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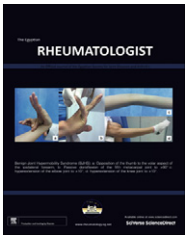

ORIGINAL ARTICLE

Clinical significance of soluble-triggering receptor expressed on myeloid cells-1 (sTREM-1) in patients with rheumatoid arthritis

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KEYWORDS

Triggering receptor expressed on myeloid cells-1; Rheumatoid arthritis; Disease activity

Abstract *Aim of the work:* To assess serum concentrations of triggering receptor expressed on myeloid cells-1 (sTREM-1) in rheumatoid arthritis (RA) patients, and correlate them with the main clinical, serological, radiological features and functional capacity of RA patients.

Patients and methods: Sera from 61 RA patients, and 30 healthy controls were assayed for sTREM-1 by Enzyme Linked Immunosorbant Assay. RA disease activity was assessed using 28-joint disease activity score (DAS-28). Assessment of patient's functional capacity was done using modified health assessment questionnaire (mHAQ). Standardized X-rays were done to all RA participants and evaluated according to Larsen score

Results: Serum levels of sTREM-1 were significantly higher in RA patients vs healthy controls (57.61 ± 28.87 and 43.72 ± 10.64 ng/ml; $p = 0.027$). These levels were higher in patients with severe disease activity (68.27 ± 36.14 ng/ml) than those with mild and moderate disease activity (43.50 ± 6.49 ng/ml and 47.52 ± 12.26 ng/ml, respectively; $p = 0.008$). On the contrary, no significant difference was found in levels of sTREM-1 in patients with extra-articular involvement or positive RF than those without. Levels of sTREM-1 showed a highly significant positive correlation with DAS-28 ($P = 0.001$), ESR ($P = 0.02$) and mHAQ ($p = 0.003$).There were no significant

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correlations between sTREM-1 level with age, disease duration, morning stiffness, nor radiological narrowing and erosion scores.

Conclusion: Levels of sTREM-1 were elevated in RA patients and correlated significantly with clinical and laboratory markers of disease activity as well as functional disability (as determined by mHAQ). To confirm our results we propose that larger scale, multicenter studies with longer evaluation periods are needed.

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory disease characterized by abnormal synovial hyperplasia and inflammatory cell infiltration into the synovium, which causes joint inflammation and subsequent destruction [1]. When synovial inflammation is initiated, RA fibroblast-like synovial cells start to proliferate and the activated inflammatory cells produce various pro-inflammatory mediators, including cytokines, chemokines and matrix metalloproteinases (MMPs), resulting in the exacerbation of synovitis and the destruction of joint integrity [2]. A large number of cytokines are found elevated in the joints of RA patients. Indeed, it is now clear that these cytokines play a fundamental role in the processes that cause inflammation, articular destruction, and the comorbidities associated with RA [3,4]. Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently identified molecule involved in monocytic activation and inflammatory response. It is expressed mainly on monocytes and neutrophils, and plays an important role in amplifying inflammatory response in acute and chronic inflammatory conditions [5,6]. TREM-1 expression is increased by various Toll-like receptor (TLR) ligands during acute inflammation [5]. Human TREM-1 is formed of an extracellular immunoglobulin (Ig)-like part, a transmembrane region with a positively charged lysine residue, and a short cytoplasmic part that does not have any signaling motifs. Its natural ligand has yet to be identified, so signaling and function of TREM-1 were studied using an agonistic antibody, which induces receptor cross-linking [7,8]. Apart from its membrane-bound form of TREM-1, a soluble form of TREM-1 (sTREM-1) is produced by the proteolytic cleavage of the extracellular, membrane-bound form. It has been reported that sTREM-1 works as a decoy receptor to prevent the binding of its ligand to membrane-bound TREM-1 and to inhibit the effect of TREM-1 activation [9].

The role of TREM-1 in producing and amplifying acute inflammatory response in many septic conditions is well established as well as in other several diseases [10–13].

Further, Kuai and colleagues have demonstrated the increased expression of functionally active TREM-1 on infiltrating leucocytes in human RA synovium [14].

In the present study we aimed to measure serum levels of sTREM-1 in RA patients and to determine its relation with disease activity, bone damage and functional capacity of RA patients.

2. Patients and methods

2.1. Study design

This is a cross sectional-observational study.

2.2. Clinical evaluation

Sixty-one patients with RA were randomly recruited from the Rheumatology and Rehabilitation Clinic Cairo University and the Rheumatology Clinic, Internal Medicine Department, Ain Shams University. All RA patients fulfilled the new EULAR/ACR criteria of 2010 [15]. Thirty healthy subjects were included as a control group and they were all age and sex matched. Patients with infection, malignancy as well as those with other autoimmune diseases were excluded from the study. All participants gave written informed consent to participate in the study, which was approved by our local Ethics Committee.

