

Study of plasma levels of soluble triggering receptor expressed on myeloid cells-1 in rheumatoid arthritis and its correlation with disease activity and tumor necrosis factor- α

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Aim of the work

The triggering receptor expressed on myeloid cells-1 (TREM-1) is a cell surface receptor expressed mainly on monocytes and neutrophils. It acts as an amplifier of inflammatory response in acute and chronic inflammatory states. The aim of this work was to study the plasma-soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in rheumatoid arthritis (RA) patients and its correlation with disease activity and tumor necrosis factor- α (TNF- α).

Patients and methods

This study included 80 patients with RA and 20 age-matched and sex-matched controls. All were subjected to demographic, clinical, laboratory, and radiological studies using the 28-joint Disease Activity Score, erythrocyte sedimentation rate, complete blood count, and radiograph of both hands. Plasma levels of sTREM-1 and TNF- α were measured with enzyme-linked immunosorbent assay.

Results

RA patients had significantly higher sTREM-1 and TNF- α levels compared with controls (206.32 ± 125.75 and 17.83 ± 11.88 ; $P < 0.001$) and (190.82 ± 69.46 and 54.75 ± 9.46 ; $P < 0.001$). In RA patients, sTREM-1 levels were found to be positively correlated with 28-joint Disease Activity Score, erythrocyte sedimentation rate, and TNF- α level ($r = 0.408$, $P = 0.001$; $r = 0.287$, $P = 0.010$; $r = 0.749$, $P = 0.001$). sTREM-1 level was significantly increasing as patients had increasing disease activity (F -test = 20.62; $P = 0.001$).

Conclusion

RA patients had higher sTREM-1 and TNF- α level compared with controls, and sTREM-1 level was correlated with disease activity, suggesting that sTREM-1 plays a role in the inflammatory process associated with TNF- α , and it may be a useful disease activity marker in RA. TREM-1 may be a safe therapeutic strategy for RA, as blocking TREM-1 signaling was found to suppress inflammatory responses without affecting the immune system to fight bacterial infection.

Keywords:

disease activity, rheumatoid arthritis, soluble triggering receptor expressed on myeloid cells-1, tumor necrosis factor- α

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Introduction

Rheumatoid arthritis (RA) is a form of autoimmune chronic inflammatory arthritis characterized by synovial hyperplasia that leads to joint damage, and its clinical manifestations are not limited to arthritis but also include various systemic problems [1].

Numerous inflammatory cells such as macrophages, monocytes, T cells, B cells, and neutrophils are involved in the inflammatory process of RA, and various cytokines and chemokines play important roles in the pathogenesis of the disease [2]. Identification of cytokines and chemokines associated with RA may reveal insights on pathogenesis, and they could be used as markers of disease activity of RA or to monitor treatment response [1].

Triggering receptor expressed on myeloid cells (TREM-1) is described as a member of the immunoglobulin superfamily and is expressed on neutrophils, monocytes, and macrophages [3]. TREM-1 has been identified as a transmembrane receptor, and the membranous domain of TREM-1 binds to DNA activation protein of 12 kDa, an immune receptor tyrosine-based activation motif containing the adapter molecule [4].

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TREM-1 serves as a critical amplifier of inflammatory signaling and is upregulated by various stimuli such as proinflammatory cytokines and microbial Toll-like receptor ligands [5].

In addition to its membrane-bound form, a soluble form of TREM-1 soluble triggering receptor expressed on myeloid cells (sTREM-1) has been detected in the blood of patients with RA. It has been reported that sTREM-1 levels are elevated in patients with RA compared with controls [6], and that synovial fluid sTREM-1 levels are greater than those in gouty arthritis, nonseptic/nonrheumatoid inflammatory arthritis, and noninflammatory arthritis [7].

Our aim was to measure the plasma sTREM-1 level in patients with RA to evaluate its usefulness as a marker of disease activity in RA patients compared with other disease activity measures and tumor necrosis factor- α (TNF- α) as the proinflammatory cytokine.

