

## Semaphorin 3A in Rheumatoid Arthritis and Systemic Lupus Erythematosus Patients

Nancy A Mohamed<sup>1</sup>, Radwa M Elkhoully<sup>1</sup>, Mohamed E Mowafy<sup>1</sup> and Wessam S Ibrahim<sup>2</sup>

<sup>1</sup>Faculty of Medicine, Tanta University, Egypt

<sup>2</sup>Clinical Pathology, Faculty of Medicine, Tanta University, Egypt

\*Corresponding author: Radwa Mostafa Elkhoully, Department of Rheumatology and Rehabilitation, Faculty of Medicine, Tanta University, Egypt, Tel: 00201223827097; E-mail: [hoully@yahoo.com](mailto:hoully@yahoo.com)

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### Abstract

The aim of this study is to evaluate Semaphorin 3A level in rheumatoid arthritis and systemic lupus and its correlation with clinical, laboratory markers of disease activity and extra articular manifestations. Fifteen (RA) patients GROUP I and fifteen (SLE) patients GROUP II with age and sex matched fifteen healthy volunteers who served as controls GROUP III were studied. Disease activity score was used to assess disease activity in RA and SLE patients, MHAQ, RF and ANTICCP were measured for RA patients. Also ANA, Anti-dsDNA was measured for SLE patients. The serum level of semaphoring 3A was assessed by (ELISA) technique for the RA, SLE patients and control group. Group II SLE patients had significant decrease in serum level of semaphoring 3A versus group I RA patients and control group also there was significant decrease in semaphorine 3 A level in group I in RA in comparison with control group. There were significant negative correlation between Semaphorin 3A and disease duration, laboratory markers of disease activity (ESR<sup>1st</sup> h,CRP) and grades of disease activity (DAS28). There were significant negative correlation between Semaphorin 3A and SLEDAI score, ANA, anti-dsDNA and renal affection (proteinuria) in SLE patients.

**Keywords** Semaphorin-3A; Rheumatoid arthritis; Systemic lupus erythematosus

**Abbreviations** ACPA: Anti Citrullinated Protein Antibody; ACR: American College of Rheumatology; ANA: Antinuclear Antibodies; Anti-dsDNA: Anti-Double Stranded DNA; BLYS: B Lymphocyte Stimulator; CBC: Complete Blood Cell Count; CRP: C-Reactive Protein; DAS: Disease Activity Score; DC: Dendritic Cell; ESR 1<sup>st</sup> h: Erythrocyte Sedimentation Rate First Hour; EULAR: European League Against Rheumatism; HAQ DI: Questionnaire Disability Index; HAQ: Health Assessment Questionnaire; HAQ-II: Health Assessment Questionnaire II; HB: Hemoglobin; MHAQ: Modified Health Assessment Questionnaire; NPs: Neuropilins; NRP1: Neuropilin-1; RA: Rheumatoid Arthritis; RF: Rheumatoid Factor; Sema 3A: Semaphorin 3A; SLE: Systemic Lupus Erythematosus; SLEDA: Systemic Lupus Erythematosus Disease Activity Index.

### Introduction

Semaphorin-3A is a chemo repulser protein, secreted by surrounding tissues to guide migrating cells and axons in the developing nervous system which is critical for the precise formation of neurons and vasculature [1]. Semaphorin 3A has multiple guidance functions, including axon path finding, cardiac and peripheral vascular patterning and branching morphogenesis. Semaphorin 3A signaling is mediated by a complex of the binding receptor neuropilin 1 and the signaling receptors plexin A1 or A3 [2]. Semaphorin-3A is well reported as a potent immuno-regulator during all immune response stages, namely, the early initiation and the late phase of inflammatory processes [3].

The expression of Sema 3A, NP-1, NP-2 and plexins were found to be increased on differentiating macrophages and on activated T cells, suggesting that they have a role in modulating inflammatory conditions [4]. In addition, it was recently shown that Sema3A is involved in the entry of dendritic cells to the lymphatic system [4].

Rheumatoid arthritis and SLE are inflammatory autoimmune disorders with articular and extra articular manifestation; many researches are trying to finding new biomarkers and factors that regulate expression of these autoimmune disease [4].

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects synovial tissue (ST) in multiple joints. Although its etiology is still unknown, RA is an autoimmune disease. RA can cause permanent joint damage quickly when it is not treated and controlled. Once damage occurs, it is irreversible and can cause significant pain and disability [5]. Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown origin affecting virtually all organ systems. Beyond genetic and environmental factors, cytokine imbalances contribute to immune dysfunction, trigger inflammation, and induce organ damage. SLE is characterized by a many of immune system aberrations that involve B cells, T cells, and cells of the monocytic lineage, resulting in polyclonal B cell activation, increased numbers of antibody producing cells, hypergamma globulinaemia, autoantibody production, and immune complex formation [6].

