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ORIGINAL ARTICLE

Elevated Serum TIM-3 Correlates with Disease Activity of Rheumatoid Arthritis

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ABSTRACT

Background: A type I transmembrane protein called T-cell immunoglobulin and mucin domain-containing molecule-3 (TIM-3) is involved in the development of several chronic autoimmune illnesses, including rheumatoid arthritis, by modulating T cell immune responses and the Th17/Treg balance.

Aim: is to detect if sTIM-3 is elevated in rheumatoid patients and correlated with disease activity.

Patients and Methods: This study was carried out on 40 RA patients and 40 apparently healthy controls attending the inpatient and outpatient clinics of the Rheumatology and Rehabilitation Department, Zagazig University Hospitals, Serum samples of all subjects were collected for routine laboratory assessment and for evaluating serum levels of TIM-3.

Results: Serum TIM-3 was elevated in RA patients (541.88 ± 389.62) compared with those in healthy subjects than the control group (150±31.61). On studying the validity of TIM-3 serum level at cut off = 207.1, the sensitivity was (97.5%) and specificity was (94.9%) while the Sensitivity of TIM 3 concentration at cut off =399.5 was (83.3%), specificity was = (70%). Different DAS 28 score gradings and TIM 3 concentrations showed statistically significant differences, with severe cases showing the highest median and moderate score gradings following.

Conclusions: By comparing RA patients and healthy controls, RA patients had considerably greater levels of circulating sTIM-3 than the healthy group. sTIM-3 was correlated with active rheumatoid patients.

Keywords: T-cell Immunoglobulin, Mucin Domain-Containing Molecule-3, Rheumatoid Arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory condition that has no known cause. If left untreated, it will destroy joints, cause disability, and perhaps shorten life through comorbidities. It is characterized by progressive and destructive joint involvement as well as extra-articular and systemic signs that range from respiratory involvement to cardiovascular illness. An ideal outcome in a managed condition is the suppression of the inflammatory process, which minimizes harm and maximizes results **(1)**.

TIM-3, a type I transmembrane protein that controls T cells' immunological responses and the Th17/Treg balance, has a role in the development of many chronic autoimmune disorders, including multiple sclerosis and rheumatoid arthritis **(2).**

An immune-mediated molecule called TIM-3 is involved in controlling immunological reactions. Upregulation of the TIM-3/galectin-9 axis may affect rheumatoid inflammatory bone destruction because TIM-3 was found in osteoclasts and their mononuclear precursors in rheumatoid synovium. Gal-9/TIM-3 pathway regulatory system controls osteoclastogenesis and inflammatory bone destruction in RA **(3).**

Aim of the work:

The present study aims to detect if sTIM-3 is elevated in rheumatoid patients and correlated with disease activity.

METHODS

After the approval of the Institutional Review Board (IRB), at 80% power and 95% CI, the estimated sample size for this case-control study

was 80 subjects. It was conducted on 40 adult-onset RA patients and 40 seemingly healthy, age- and sex-matched controls attending the inpatient and outpatient clinics of the Rheumatology and Rehabilitation Department, Zagazig University Hospitals.

Inclusion criteria

Group 1: included 40 RA patients; they were (38) females and (2) males, and their ages ranged between (27 - and 65) years; All patients met the 2010 European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) criteria for the diagnosis of RA **(4).**

Group 2: 40 healthy volunteers who were matched by age and sex served as the control group.

Exclusion criteria: Other autoimmune diseases, patients with a history of infection at the time of study, and known cases of malignancy**.**

Measurements of clinical disease activity:

Cases were subjected to complete history taking, general examination, locomotor examination (inspection, palpation, and range of motion), and other systems examination.

Disease activity: It was done by measuring **DAS-28**. The DAS includes the total number of sore and swollen joints (28), including both shoulders, both elbows, both wrists, and both MCPs of both hands (10), PIPs of both hands (10), and both knees), the ESR, and the patient's global assessment of general health (indicated by marking a 100 mm line between zero, which represents very good health, and 100 mm, which represents very bad health) **(5).**

Routine laboratory investigations: only for patients group (RA), (ESR), Complete blood count (CBC), C-reactive protein (CRP), Liver function tests, Kidney function tests, RF titer, and anti-CCP titer.

Enzyme-linked immunosorbent assay (ELISA) methods

A commercially available Human TIM-3 in vitro Simple ELISA was used to test the amounts of sTIM-3 in the serum.