Patients and controls were subjected to the following: full medical history and thorough clinical examination (general, systemic and musculoskeletal). Assessment of RA disease activity by using 28 tender and swollen joint count disease activity score (DAS-28) [16] and patients were further divided according to their DAS-28 into severe, moderate, and mild disease activity (DAS >5.1, 3.2–5.1 and <3.2, respectively). Standardized X-rays were done to all participants and joint damage in RA patients was assessed by Larsen score [17]. As well, the functional assessment of RA patients was done using a modified health assessment questionnaire (mHAQ) [18].

2.3. Laboratory assessment

Routine biochemistry tests were collected from patients' records. Complete blood count was done using a Coulter counter (T660) and ESR was done by the Westergren method.

2.4. Rheumatoid factor (RF)

RF was determined by the latex fixation method. A suspension of uniform polystyrene particles sensitized in glycine buffer with heat altered human IgG (BD Diagnostic Systems, Sparks, Maryland, USA) was incubated with progressive dilutions of human sera in microtiter wells. After incubation, the plates were inspected for observable agglutination.

2.5. Serum sTREM level determination

Peripheral venous blood samples were obtained from patients and controls. After centrifugation at 1000*g* for 10 min, the serum was frozen and stored at −20 °C until assayed on the same day to eliminate the day to day interassay variability. A commercial Enzyme Linked Immunosorbant Assay (ELISA) kit (WKEA Med Supplies Corporation; NY; USA) was used for assaying sTREM levels. This assay employed an antibody specific for human TREM coated on a 96-well plate. In brief, each serum sample was directly transferred to the wells of the

ELISA plate and then assayed according to the manufacturer's instructions. The absorbance was measured at 450 nm in a microtest plate spectrophotometer, and TREM levels were quantified with a calibration curve using human TREM as a standard. Both standards and samples were evaluated in duplicates and the inter-assay variations were shown to be within the range given by the manufacturer.

Statistical analysis. The Statistical Package for Social Sciences (SPSS) version 10 (LEAD Technology Inc., Charlotte, NC, USA) was used to analyze the data. Continuous variables were summarized using the mean ± standard deviation (SD) values and categorical variables using absolute values and percentages. Significant differences were calculated using the Mann–Whitney U test for continuous variables. Spearman's rank correlation was used to examine the relationship between two continuous variables. A difference was considered to be statistically significant when the probability (p) value was <0.05.

3. Results

Sixty-one RA patients were included in the study. The mean age was 44.7 ± 10.7 years and 45 were females. Thirty age and gender matched healthy volunteers were considered as the control group with a mean age of 44 ± 11.2 years and 21were females. In RA patients, 72% had positive RF and 28% were sero-negative. Other characteristics of RA patients are displayed in Table 1.

RA patients had higher serum levels of sTREM-1 than controls, being 57.61 ± 28.87 ng/ml and 43.72 ± 10.64 ng/ml, respectively; $p = 0.027$ (Fig. 1).

Patients with RA were subsequently divided according to their grade of disease activity by DAS-28. Seven patients (11.5%) had mild disease activity, 23 patients (37.7%) had moderate disease activity while 31 patients (50.8%) had severe disease activity. Serum levels of sTREM-1 were higher in patients with severe disease activity (68.27 ± 36.14 ng/ml) than those with mild and moderate disease activity (43.50 ± 6.49 ng/ml and 47.52 ± 12.26 ng/ml, respectively; $p = 0.008$) (Fig. 2).

Twenty-six RA patients suffered from extra-articular manifestations (42.6%). Among them, 11 patients had SC nodules, 10 with Sicca Syndrome, neuropathy was found in five and interstitial pulmonary disease in six patients. Comparing serum levels of sTREM-1 in patients who had extra-articular manifestations with those without showed no significant difference (64.28 ± 36.92 ng/ml and 52.65 ± 20.22 ng/ml, respectively; $p = 0.165$). Similarly, no significant difference was found on comparing serum levels of sTREM-1 in patients with positive RF and those without (61.43 ± 32.17 ng/ml and 47.71 ± 14.09 ng/ml, respectively; $P = 0.08$).