Patients and methods

Patients

Eighty RA patients who fulfilled the 2010 revised American College of Rheumatology/European League Against Rheumatism classification criteria [8] were selected from the Physical Medicine, Rheumatology and Rehabilitation Outpatient Clinic, Menoufia University Hospitals, in the years 2013–2014. Only patients older than 16 years were selected. They were classified into two groups: group 1, comprising RA patients with disease duration less than 6 months (early RA), and group 2, comprising RA patients with disease duration more than or equal to 6 months (established RA). Written consent was obtained from all patients and controls after a full explanation of the study. This study was approved by the Ethical Committee of the Faculty of Medicine, Menoufia University.

Exclusion criteria

Patients with a history of diabetes, hypertension, congestive heart failure, pregnancy, or concomitant renal, hepatic, cardiac, or infectious processes were excluded from the study.

Controls

Twenty healthy volunteers matched for age and sex with the patient group were enrolled in the study as controls.

Methods

All patients were subjected to the following:

(1) Demographic data recording.

(2) Clinical examination.

The locomotor system was examined and local examination of joint tenderness and swelling was carried out.

Chest, heart, and abdominal examination to detect extra-articular manifestations of RA was carried out for all patients.

(3) Disease activity assessment: this was achieved using the Disease Activity Score (DAS28) with three variables [erythrocyte sedimentation rate (ESR), number of swollen joints, and number of tender joints] [9].

The level of disease activity was interpreted as follows:

(a) Low: $\text{DAS28} \leq 3.2$.

(b) Moderate: $3.2 < \text{DAS28} \leq 5.1$.

(c) $\text{DAS} < 2.6$: remission according to the American College of Rheumatology criteria.

(4) Laboratory investigations: complete blood count, ESR [10], rheumatoid factor (RF) [11], anti-cyclic citrullinated peptide (ACCP) level [12], C-reactive protein (CRP) [13], fasting and 2 hours' postprandial blood glucose, liver function tests (serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase), renal function tests (serum creatinine and blood urea), and serum levels of human TREM-1 [14] and human TNF α [15] by enzyme-linked immunosorbent assay were measured.

(5) Radiographic assessment: posterior–anterior radiographs of the hands were obtained within 1 month of blood sampling. The degree of RA progression was assessed by Larsen scoring of the metacarpophalangeal joints on a scale of 0–5.

Data obtained were tabulated and analyzed with SPSS (SPSS Inc., Chicago, Illinois, USA) statistical package, version 18, on an IBM compatible computer. Quantitative data were expressed as $\bar{X} \pm \text{SD}$. Qualitative data were expressed as number and percentage and analyzed by applying the χ^2 -test. Comparisons between groups were made using the unpaired t -test. Pearson's correlation (r) was used to detect the association between quantitative variables. P values greater than 0.05 were considered nonsignificant, values less than 0.05 were considered significant, and values less than 0.001 were considered highly significant.

Results

The clinical data of the studied RA patients are shown in Table 1. The study revealed a statistically highly significant difference between RA patients and

Table 1 Clinical data of the studied RA patient groups (N=80)

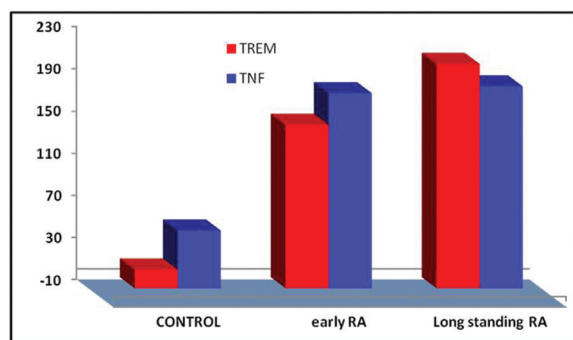
Parameter	Groups	Mean±SD	Range
Disease duration (years)	Group 1 (9)	0.333±0.106	0.25–30
	Group 2 (71)	11.07±5.88	
Tender joints (of 28)	Group 1 (9)	4.56±3.909	0–24
	Group 2 (71)	5.25±4.535	
Swollen joints (of 28)	Group 1 (9)	1.22±1.394	0–20
	Group 2 (71)	2.96±3.454	
DAS28	Group 1 (9)	3.755±0.817	2.38–8.34
	Group 2 (71)	4.709±1.264	

DAS28, 28-joint Disease Activity Score; RA, rheumatoid arthritis.