STATISTICAL ANALYSIS

Using SPSS version 26 database software, the obtained data were coded, entered, presented, and analyzed electronically. For data that were not regularly distributed, quantitative variables were estimated as median with interquartile range and mean with standard deviation (SD). The Chi-square (X2) test was performed to find relationships between various qualitative variables. Qualitative data were given as frequencies and percentages. While nonparametric data was assessed using the Mann-Whitney U test, independent t-tests (t) were employed to identify differences between various quantitative variables. The different variables were correlated using Pearson and Spearman's correlation (r) coefficients. Always a value between (-1 and 1), correlation r is a number. A positive r number shows that the variables are positively associated, and a negative r value that they are negatively associated.

This study carried out 40 rheumatoid patients and 40 controls. Table 1 displays the demographic and clinical traits of people with rheumatoid arthritis**.** Among 40 patients with RA, 38 (95%) were females and their mean age was 42.88±9.34 years. All patients were taking immunosuppressive drugs, mostly methotrexate (84.6%), and hydroxychloroquine (72.5%).

Table (3) found that sTIM-3 levels in rheumatoid patients were substantially greater than those in healthy subjects. **Table (4)** shows the validity of TIM-3 serum level at cut off = 207.1, the sensitivity was (97.5%) and specificity was (94.9%) **(fig.1)** while **table (5)** sensitivity of TIM 3 concentration at cut off =399.5 was (83.3%), specificity was = (70%). As severe cases show the highest median followed by moderate score grading, there were statistically significant differences between the various DAS28 score gradings and TIM 3 concentration. **(table 6)**. **Table (7)** showed sTIM-3 and rheumatoid inflammatory indicators like ESR to have substantial positive associations., CRP, DAS28, and negative correlation with hemoglobin level.

Table 1: Demographic and clinical characteristics of the studied groups:

Variable		**RA Group (n=40)**		**control group (n=40)**		**Tests**	
						t	***P* value**
Age (years) Mean± SD		42.88±9.34		39.97±10.23		1.3	0.190
Variable		No	(%)	No	(%)	x^2	P value
Sex	**Female**	38	95	29	72.5	7.44	0.006*
	Male	2	5	11	27.5		

Characteristic		Rheumatoid group (n=40)	
Duration	Mean± SD Median (IQR)	7.93±7.21 4 (2-14.75)	
		No.	**%**
Clinical Extra-articular manifestations	**Arthritis**	37	92.5
	Subcutaneous nodules	3	7.5
	Eye dryness	4	10
	Mouth dryness	1	2.5
Drugs	**Corticosteroids**	27	69.2
	Methotrexate	33	84.6
	Hydroxychloroquine	29	72.5
	Leflunomide	19	47.5
	Sulfasalazine	2	5
	Folic acid	28	70
	Biological treatment	2	5

Table 2: Laboratory investigations of the rheumatoid group

Variables		Study group (n=40)	
CRP levels Mean ±SD **Median (IQR)**		21.86±22.8 12 (6.6-36)	
Blood picture Mean ±SD	**Hemoglobin (g/dl)** Range	11.90±1.34 (9.7-16)	
	WBCs Range	7.64±2.27 (3.5-14)	
	Platelets (10^3/ul) Range	293.6±99.51 (139-680)	
Liver function Mean ±SD	**ALT(u/l)** Median (IQR)	19.22±8.95 18 (12.9-25.9)	
	AST(u/l) Median (IQR)	23.95±25.33 18.75 (14.25-28.4)	
	Albumin Range	4.29±0.90 (4-4.3)	
Renal function Mean ±SD	**BUN** Median (IQR)	8.55±3.07 8 (6.58-10)	
	Serum Creatinine (mg/dl) Median (IQR)	0.69±0.17 0.7 (0.6-0.8)	
		No.	**%**
Rheumatoid Factor	**Positive**	28	70
	Negative	12	30
Anti-CCP	**Positive**	11	27.5
	Negative	29	72.5

Table 3: TIM3 concentration of the studied groups:

Variable	RA Group (n=40)	control group (n=40)	tests	
			z	***P* value**
TIM 3 concentration (pg\ml) Mean±SD Median (IQR)	541.88±389.62 484.9 (377.9-554.3)	150±31.61 147.26 (125.3-170.1)	-7.570	**<0.001***

Table 4: Validity of TIM- 3 concentration (pg/ml) at cut off= 207.1 as a predictor for the rheumatoid group as compared to the control

Variables	AUC	95%CI	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
TIM 3 concentration	0.995	0.986-1.000	207.1	97.5%	94.9%	95.1%	97.4%	96.25%

Table 5: Validity of TIM 3 concentration (pg/ml) at cut off= 399.5 as a marker of activity for RA patients

Variables	AUC	95%CI	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
TIM 3 concentration	0.840	0.701-0.979	399.5	83.3%	70%	89.3%	58.3%	80%