Using the Spearman rank correlation analysis (Table 2, Fig 3), serum levels of sTREM-1 showed a highly significant positive correlation with DAS-28 ($P = 0.001$), ESR ($P = 0.02$) and mHAQ ($p = 0.003$). On the other hand, there were no significant correlations between sTREM-1 level and age, disease duration, morning stiffness, nor radiological narrowing and erosion scores of the hand joints ($p > 0.05$). Collectively, elevated sTREM levels were associated with clinical and laboratory measures of disease activity in RA patients as well as functional disability.

Table 1 Characteristics of rheumatoid arthritis (RA) patients (No = 61).

Parameter	Mean	±SD	Range
Disease duration (years)	8.1	7.1	1–30
Morning stiffness (min)	38.5	69.6	0–300
Tender joints (of 28)	9.1	4.2	0–28
Swollen joints (of 28)	4.6	5.5	0–24
DAS-28	5	1.5	2.21–8.2
mHAQ	0.9	0.7	0–2.75
Hemoglobin (g/dl)	11.7	1.1	9.6–14.6
Leucocytes (×1000/μl)	7.9	2.8	2.4–19.4
Platelets (×1000/μl)	330.3	119.5	153–755
ESR (mm/hr)	52.8	30.9	8–135

DAS: disease activity score, mHAQ: modified health assessment questionnaire, ESR: erythrocyte sedimentation rate.

4. Discussion

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently identified immunoglobulin-like cell surface receptor mainly expressed on neutrophils and its expression is increased by various Toll-like receptor (TLR) ligands during acute inflammation [8,19,20]. In this study sTREM-1 levels were found to be significantly elevated in patients with RA as compared to healthy controls. The data presented here demonstrate that sTREM-1 has an important role in RA, a disease in which persistent inflammation with subsequent progressive destruction of the bone and joint appears to play an indispensable role in the disease pathogenesis. The role of TREM-1 in mediating inflammatory response has been confirmed in acute and chronic inflammatory conditions [10,21–23]. TREM-1 is expressed in high levels in acute infectious lesions caused by bacteria and fungi [10]. Furthermore, it has been found elevated in murine models of septic shock, experimental acute pancreatitis and experimental inflammatory bowel diseases [21–23]. As well, the role of TREM-1 has been studied in human autoimmune diseases [24–27]. Plasma TREM-1 was elevated in patients with Behcet's and Crohn's disease [13,24]. Furthermore, genetic polymorphism in TREM-1 has been found to be associated with inflammatory bowel diseases in the Korean population [25]. Tomita et al. [26] studied the clinical significance of TREM-1 in patients with systemic sclerosis (SSc) and found that serum sTREM-1 levels were elevated in patients with diffuse cutaneous systemic sclerosis than those with the limited sub type. They were able to correlate these data with the severity of pulmonary fibrosis, suggesting that serum sTREM-1 is a novel serological marker for the disease severity of SSc. The possible role of sTREM-1 in ankylosing spondylitis (AS) had been studied and sTREM-1 seems to be a new mediator involved in patients with AS, particularly in the early stages of disease [27].

Here we found that sTREM-1 correlated significantly with disease activity, both clinically and the laboratory. Serum levels of sTREM-1 were higher in patients with elevated ESR. As well, sTREM-1 levels were elevated in patients with severe disease activity (DAS > 5.1) than those with mild disease activity (DAS < 3.2), a finding which suggests that sTREM-1 may reflect a degree of systemic inflammation. TREM-1 expression is

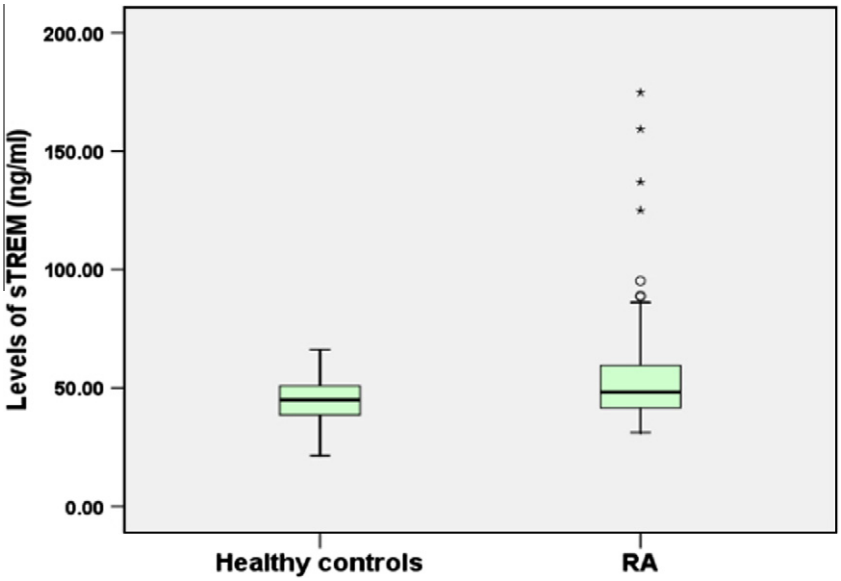

Figure 1 Serum levels of sTREM-1 (ng/ml) in RA patients and healthy controls.

