TCR-CD3ζ gene expression profile in patients with rheumatoid arthritis and correlation with disease activity

Abeer Abdelati[a], Rehab Elnemr[b], Ahmed Ismail[a], Marwa Gamal-Eldeen[c]

[a]Rheumatology Unit, Department of Internal Medicine, [b]Department of Physical Medicine, Rheumatology and Rehabilitation, [c]Department of Clinical and Chemical Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Correspondence to Rehab Elnemr, Lecturer Department of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Alexandria University, Alexandria, Egypt. Tel: +20 128 151 0109; e-mail: hobaelnemr@hotmail.com

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Objective
To measure the T-cell receptor-CD3 zeta chain (*TCR-CD3ζ*) gene expression profile in a cohort of patients with rheumatoid arthritis (RA).
Patients and methods
A case–control study on 150 consecutive RA patients diagnosed according to 2010 ACR/EULAR criteria and 150 matched healthy controls without a family history of RA or other autoimmune diseases. RA patients with other autoimmune diseases, viral hepatitis B or C, malignancy or hematological disorders were excluded from the study. All participants were subjected to history taking, clinical examination, assessment of disease activity (in RA patients) using Disease Activity Score-28 and Health Assessment Questionnaire, routine laboratory investigations, inflammatory marker levels, serological tests, as well as molecular analysis for *TCR-CD3ζ* mRNA expression by quantitative real-time PCR.
Results
TCR-CD3ζ gene expression was significantly lower in RA cases than in controls ($P<0.05$). Expression of *TCR-CD3ζ* has shown a significant negative correlation with RA disease duration, rheumatoid factor, and erythrocyte sedimentation rate ($P<0.05$) in RA cases. The level of *TCR-CD3ζ* also showed a significantly less expression in patients with positive rheumatoid factor.
Conclusion
Our results demonstrated a lower expression of TCR-CD3ζ in RA patients than in healthy controls. We suggested that CD247 gene downregulation might contribute in the susceptibility to RA and help understanding the pathways responsible for deficient T-cell responses in RA patients.

Keywords:
CD247, gene expression, PCR, rheumatoid arthritis, T-cell receptor-CD3 zeta chain

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Introduction

Rheumatoid arthritis (RA) is the most common systemic inflammatory disease with articular and extra-articular manifestations [1,2]. It affects 1–2% of the population worldwide, with women affected 2–3 times more commonly than men [3]. Regarding the etiology of RA, there are many genetic and environmental factors that have associations with the occurrence of RA [4]. Detecting the genetic susceptibility to RA is very challenging. However, many studies identified genetic loci linked to RA such as HLA-DRB1 [5] and cytotoxic T-lymphocyte protein 4 [6]. The initiation and progression of the inflammatory disorder is controlled through the activation and signaling of specific cell surface chemoattractant receptors by their cognate protein ligands, termed chemokines [7].

Over the past two decades, T cells have become the scope of researches in immunology [8,9]. T cells are the doorkeepers of immune responses toward both foreign and self-antigens. Advances in understanding the pathogenesis of RA confirm the contribution of T cells in the disease process through the recognition of the arthritogenic antigen and maintenance of chronic inflammatory response [10]. It is well recognized that T cells respond to antigen through T-cell receptor (TCR) recognition of MHC-bound peptides on antigen-presenting cells. After identification of a peptide as foreign, a cascade of intracellular signaling takes place, producing both costimulatory molecules and cytokines that will subsequently activate other immune cells. The *TCR-CD3* is a multisubunit complex, made up of at least eight transmembrane units. The TCR α-chain and β-chain are essential for antigen-specific recognition [11], whereas CD3 complex (δ, ε, and γ) is involved in signal-transduction pathways through the immunoreceptor tyrosine-based activation motifs [12].

T-cell receptor zeta chain (*TCR-CD3ζ)*, also known as CD247, is responsible for surface expression and signaling steps of the TCR–CD3 complex. CD247 encodes the TCR ζ, a subunit of the T-cell receptor–CD3 complex that may cause inflammatory arthritis in mice if mutated [13]. The zeta chain has a distinguished role in coupling antigen recognition to intracellular signal-transduction pathways. These data were supported by the reported data on T-cell activation and differentiation in RA pathogenesis [14].