The aim of the work is to evaluate Semaphorin3A level in rheumatoid arthritis, and systemic lupus and its correlation with clinical, laboratory markers of disease activity and extra articular manifestations.

## Methods

This study included fifteen RA patients, fifteen SLE patients and fifteen controls. They were collected from the outpatient clinic of Physical Medicine, Rheumatology and Rehabilitation department of Tanta University Hospitals. The subjects subdivided into 3 groups.

### Inclusion Criteria

#### RA group (Group I)

Fifteen rheumatoid arthritis patients diagnosed according to the American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) 2010 criteria for diagnosis of rheumatoid arthritis [7].

#### SLE group (Group II)

Fifteen SLE patients, diagnosed according to the American College of Rheumatology revised criteria for classification of SLE [8].

#### Control group (Group III)

This group included fifteen apparently healthy volunteers.

This study was approved by Local Research Ethics Committee of faculty of medicine Tanta University. All tests were explained to RA patients, SLE patients and control subjects before having their written informed consents to participate in this study. All laboratory investigation was performed in clinical pathology department, Tanta university hospital we excluded patients with degenerative, metabolic, infectious, or other inflammatory arthritis.

All patients were subjected to the following: Complete history taking, Thorough clinical examination (general and locomotor examination was done to detect articular and extraarticular involvement). Rheumatoid arthritis patients were assessed for disease activity: using DAS28 from this score the patients were classified as following: mild activity  $DAS \leq 3.2$  moderate activities, DASE 3.2 TO 5.2 and severe activity  $\geq 5.1$  [9]. Functional assessment: using Modified Health Assessment Questionnaire from this score the patients were classified as following: Mild ( $0.3 \leq MHAQ < 1.3$ ), Moderate ( $1.3 \leq MHAQ < 1.8$ ) and Severe ( $MHAQ > 1.8$ ) [10].

Laboratory assessment using the following: complete blood picture [11], Erythrocyte sedimentation rates (ESR) by westgren methods [12], C-reactive protein (CRP) [12], Rheumatoid factor (RF) and Anticyclic citrullinated peptide antibodies (anti-CCP) [13].

Systemic lupus patients were assessed for Disease activity according to the SLE disease activity index (SLEDAI) and distributed into categories according to the scores: No activity (SLEDAI=0), mild activity (SLEDAI=1 to 5), moderate activity (SLEDAI=6 to 10), high activity (SLEDAI=11 to 19) and very high activity (SLEDAI>20) [14].

Laboratory investigations of SLE patients; complete blood picture [11], Erythrocyte sedimentation rates (ESR) by westgren methods [12], C-reactive protein (CRP) [12], Antinuclear antibody (ANA) and anti- double stranded DNA(Anti-dsDNA) [15].

### For all groups

Serum Semaphorin 3A was assessed by enzyme-linked immunosorbent assay (ELISA) technique [16].

## Principle of the Assay of Semaphorin 3A

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for Sema 3A has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Sema 3A present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Sema 3A is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Sema 3A bound in the initial step. The color development is stopped and the intensity of color is measured.

### Statistical analysis

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and Student t-test (Unpaired), and Linear Correlation Coefficient (r) Chi-square ANOVA and ROC curve by SPSSV17.

## Results

This study conducted on fifteen RA patients (group I) in addition to fifteen SLE patients (group II) and fifteen apparently healthy Controls (group III).

In this study as regards sex all RA patients were females whereas in control and the age of RA patients ranged from (32 to 67) years with the mean age of ( $44.2 \pm 10.54$ ) years. In SLE patients group II out of 15 patients 12 were females and 3 were males. The age of our SLE patients ranged from 22 to 46 years with the mean age of  $33.2 \pm 7.50$ .

GROUP III included 15 healthy volunteers served as control. Their age ranged from 25 to 65 years with the mean age of  $39.267 \pm 9.68$  years. Thirteen persons were females and two were males. The control group was matched with RA and SLE patients groups as regard age and sex. Included 15 healthy volunteers served as control with age and sex matched. The main demographic characteristics of RA, SLE patients and controls are summarized in Table 1.

The functional assessment of our RA patients was done by MHAQ with mean value of  $1.9 \pm 0.55$  most of our RA patients (66.67%) had moderate functional Impairment whereas (33.33%) had severe functional impairment.

As regards DAS28 in our RA patients, it was ranged from 3.66 to 6.42 with mean DAS28 of  $5.25 \pm 0.75$  eight RA patients (53.33%) had high disease activity but Seven RA patients (46.67%) had moderate disease activity. All RA patients studied had active RA.