Table 2 Comparison between studied RA patients and controls regarding TREM-1 and TNF- α serum levels

Characteristic	RA patients (n=80) (mean±SD)		Control (n=20) (mean±SD)	F-test	P value
	Early RA (n=9)	Established RA (n=71)			
TREM-1	155.0±79.38	212.8±129.40	17.83±11.88	23.55	<0.001
TNF- α	185.11±79.89	191.54±68.34	54.75±9.46	37.87	<0.001

RA, rheumatoid arthritis; TNF- α , tumor necrosis factor- α ; TREM-1, triggering receptor expressed on myeloid cells-1. * P <0.05 is significant. ** P <0.001 is highly significant.

Figure 1

Comparison between studied RA patients and controls regarding TREM-1 and TNF- α . RA, rheumatoid arthritis; TNF- α , tumor necrosis factor- α ; TREM-1, triggering receptor expressed on myeloid cells-1.

controls regarding the level of TREM-1 and TNF- α (Table 2 and Fig. 1). There was no statistically significant difference in serum levels of sTREM-1 and TNF- α between RA patients with positive RF and those who were seronegative (Table 3) or between patients with positive ACCP and those with negative ACCP (Table 4). There was a statistically highly significance difference (P <0.001) regarding serum sTREM-1 and TNF- α levels in RA patients according to disease activity (Table 5 and Fig. 2). Pearson's correlation revealed a statistically highly significant positive correlation between sTREM level and DAS28 (r =0.408, P <0.001), TNF- α (r =0.749, P <0.001), and ESR (r =0.287, P <0.010). There was a statistically significant negative correlation between sTREM level and hemoglobin (Hb) (r =−0.228, P <0.435), whereas there was no statistically significant correlation between sTREM level and age, disease duration, leukocytic count, platelet

count, and radiological findings (Table 6 and Fig. 3). Pearson's correlation revealed a statistically highly significant positive correlation between TNF- α level, DAS28 (r =0.520, P <0.001), sTREM (r =0.749, P <0.001), and ESR (r =0.317, P <0.004) and statistically significant negative correlation between TNF- α level and Hb (r =−0.345, P <0.002). There was no statistically significant correlation between TNF- α level and age, disease duration, leukocytic count, platelet count, and radiological findings (Table 7 and Fig. 4).

Discussion

RA is a systemic autoimmune disease characterized by chronic inflammation of the synovial joints, which leads to progressive destruction of cartilage and bone. It is now clear that a large number of inflammatory cytokines such as TNF- α , interleukin-1b (IL-1b), IL-6, IL-12, IL-23, and IL-17A play an important role in persistent inflammation and progressive destruction of cartilage and bone [16].

TREM-1 is known as a cell surface receptor mainly expressed on monocytes and neutrophils; it is expressed in acute and chronic inflammatory conditions and is upgraded by various Toll-like receptor ligands [2].

TREM-1 is expressed in many diseases, including infections, and in acute and chronic noninfectious inflammatory disorders, such as inflammatory bowel disease and ankylosing spondylitis. More specifically, TREM-1 is upregulated in RA synovium, and its activation induces many proinflammatory cytokines in patients with RA [5].

Table 3 Comparison between serum TREM-1 and TNF- α level in the studied RA patients regarding RF status

Variable	Positive RF ($n=69$) (mean \pm SD)	Negative RF ($n=11$) (mean \pm SD)	<i>t</i> -test	<i>P</i>
TREM-1	211.30 \pm 131.37	175.08 \pm 79.44	0.886	0.378
TNF- α	195.23 \pm 69.35	163.12 \pm 64.44	1.439	0.154

RA, rheumatoid arthritis; RF, rheumatoid factor; TNF- α , tumor necrosis factor- α ; TREM-1, triggering receptor expressed on myeloid cells-1.