Lower expression of CD247 has been found in different diseases including some infection, inflammatory disorders, and malignancy [15,16]. As the depressed levels of *TCR-CD3ζ* are not specific to one particular disease, it remains unclear whether *TCR-CD3ζ* dysfunction is a primary inciting event or arises as a consequence of chronic inflammatory responses. The initiation and progression of the inflammatory disorder is controlled through the activation and signaling of specific cell surface chemoattractant receptors by their cognate protein ligands, termed chemokines.

Past researches on gene association have reported that TCR-CD3ζ gene polymorphisms and TCR-CD3ζ downregulation are associated with various diseases including Crohn's disease, systemic lupus erythematosus [12,17], and systemic sclerosis [18,19]. As regards RA, this relationship has not been fully clarified yet as well as its correlation with disease activity was not studied [14,20,21].

Accordingly, we aimed to measure the TCR-CD3ζ gene expression profile in a cohort of patients with RA and correlating its expression with disease activity.

Patients and methods

The study cohort included 150 consecutive RA patients diagnosed according to 2010 ACR/EULAR criteria [22] and recruited between 1 April 2017 and 31 March 2018 from the Rheumatology Outpatient Clinic and Rheumatology Division of the Main Alexandria University Hospital; and 150 matched healthy controls (HC) with no family history of RA or other immune diseases.

Exclusion criteria were RA patients with other autoimmune diseases, viral hepatitis B or C, underlying malignancy, or hematological disorders.

All participants were subjected to full history taking and physical examination, routine laboratory investigations including complete blood count, fasting blood sugar, aspartate transaminases, alanine transaminases, blood urea nitrogen and serum creatinine, inflammatory markers [erythrocyte sedimentation rate (ESR) and quantitative C-reactive protein (CRP)], serological tests [rheumatoid factor (RF) and anti-citrullinated peptide protein antibody (ACPA)], and molecular analysis. Furthermore, RA cases were subjected to disease activity assessment using Disease Activity Score (DAS)-28 and Health Assessment Questionnaire (HAQ) ([23,24].

RF was measured by nephelometry on BN-ProSpec which identifies IgM-RF and the reference range was from 0 to 20 IU/ml [25], whereas ACPA was tested using enzyme-linked immunosorbent assay and the cutoff for positivity was up to 25 U/ml. ACPA levels then were classified in titer as low for values 25–50 U/ml, moderate for values 50–75 U/ml, and high for values more than 75 U/ml [26].

Quantitative real-time PCR

Total RNA was extracted from whole venous blood samples immediately using Pure-Link RNA Mini Kit (Invitrogen Life Technologies, Carlsbad, California, USA).

The extraction procedure was done according to the manufacturer's instructions. Homogenization of the frozen cell pellet was done, followed by binding, washing, and elution of RNA. The quantity and quality were determined by NanoDrop2000 Spectrophotometer (Thermo Scientific, State of California, United States of America) UV absorbance at 260, 280, and 230 nm. A260 : A230 ratio greater than 1.7 and A260 : A280 ratio greater than 2.0 were considered indicators for highly pure RNA.

Reverse-transcription PCR for cDNA synthesis was carried out using Invitrogen High Capacity cDNA RT kit (Thermo Fisher Scientific, State of California, United States of America) according to the manufacturer's instructions.

TCR-CD3ζmRNA expression was then studied by quantitative real-time PCR using TaqMan Gene Expression assay probes on Rotor-Gene Q real-time PCR amplifier (Qiagen, Hilden, Germany), with co-amplification of the reference gene B-Actin from the same blood sample as an internal control (TCR-CD3ζ: Hs00609515_m1, B-actin: Hs99999903_M1).

The PCR mixture was 20 µl reaction composed of 4 µl of cDNA, 10 µl TaqMan Gene Expression Master

Mix, 1 µl assay, and 5 µl water. The thermal cycling conditions were composed of incubation for 2 min at 50°C followed by incubation at 95°C for 10 min and then 40 PCR cycles of 95°C for 15 s and 60°C for 1 min. Each sample on RT-qPCR was analyzed in duplicate. Relative *TCR-CD3ζ* mRNA expression was calculated as $2^{-\Delta\Delta C_t}$ ($\Delta C_t = C_t$ target-C_t β-actin) [27].

Statistical analysis

Data were analyzed using IBM SPSS version 20 [28]. Qualitative data were illustrated using number and percent. Quantitative data were presented using range (minimum and maximum), mean, SD, and median. *P* value less than 0.05 was accepted as statistically significant. The used statistical tests were: χ^2-test (for categorical variables), Student's *t*-test (for normally quantitative variables), Mann–Whitney test (for abnormally quantitative variables), and Spearman's coefficient (to correlate between two abnormally quantitative variables). *P* value less than 0.05 was interpreted as statistically significant.