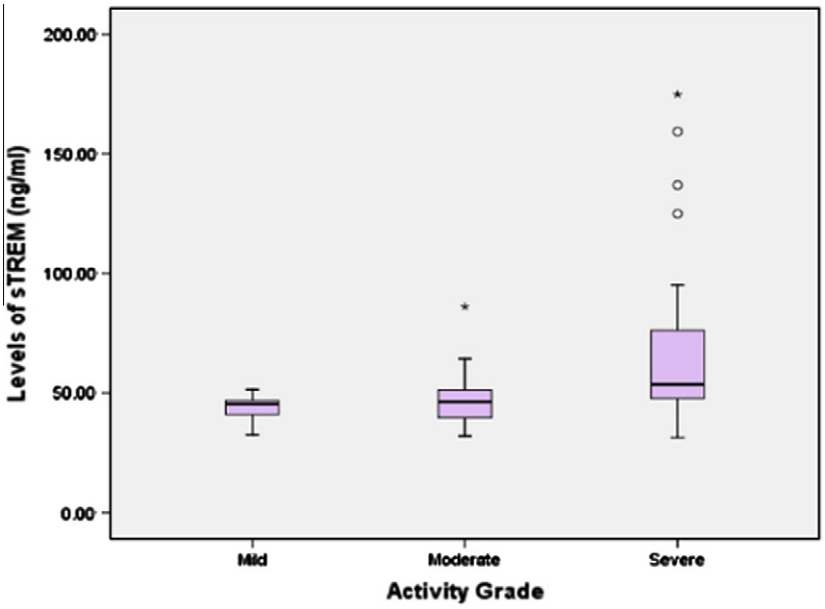

Figure 2 Serum sTREM-1 levels (ng/ml) in RA patients with different grades of disease activity.

increased by various TLR ligands during acute inflammation with subsequent increase in the production of pro-inflammatory cytokines [8,19,20]. This was confirmed by in vitro studies which have revealed that stimulation of synovial cells, isolated from synovial tissue specimens of RA patients, by TREM-1 showed increased cytokine production such as; TNFα, IL-8, IL-1β, GM-CSF as compared to controls. This suggests that TREM-1 contributes in amplifying inflammation in arthritis [14]. Similarly, Collins and coworkers have assessed the TREM-1 synovial expression in patients with distinct types of inflammatory and non-inflammatory arthritis [11]. They have reported comparable increase of sTREM-1 levels in septic arthritis and RA which were greater than those in gouty arthritis and non-inflammatory arthritis [11].

Two recent studies have found elevated sTREM-1 levels in RA patients than controls with significant association to clinical and laboratory parameters of disease activity, although sTREM-1 relation with the radiological damage or the functional capacity have not been discussed [14,28]. In the present study, elevated serum sTREM-1 was significantly higher in RA patients with deteriorated functional capacity. On the other hand, sTREM-1 was not correlated with radiological damage, neither narrowing score nor erosion score.

Biologic agents blocking the action of inflammatory cytokines have been able to suppress progression inflammation and joint destruction in RA patients [3]. However, some RA patients, about one third, fail to achieve complete remission. As well, serious bacterial infection and reactivation of

Table 2 Correlation between sTREM-1 levels (ng/ml) and some RA disease parameters.

Variable	*r*	*P*
Age (years)	−0.019	0.860
Disease duration (years)	−0.118	0.366
Morning stiffness (minutes)	0.209	0.107
DAS-28	0.405	0.001**
mHAQ	0.372	0.003*
ESR (mm/hr)	0.297	0.020*
Hemoglobin (g/dl)	0.078	0.551
Leucocytes (×1000/μl)	0.182	0.161
Platelets (×1000/μl)	0.093	0.478

DAS: disease activity score, mHAQ: modified health assessment questionnaire, ESR: erythrocyte sedimentation rate.
* High significant difference.
** Very high significant difference.

tuberculosis, as with treatment with TNF inhibitors, have been observed [4]. Targeting TREM-1 signaling may be used as a therapeutic strategy in RA. The administration of TREM-1 fusion protein in experimental collagen type II induced arthritis in mice has been associated with a significant reduction of clinical signs in a dose-dependent manner [29]. Further, it was hypothesized that modulating the actions of TREM-1 may be a safe therapeutic strategy for RA, as blocking TREM-1 signaling was found to suppress inflammatory responses without affecting the ability of the immune system to fight bacterial infection [30,31].