Table 4 Comparison between serum TREM-1 and TNF- α level in the studied RA patients regarding ACCP status

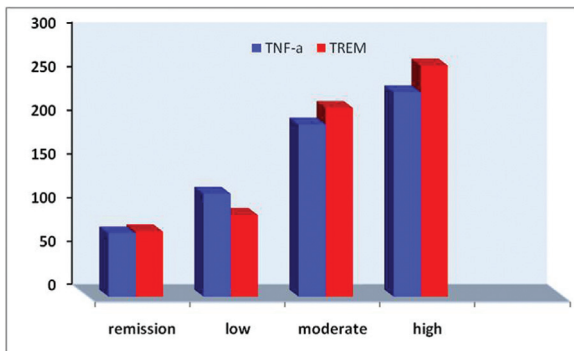
Variable	Positive ACCP ($n=55$) (mean \pm SD)	Negative ACCP ($n=25$) (mean \pm SD)	<i>t</i> -test	<i>P</i>
TREM-1	209.56 \pm 115.61	199.2 \pm 147.99	0.340	0.735
TNF- α	200.35 \pm 69.01	169.85 \pm 66.21	1.855	0.067

ACCP, anti-cyclic citrullinated peptide; RA, rheumatoid arthritis; TNF- α , tumor necrosis factor- α ; TREM-1, triggering receptor expressed on myeloid cells-1.

Table 5 Comparison between serum TREM-1 and TNF- α level in RA patients according to disease activity

Variable	Remission (group 1) (DAS \leq 2.6) ($n=4$)	Low (group 2) (2.6<DAS \leq 3.2) ($n=12$)	Moderate (group 3) (3.2<DAS \leq 5.1) ($n=38$)	High (group 4) (DAS>5.1) ($n=26$)	<i>F</i> -test	<i>P</i>
TNF	72.25 \pm 15.392	117.75 \pm 44.89	196.95 \pm 50.55	233.82 \pm 60.36	20.62	<0.001**
TREM	74.50 \pm 0.57	93.36 \pm 27.02	216.06 \pm 119.07	264.50 \pm 126.86	8.51	<0.001**

DAS, Disease Activity Score; RA, rheumatoid arthritis; TNF- α , tumor necrosis factor- α ; TREM-1, triggering receptor expressed on myeloid cells-1. * P <0.05 is significant. ** P <0.001 is highly significant.

Figure 2

TREM-1 and TNF- α level in RA patients according to disease activity. RA, rheumatoid arthritis; TNF- α , tumor necrosis factor- α ; TREM-1, triggering receptor expressed on myeloid cells-1.

Therefore, our aim was to study the plasma level of sTREM-1 in RA patients and its correlation with disease activity and TNF- α as a proinflammatory cytokine.

This study showed that patients with RA have significantly higher plasma sTREM-1 and TNF- α levels compared with controls. This was in agreement with the results of Kuai *et al.* [6], El Bakry Samah *et al.* [2], and Sung *et al.* [5], who found that the average amount of soluble TREM-1 in RA patients was higher than that of controls. The data showed that sTREM-1 has a major role in RA.

In this study, sTREM-1 correlated significantly with disease activity. Serum levels of sTREM-1 were significantly higher in patients with elevated ESR and CRP. sTREM-1 levels were elevated in patients with

Table 6 Correlation between sTREM-1 levels and demographic, clinical, and laboratory parameters of RA patients