The duration of disease ranged from 3 to 16 years with mean disease duration of  $7.06 \pm 4.11$  years. There was significant increase in laboratory parameter of disease activity (ESR 1sthr and CRP) with mean values of  $45.20 \pm 21.05$  and  $17.92 \pm 9.87$  respectively with significant increase when compared with the control group.

In our RA patients, 86.66% were positive Anti-CCP while 80% had positive RF. As regards hemoglobin levels in our RA patients, their hemoglobin levels ranged from 8.8 to 13.2 g/dl with mean hemoglobin levels of 11.42 & 1.35 with significant decrease of Hb levels when compared with controls. The clinical and laboratories characteristics of RA patients are summarized in Table 2.

		Groups						Chi-Square	
		RA		SLE		Controls			
		N	%	N	%	N	%	X <sup>2</sup>	P-value
Sex	Male	0	0.00	3	20.00	3	20.00	5.317	0.070
	Female	15	100.00	12	80.00	12	80.00		
Age	Range	22 - 67		22- 46		22- 65		4.589	0.016
	Mean ± SD	44.2 ± 10.54		33.2 ± 7.5		39.26 ± 9.68			

Table 1: Sex and age in RA, SLE patients and controls.

Variables	RA (N=)	CONTROL(N=)	P
Age	44.2 ± 10.54	39.26 ± 9.68	0.016
FEMALE N%	15 (100.00)	12 (80.00)	0.070
Duration of illness (Years)	7.067+4.114		
Morning stiffness (min)	45.35+30.55		
No of tender joint	11.933+5.675		
No of swollen joint	5.533+2.875		
Visual analogue scale (VAS) (1-100)	46.42+23.67		
Moderate disease activity (3.2<DAS28<5.1)	7 (46.67%)		
High disease activity (DAS28>5.1)	8 (53.33%)		
Moderate functional loss (1.3 ≤MHAQ<1.8)	10 (66.67%)		
Severe functional loss (MHAQ>1.8)	5 (33.33%)		
Anti CCP(IU)	72.193 ± 31.376	8.5+4.878	<0.001*
ESR1 <sup>st</sup> h (mm/h)	45.200 ± 21.058	12.333 ± 4.435	<0.001*
CRP	17.923 ± 9.878	2.8 ± 1.483	0.004*
RF	12 (80%)	0 (0)	<0.001*
HB	11.420 ± 1.354	11.287 ± 1.145	0.773

Table 2: Clinical and laboratory manifestations of RA patients.

It revealed that there was no statistically significance between both groups disease activity assessed by SLEDAI score showed that most of our SLE patients (73.33%) had moderate disease activity while (20.0%) had high activity and (6.67%) had Mild disease activity. The ESR 1<sup>st</sup> h ranged from 30 to 112 mm/h with mean ESR of 62.40 ± 24.42. There

was significant increased ESR 1<sup>st</sup> h levels in our patients when compared to controls.

As regards C - reactive protein (CRP) in our SLE patients 14 patients had CRP negative (93.33%) and 1 patients had CRP positive (6.67%) there was significant difference between, SLE patients and controls as regard CRP. In our SLE patients all patients were positive ANA and 88% were Anti-dsDNA positive. The clinical and laboratories characteristics of SLE patients are summarized in Table 3.

Variables	SLE (N=)	CONTROL(N=)	P
Age	44.2 ± 10.54	39.26 ± 9.68	0.016
FEMALE N%	15 (100.00)	12 (80.00)	0.070
Duration of illness (Years)	7.067+4.114		
Morning stiffness (min)	45.35+30.55		
No of tender joint	11.933+5.675		
No of swollen joint	5.533+2.875		
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Moderate disease activity (3.2<DAS28 <5.1)	7 (46.67%)		
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Anti CCP (IU)	72.193 ± 31.376	8.5+4.878	<0.001*
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CRP	17.923 ± 9.878	2.8 ± 1.483	0.004*
RF	12 (80%)	0 (0)	<0.001*
HB	11.420 ± 1.354	11.287 ± 1.145	0.773

Table 3: Clinical and laboratory manifestations of SLE patients.

The mean values of Semaphorin 3A in RA group and the control group were (1.15 ± 0.358) and (2.760 ± 0.945) respectively (Table 4). The results of this study showed that there was significant decrease of mean value of serum Semaphorin 3A in RA group when compared with mean value in the control group and the difference was statistically significant (p<0.001\*).

In the present study there were no significant correlation between Semaphorin 3A and age, clinical parameters of disease activity (morning stiffness, number of tender joint, number of swollen joint and the visual analogue scale (VAS), MHAQ, R, Anti-CCP and haemoglobin levels in our RA patients (Table 5). Whereas there were significant negative correlation between Semaphorin 3A and disease

duration, laboratory markers of disease activity (ESR<sup>1st</sup> h,CRP) and grades of disease activity (DAS28).