The receiver operating characteristic (ROC) curves with the area under the curve (AUC) were used to assess the diagnostic performance of TCR-CD3ζ level as a predictor of RA, 'an area=1.00 (100%) denoting (a gold standard-like) performance, while an area=0.5 (50%) denoting (a chance-like) performance. Significant areas ($P<0.05$) indicate that the diagnostic performance is significantly better than chance. The cutoff value was chosen as the point that maximizes sensitivity and specificity.

Results

Participants' demographic data and T-cell receptor-CD3 zeta chain gene expression

This study was conducted on 150 RA patients (117 women and 33 men) with a mean age of 30.04±10.26 years (RA cases), and 150 (123 women and 27 men) HCs with a mean age of 35.14±9.12 years. There was no statistically significant difference between the two groups (RA cases and HC) regarding sex and age ($P<0.05$), but there was statistically significant difference between both groups regarding *TCR-CD3ζ* gene expression being lower in the RA group (mean was 0.663±1.90) than the HC group (mean was 0.816±1.64) as shown in Table 1.

Correlations

Measuring the correlation between *TCR-CD3ζ* level and different clinical and laboratory variables assessed in RA patients are shown in Table 2. Assessment of disease activity was performed by applying the DAS (DAS-28/ESR version) and the mean was 3.6±1.13, whereas functional state by applying the HAQ and its mean was 0.98±0.45. *TCR-CD3ζ* level did not correlate with either DAS-28/ESR, HAQ, age of the patients, or duration of morning stiffness.

Although a correlation between two variables does not imply causation, our data showed significant negative correlation between *TCR-CD3ζ* and disease duration,

Table 2 Correlation between the T-cell receptor-CD3 zeta chain level with different variables in rheumatoid arthritis cases

Disease variables	CD247	
	R	*P*
Age (years)	0.138	0.338
Disease duration (years)	0.279	0.049*
Duration of morning stiffness (min)	0.214	0.137
Erythrocyte sedimentation rate (mm/h)	–0.301	0.034*
C-reactive protein (mg/l)	–0.228	0.111
Disease Activity Score-28	0.269	0.059
Health Assessment Questionnaire score	0.057	0.696
Rheumatoid factor (IU/ml)	–0.314	0.026*
Anti-citrulinated peptide protein antibody (U/ml)	–0.257	0.072

*$P \leq 0.05$, statistically significant.

Table 1 Comparison between rheumatoid arthritis cases and healthy controls regarding demographic characteristics and T-cell receptor-CD3 zeta chain gene expression

Demographic characteristics	RA cases (*n*=150) [*n* (%)]	HC (*n*=150) [*n* (%)]	Test of significance	*P*
Sex				
Male	33 (22.0)	27 (18.0)	χ^2=0.250	0.617
F emale	117 (78.0)	123 (82.0)		
Age (years)				
Minimum–maximum	20–58	22–56	*t*=–0.464	0.644
Mean±SD	30.04±10.26	35.14±9.12		
TCR-CD3ζ				
Minimum–maximum	0.037–12.467	0.034–11.314	*Z*=–3.047*	0.002*
Mean±SD	0.663±1.90	0.816±1.64		
Median (Q1–Q3)	0.178 (0.109–0.314)	0.315 (0.198–0.922)		

HC, healthy control; RA, rheumatoid arthritis; TCR-CD3ζ, T-cell receptor-CD3 zeta chain. *$P \leq 0.05$, statistically significant.

Table 3 Multivariate linear regression for factors affecting T-cell receptor-CD3 zeta chain level in the rheumatoid arthritis group

Disease parameters	*B*	SE	*β*	*t*	*P*
Disease duration (years)	0.047	0.065	0.093	0.716	0.478
Erythrocyte sedimentation rate (mm/h)	0.010	0.010	0.150	0.999	0.323
Rheumatoid factor (IU/ml)	0.005	0.002	0.469	2.879	0.006*

*$P \leq 0.05$, statistically significant.