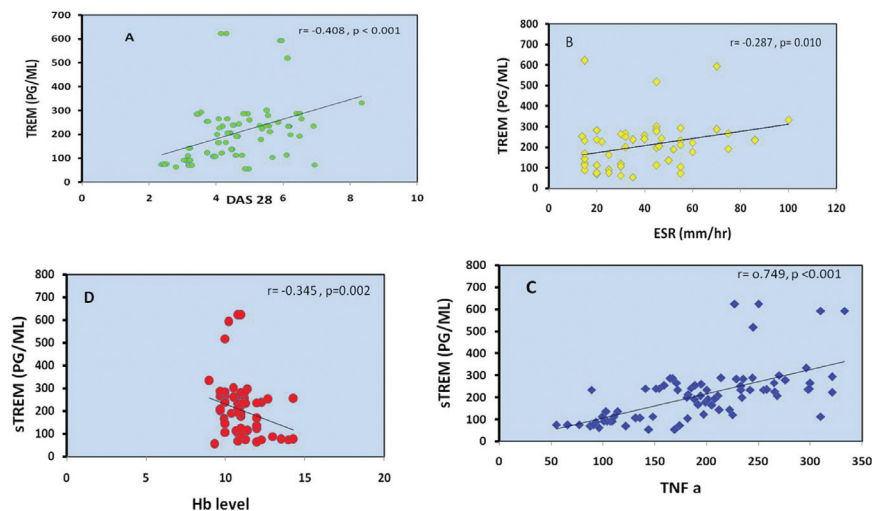
Variable	<i>r</i>	<i>P</i>
Age (years)	0.065	0.522
Disease duration (years)	0.101	0.375
DAS28	0.408	<0.001**
ESR (mm/h)	0.287	0.010**
TNF- α	0.749	<0.001**
Hemoglobin (g/dl)	-0.228	0.042*
Leukocytes	0.088	0.435
Platelets	0.098	0.386
Larsen score	0.087	0.441

DAS28, 28-joint Disease Activity Score; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; sTREM-1, soluble triggering receptor expressed on myeloid cells-1; TNF- α , tumor necrosis factor- α . * P <0.05 is significant. ** P <0.001 is highly significant.

severe disease activity (DAS>5.1) compared with those with mild disease activity (DAS<3.2). This was in agreement with the findings of Kuai *et al.* [6], El Bakry Samah *et al.* [2], and Sung *et al.* [5], who found that sTREM-1 levels were found to be significantly elevated in patients with RA and correlated significantly with disease activity. These results give an index that sTREM-1 contributes to the inflammatory process of RA, and that plasma sTREM-1 levels may be used as an activity marker in RA.

In this study, RA patients had higher TNF- α levels, which were correlated with plasma sTREM-1 levels; this is supported by the work of Collins *et al.* [7], who have assessed the TREM-1 synovial expression in patients with certain types of inflammatory and

Figure 3



Correlation of serum sTREM levels with (a) DAS28, (b) ESR, (c) TNF, and (d) Hb. DAS28, 28-joint Disease Activity Score; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; sTREM-1, soluble triggering receptor expressed on myeloid cells-1; TNF, tumor necrosis factor.

Table 7 Correlation between TNF- α level and demographic, clinical, and laboratory parameters of RA patients

Variable	<i>r</i>	<i>P</i>
Age (years)	0.158	0.116
Disease duration (years)	0.142	0.209
DAS28	0.520	<0.001**
ESR (mm/h)	0.317	0.004**
TREM	0.749	<0.001**
Hemoglobin (g/dl)	-0.345	0.002**
Leukocytes	0.143	0.206
Platelets	0.137	0.224
Larsen score	0.125	0.270

DAS28, 28-joint Disease Activity Score; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; TNF- α , tumor necrosis factor- α ; TREM-1, triggering receptor expressed on myeloid cells-1. * $P < 0.05$ is significant. ** $P < 0.001$ is highly significant.

noninflammatory arthritis. They have reported an increase in TNF- α and sTREM-1 levels in septic arthritis and RA compared with gouty arthritis and noninflammatory arthritis.

Although the biologic agents used to block inflammatory cytokines have the ability to suppress the progression of inflammation and joint destruction in RA patients [17], about one-third of RA patients fail to achieve complete remission and there are serious side effects of bacterial infection and reactivation of tuberculosis with those biologic treatments [18]. Targeting of TREM-1 signaling may be a therapeutic modality in RA [19]. Further, it was hypothesized that modifying 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 [20].