Table 6: Comparing TIM 3 concentration and with DAS28 score grading

Characteristic	score grading				Kruskal-Wallis H	P value
	No activity	Mild	Moderate	severe		
TIM 3 concentration (pg\ml) Median (IQR)	349.34 (210.5-466.4)	401 (231-401)	523.27 (391.1-559.5)	540.01 (463.3-634.3)	14.981	**0.002***

Kruskal Wallis Test

Table 7: Correlation between TIM 3 concentration and different parameters

Variables		TIM 3 concentration (pg/ml)
Age	**R**	-0.073
	P	0.655
Duration	**R**	-0.064
	P	0.694
ESR	**R**	0.460**
	P	0.003
DAS28	**R**	0.647**
	P	0.000
CRP	**R**	0.408*
	P	0.019
Hemoglobin gm/dl	**R**	-0.417**
	P	0.007
WBCs	**R**	-0.066
	P	0.685
Platelets	**R**	0.107
	P	0.512
RF titer	**R**	0.034

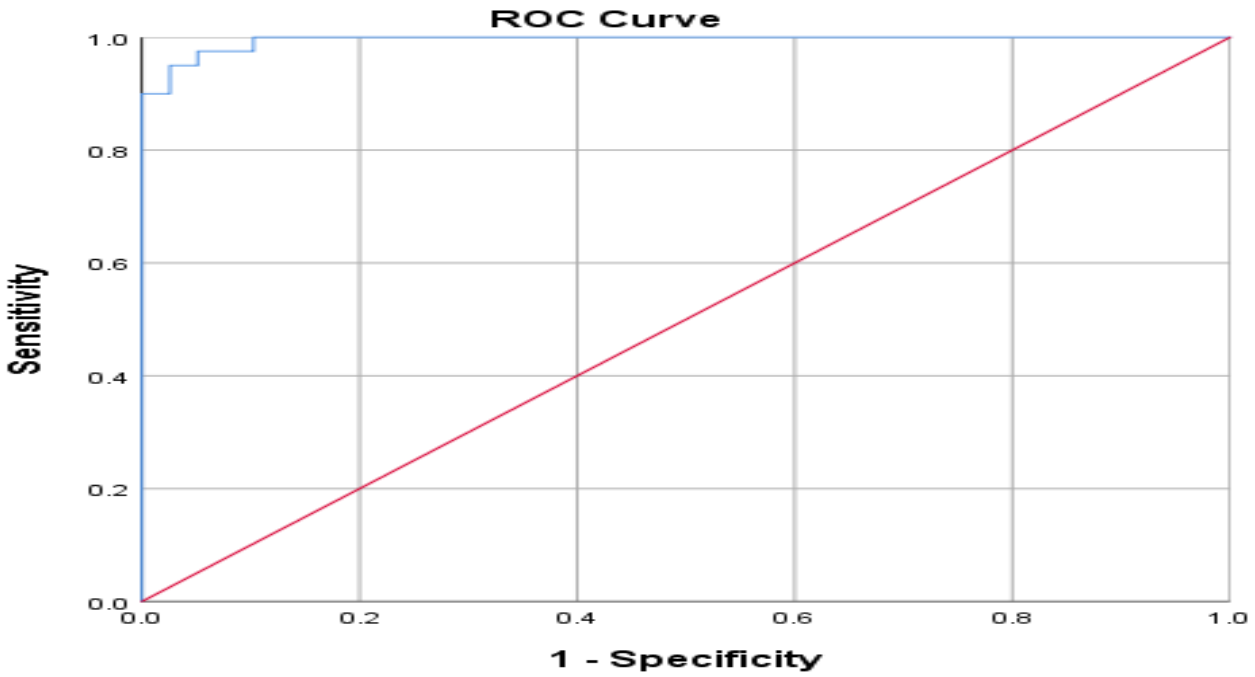

Figure 1: ROC curve of TIM-3 concentration (pg/ml) at cut-off= 207.1 as a predictor for rheumatoid arthritis