In conclusion, we have demonstrated high levels of sTREM-1 in the sera of RA patients compared to healthy controls. In addition, we have reported that serum levels of sTREM-1 are related to symptoms and signs of disease activity, as well as functional scores of RA patients. To confirm our results we propose that larger scale, multicenter studies with longer evaluation periods are needed.

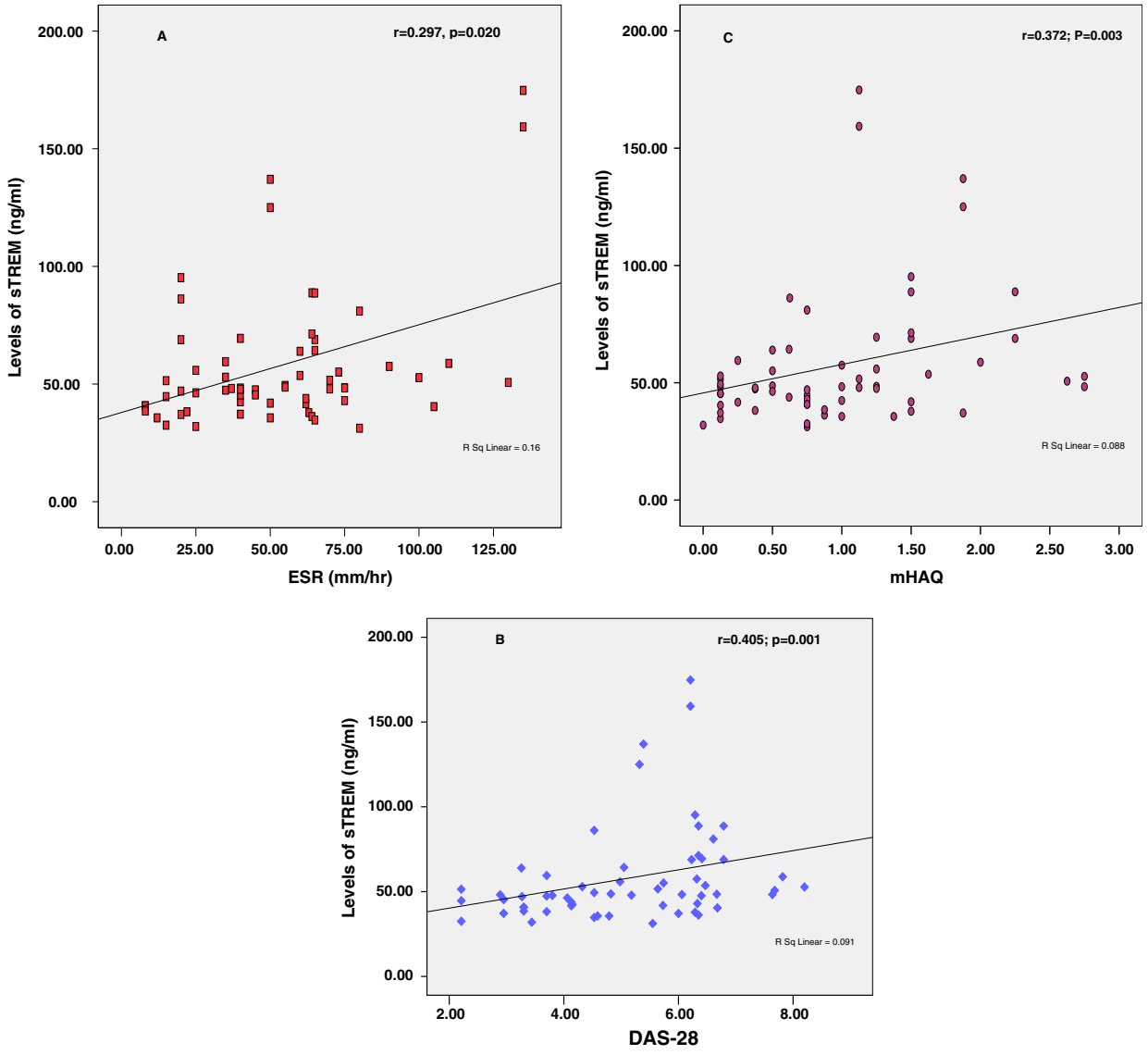

Figure 3 Scatter plot demonstrating correlation of serum sTREM levels with: (A) ESR, (B) DAS-28, (C) mHAQ.

5. Conflict of interest

No conflict of interest exists.

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