Table 4 Relation between the T-cell receptor-CD3 zeta chain level and different variables in the rheumatoid arthritis group

Parameters	TCR-CD3ζ (mean±SD)	Test of significance (*Z*)	*P*
Sex			
Male	0.849±1.685	−0.023	0.981
Female	0.609±1.976		
Rheumatoid factor			
Positive	0.092±0.065	−2.235	0.025*
Negative	0.712±1.977		
Anti-citrulinated peptide protein antibody			
Positive	0.330±0.256	−0.791	0.429
Negative	0.708±2.024		
C-reactive protein			
Positive	0.337±0.532	−1.006	0.314
Negative	0.777±2.186		

TCR-CD3ζ, T-cell receptor-CD3 zeta chain. *$P \leq 0.05$, statistically significant.

RF, and ESR ($P<0.05$). This means, the longer the duration of RA lower the level of *TCR-CD3ζ* gene expression, and the higher the value of RF and ESR the lower the level of *TCR-CD3ζ* gene expression. Moreover, the level of *TCR-CD3ζ* was negatively correlated with CRP and ACPA, but did not reach a significant value ($P=0.111$ and 0.072), respectively.

Multivariate linear regression for factors that showed significant negative correlation with the *TCR-CD3ζ* level in the RA group including disease duration, ESR, and RF revealed that RF positivity is a predictor of lower level of *TCR-CD3ζ* ($P<0.05$) as shown in Table 3.

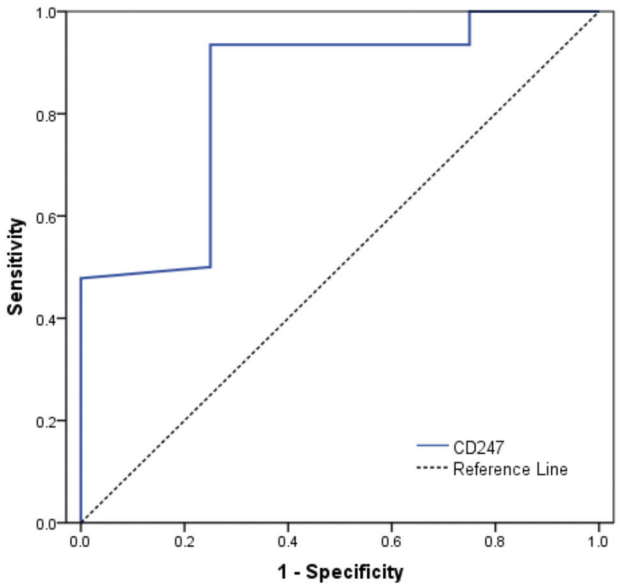
Relations

In an attempt to find a causal relation between *TCR-CD3ζ* level and different variables in RA, Mann–Whitney test was calculated and the data are demonstrated in Table 4. There was a statistically significant relation between the expression of *TCR-CD3ζ* gene and RF positivity ($P=0.025$) being less expressed in patients with positive RF. This means that the lower level of *TCR-CD3ζ* gene expression could be a cause of RF positivity in RA patients. Moreover, *TCR-CD3ζ* level was less expressed in ACPA positive and CRP positive cases but did not reach a significant level.

Receiver operating characteristic analysis

Values of TCR-CD3ζ were used to construct the ROC curve as shown in the area under the ROC (AUC) curve and the confidence intervals were estimated to examine the accuracy of TCR-CD3ζ gene level in discriminating patients with RA from those without RA as shown in Fig. 1. The AUC of the TCR-CD3ζ level showed a moderate level of accuracy (AUC=0.840, $P=0.025$) and the calculated cutoff value (≤0.077) can precisely discriminate patients with RA from those without RA with 93.5% sensitivity and 75% specificity as shown in Table 5.

Figure 1



Receiver operating characteristic curve for CD247 (T-cell receptor-CD3 zeta chain) level as a predictor of rheumatoid arthritis.

Table 5 Analysis of receiver operating characteristic curve for T-cell receptor-CD3 zeta chain level as a predictor of rheumatoid arthritis

Variable	Cutoff point (ng/ml)	AUC	*P*	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy (%)
TCR-CD3ζ	≤0.077	0.840*	0.025**	93.5	75.0	78.9	92.0	84.3

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; TCR-CD3ζ, T-cell receptor-CD3 zeta chain. *AUC≥0.5. **$P \leq 0.05$, statistically significant.

Discussion

The CD247 codes for CD3ζ, which is a component of the TCR/CD3 signaling complex on T cells, and tyrosine phosphorylation of CD3ζ is one of the first events occurring after TCR engagement [4]. The role of zeta chain is to combine antigen recognition to intracellular signal pathways. Reduced expression of the antigen leads to impaired immune response. Previous studies have suggested that there may be potential errors in the proximal signal-transduction pathway around the TCR–CD3 complex. To date, reviewing the literature has shown limited reported data on TCR-CD3ζ gene expression in RA. In this study, we have detected that the expression of the TCR-CD3ζ gene is significantly reduced in RA patients compared with HC.