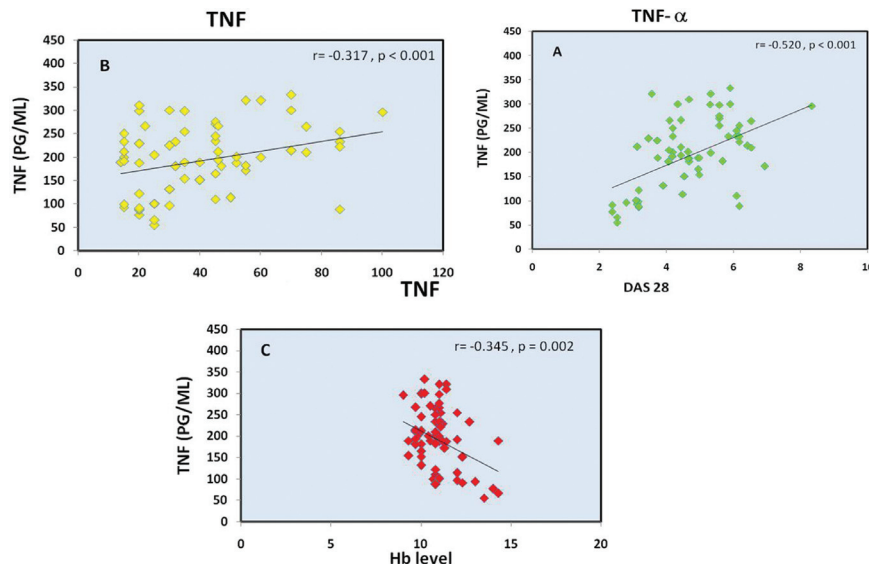
In this study, there were no correlations between sTREM-1 and TNF- α level in RA patients according to RF status. This was in agreement with the findings of Kuai *et al.* [6], El Bakry Samah *et al.* [2], and Sung *et al.* [5], who found that the average amount of soluble TREM-1 and TNF- α level in RA patients was not correlated with RF status.

In this study, there were no correlations between sTREM-1 and TNF- α level in RA patients according to ACCP status. This was in agreement with the results of Kuai *et al.* [6], El Bakry Samah *et al.* [2], and Sung *et al.* [5], who found that the average amount of soluble TREM-1 and TNF- α level in RA patients was not correlated with ACCP status.

In this study, there were no correlations between sTREM-1 level and age, disease duration, platelets, and radiological findings. This was in agreement with El Bakry Samah *et al.* [2], who found that there were no significant correlations between sTREM-1 level and age, disease duration, morning stiffness, radiological narrowing, and erosion scores of the hand joints ($P > 0.05$).

In this study, there were no correlations between sTREM-1 level and white blood cells (WBC). This was in agreement with the results of El Bakry Samah *et al.* [2], who found no significant correlations between sTREM-1 level and WBC and platelets. However, this is in contradiction to the results of Sung *et al.* [5], who found that sTREM-1 levels were significantly correlated with serum WBC and neutrophil counts. He found that TREM-1 is expressed on neutrophils, monocytes, and macrophages [21], and therefore plasma

Figure 4



Correlation of serum TNF- α levels with (a) DAS28, (b) ESR, and (c) Hb. DAS28, 28-joint Disease Activity Score; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; TNF- α , tumor necrosis factor- α .

sTREM-1 might be released by macrophages/monocytes or neutrophils in RA patients.

In this study, there was a negative correlation between sTREM-1 and TNF- α level with Hb. It is reported that the blunting of bone marrow response to erythropoietin may be mediated by IL-1, TNF- α , and transforming growth factor β , which affect erythropoietin mRNA transcription [22]. It has also been demonstrated that IL-1, which is increased during inflammation, inhibits erythroid colony-forming units, which later develop into erythroblasts [23].

The present study demonstrated that RA patients had higher plasma sTREM-1 and TNF- α levels compared with controls, and plasma sTREM-1 levels were correlated with disease activity indices, including DAS28, ESR, and CRP, suggesting that plasma sTREM-1 could play a role in the inflammatory process associated with TNF- α , and it may be a useful disease activity marker in RA. Targeting of TREM-1 signaling may be a therapeutic strategy in RA, as blocking TREM-1 signaling was found to suppress inflammatory responses without affecting the ability of the immune system to fight bacterial infection.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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