentration of PD-1 and PD-L1 seen in RA SF [10]. Again, in contrast to our results, in the study of Li et al. [35] it has been found that levels of sPD-1 were downregulated in RA cases and negatively correlated with the increased CRP and disease activity score.

Grisen et al. [32] suggested a relationship between the production of autoantibodies and the shedding of PD-1, this was confirmed in our study as we found that plasma sPD-1 is strongly associated with the autoantibodies IgM-RF and anti-CCP and the SF sPD-1 with anti-CCP raising questions about the role of sPD-1 in autoantibody formation.

We found no significant correlation between the plasma or synovial sPD-1 levels and radiographic total sharp score. This was in contrast to that found by Grisen et al. [32] who demonstrated an inverse association between the relative change in sPD-1 and radiographic progression and showed that sustained high levels of sPD-1 after treatment initiation were associated with less radiographic progression in the future. Grisen et al. [32] explained that the T-cell co-stimulatory factors PD-1 act as CTLA-4, both can interfere with osteoclastogenesis but not with osteoclast function and the surplus of sPD-1 could modulate the activation of the T cells, decrease osteoclastogenesis and thereby modulate osteoclast function and on that base they claimed that inflammation and bone erosion can be independent events.

In conclusion, RA patients have significantly elevated levels of plasma and SF sPD-1 that are correlated with DAS28 suggesting sPD-1 to be a useful marker that reflects RA disease activity. The considerable association of sPD-1 with autoantibodies production implies a possible role in the pathogenesis of RA.

Conflict of interest

All the authors responsible for this work declare no conflict of interest.

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References


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