This is in agreement with Matsuda *et al*. [29], who reported a low expression of CD3ζ in RA peripheral blood mononuclear cells compared with HC. Moreover, Peng *et al*. [30] found that CD3ζ mRNA levels were downregulated in peripheral blood mononuclear cells of patients with RA when compared with HCs, but partly contradicts the study by Berg *et al.* [31] who demonstrated through stimulation with anti-CD3 antibodies, a low expression of CD3ζ in all synovial fluids T cells of RA cases when compared with peripheral blood T cells, while there was no difference in CD3ζ expression in T cells of the peripheral blood between RA patients and HC.Moreover, Maurice *et al.* [32] found that some events in TCR signaling was clearly reduced in RA synovial fluid T cells with diminished tyrosine phosphorylation of the TCR 5-chain. This decrease in tyrosine phosphorylation was linked to decreased levels of 5-protein within synovial fluid T cells. They suggested that a defective TCR signaling contributes the impaired responsiveness of T cells in the synovial fluid of RA.

Only a few studies had correlated the level of TCR-CD3ζ in RA patients with the disease parameters including the study of Matsuda *et al.* [29], who reported no association between the patient's CRP and CD3 expression in mononuclear cells of peripheral blood when tested with serum samples in three occasions with 1-month intervals from 10 patients with RA. Their results were in concordance with our results regarding CRP as we found a negative correlation between TCR-CD3ζ and CRP but did not reach a significant value. However, we reported a significant negative correlation between TCR-CD3ζ level and RA disease duration, RF, and ESR with higher levels of the variables associated with lower levels of TCR-CD3ζ mRNA. In this study, the level of *TCR-CD3ζ* gene expression being negative correlates with, and at the same time significantly relates to RF positivity, which means that the lower expression could be a cause of RF positivity. This might help in understanding the immunopathogenesis of the RF positive/ACPA negative RA patients. In contrast, a previous study of Mirza *et al.* [33] concluded that the impaired CD3 in T cells of RA patients showed no correlation with disease duration.

Since changes in the CD3ζ chain may affect negatively the internal signaling cascade of the TCR and lead to defective T-cell activation, our observations suggested that CD247 gene downregulation in our cohort of RA cases may contribute in the susceptibility to RA and help comprehending the mechanisms that may lead to deficient T-cell responses in RA patients as well as this downregulation is correlated with disease activity and functional status. Further, we proposed a cutoff point for TCR-CD3ζ expression that had good diagnostic accuracy and sensitivity for predicting RA disease. To our knowledge, there are no specific reported values that could be used as reference for TCR-CD3ζ expression in RA. We suppose that the presented values could be considered as reference; however, further studies with a larger population should be considered to confirm the current values.

Clinical implementation

The T-cell receptor T3 zeta chain, CD247, is essential for assembly, surface expression, and signaling cascade of the TCR/CD3 complex. Abnormalities in this pathway can result in T-cell dysfunction and development of autoimmune disorders. Regarding this, previous reports have demonstrated an association of CD247 variants with different autoimmune disorders. This study demonstrated that CD247 was underexpressed among RA than healthy

patients and detected a cutoff point for TCR-CD3ζ expression that had good diagnostic accuracy and sensitivity for predicting RA disease, thus CD247 can be used as a predictor factor for RA occurrence as well as help in predicting the disease course.

Limitations

This study has some limitations. First, we studied only the CD247 expression not genotyping, so, further studies are needed to assess CD247 genotyping and correlating them with RA activity. Second, we did not assess the changes in the level of TCR-CD3ζ with treatment to evaluate its role as a prognostic factor in RA patients. So, longitudinal studies are needed to clarify this point.

Conclusion

Our results demonstrate a lower level of TCR-CD3ζ (CD247) gene expression in RA patients and also correlate with some parameters of the disease activity. The cutoff values for CD247 in RA patients in this study had good diagnostic value in predicting RA patients. These results highlight a pathway in the immunopathogenesis of RA and may open the door for new therapeutic era in disease management that tackles this limb in the pathogenesis. Further multicenter studies with a larger sample size are recommended to validate the exact role of CD247 gene expression in the pathogenesis of RA.

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Conflicts of interest

There are no conflicts of interest.

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