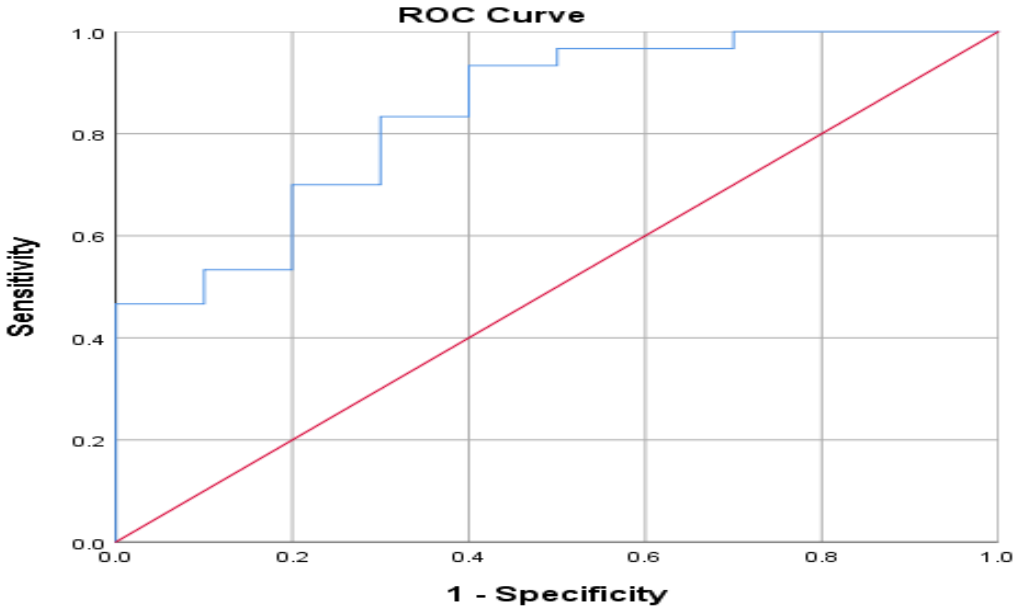

Figure 2: ROC curve of TIM-3 concentration (pg/ml) at cut off= 399.5 as a marker of activity for rheumatoid arthritis

DISCUSSION

An autoimmune condition with an unknown exact origin is rheumatoid arthritis. It is characterized by extra-articular and systemic indications, such as involvement in the respiratory system and cardiovascular disease, in addition to the progressive and devastating joint involvement that is its most well-known feature **(6).**

In the absence of treatment, it will cause damage to the joints, which may result in disability, and it may also shorten a person's life due to the development of comorbid conditions. On the other hand, stopping the inflammatory process enhances results and prevents injury (**7).**

Osteoclasts, dendritic cells, and activated T cells, especially CD4+ and CD8+ T cells, are the

main sources of TIM-3 expression. When compared with healthy participants, the levels of TIM–3 in RA patients were considerably higher **(2).**

While there was a statistically significant difference between the 2 study groups (RA and controls) in terms of age, there was not a statistically significant difference between them in terms of sex. The current study illustrated a statistically significant increase in TIM3 concentration in the rheumatoid arthritis group (541.88 ± 389.62) with a median of 484.9 (377.9 - 554.3) than the control group.

In agreement with our results, using the ANOVA test, **Soliman et al. (8)** indicated that rheumatoid patients had considerably greater serum sTIM-3 levels than healthy people. In agreement with our results, **Skejoe et al.(9)** showed that Joint swelling (count 28), joint discomfort (count 28), and plasma sTim-3 levels were all linked with disease activity as measured by DAS28 CRP. After 24 months of therapy, the connection between the starting sTim-3 and DAS28CRP remained constant. In keeping with findings from SLE, no other relationships between baseline sTim-3 concentration and clinical outcomes were seen, neither for anti-CCP levels nor RF titer, indicating that sTim-3 is elevated in conditions with chronic immunological burden **(10).**

The current study showed that the sensitivity of TIM 3 concentration at cut-off =207.1 as a predictor for rheumatoid arthritis activity between cases and control groups was (97.5%). As a marker of activity for rheumatoid arthritis, the value of Sensitivity of TIM 3 concentration at cut-off =399.5 was (83.3%), and specificity was = (70%).

There was a statistically significant positive link, as this study has demonstrated between TIM 3 concentration and each of the DAS score, CRP, and ESR, and shows a significant negative correlation between TIM 3 concentration and hemoglobin level. This research revealed statistically significant variations between different DAS score grading and TIM 3 concentrations as severe cases show the highest median followed by moderate score grading.

In agreement with our results, **Matsumoto et al. (11)** reported that sTIM-3 and inflammatory indicators like ESR had demonstrable positive associations, matrix metalloproteinase-3, and anti-citrullinated peptides antibodies.

Advanced joint damage in RA patients was associated with significantly greater levels of sTIM-3 than with advanced joint damage without RA. MMP-3 but not ESR is substantially associated with serum sTIM-3 in RA patients with extensive joint degeneration **(11)**.

In a study by **Soliman et al. (8)**, a significant positive association between Tim-3 + CD4 + CD3 + and Tim-3 + CD8 + CD3 + cells percentages.

Li et al. (**12)** concluded that TIM-3 may be involved in the prognosis of RA and may be substantially linked with disease activity.

TIM-3 / Gal-9 is associated with rheumatoid arthritis activity and might be crucial in rheumatoid arthritis etiology **(13).**

CONCLUSION

By comparing RA patients and healthy controls, rheumatoid patients had considerably greater levels of circulating sTIM-3 than the healthy group. sTIM-3 was correlated with active rheumatoid arthritis.

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