The results of this study showed that there was significant decrease of mean value of serum Semaphorin 3A in SLE group ( $0.483 \pm 0.109$ ) when compared with control group ( $2.76 \pm 0.94$ ) and the difference was statistically significant ( $p < 0.001^*$ ). There was significant negative correlation between Semaphorin 3A and SLEDAI score, ANA, anti dsDNA and renal affection (proteinuria) in SLE patients. In this study there was no significant correlation between Semaphorin 3A and duration of the disease (Years), Age, ESR and CRP (Table 6).

Groups	Semaphorin 3A					
	Range			Mean	±	SD
SLE	0.265	-	0.720	0.483	±	0.109
RA	0.435	-	2.190	1.151	±	0.358
Controls	0.870	-	3.866	2.760	±	0.945
T test	t			P values		
P1	6.9133			0.022 <sup>*</sup>		
P2	6.1666			<0.001 <sup>*</sup>		
P3	9.2706			<0.001 <sup>*</sup>		

**Table 4:** Semaphorin 3A in RA, SLE patients and controls.

Clinical data	Serum semaphorin 3A.	
	r	P-value
Age	0.153	0.544
Duration of the disease(years)	-0.527	0.043 <sup>*</sup>
morning stiffness(min)	0.375	0.092
No. of tender joint	0.038	0.894
No. of swollen joint	0.048	0.884
VAS	-0.070	0.804
DAS28 score	-0.899	<0.001 <sup>*</sup>
ESR(mm/h)	-0.364	0.048 <sup>*</sup>
CRP(mg/L)	-0.042	0.887 <sup>*</sup>
Haemoglobin Levels (g/dl)	0.726	0.118
Rheumatoid factor (IU)	0.089	0.784
Anti-CCP (IU)	-0.465	0.081
MHAQ	0.036	0.899
Larsen Score	0.384	0.158

**Table 5:** Correlation of clinical and laboratory data of RA patients with serum semaphorin 3A.

Our results showed that there was significant decrease of Semaphorin 3A in RA and SLE group when compared with controls.

Also there was significant reduction of Semaphorin 3A level in SLE group when compared with RA patients.

SLE	Serum semaphorin 3A	
	r	P-value
Age	0.102	0.718
Duration of illness (Years)	0.339	0.217
SLE DAI	-0.850	<0.001 <sup>*</sup>
Renal affection(Proteinuria)	0.658	0.000 <sup>**</sup>
ANA	-0.549	0.027 <sup>*</sup>
Anti dsDNA	-0.425	0.032 <sup>*</sup>
ESR	0.223	0.425
CRP	0.196	0.483
Hb	0.363	0.184

**Table 6:** Correlation of clinical and laboratory data of SLE patients with serum semaphorin 3A.

## Discussion

In our study we evaluate the Semaphorin 3A level in RA and SLE patients and its correlation with different clinical and laboratory parameters of diseases.

In the current study there was significant decrease of mean value of serum Semaphorin 3A in RA group when compared with mean value in the control group.

These results confirm the finding of Vadas et al. who studied the level of Semaphorin 3A in 24 RA patients and 40 healthy controls. Their results showed that serum level of sema3A was lower in RA patients when compared with the control group [17].

Shu Takagawa et al. studied Synovial level of Semaphorin 3A in 30 RA and 23 OA patients they found that synovial level of semaphorin 3A was significantly lower in RA patients when compared with OA group [18].

Semaphorin 3A have a role in modulation of inflammation in RA, it inhibits Th1 mediated inflammatory response in RA patients that is reducing the pro inflammatory cytokines such as IFN- $\gamma$  and IL17 also enhance the suppressive ability of CD4+NP-1+T cells by increasing their IL-10 expression and their regulatory function on effector CD4+T cells. Their altered expression on T cells was shown to correlate with the progression of RA [19].

Also in our study there was significant negative correlation of serum level of Sema 3A and laboratory markers of disease activity (ESR 1<sup>st</sup> h, CRP) and grades of disease activity (DAS28). The previous results were supported by Shu Takagawa et al. found significant negative correlation between Semaphorin 3A and ESR, CRP and (DAS28) [18]. Kurosaka et al. reported that NRP1 is a common receptor for Sema3A and VEGF165, the efficacy of VEGF165 is attributed to Sema3A expression. Sema3A expression and/or the balance of Sema3A and VEGF165 expression may regulate the disease activity of RA including inflammation, angiogenesis and proliferation of synovial cells. So, the



Sema3A expression level was also significantly associated with the RA pathological score and disease activity score [19,20].

Also in our study there was no significant correlation between serum Semaphorin 3A levels and RF, Anti-CCP and haemoglobin levels in our RA patients.

In the present study there was significant decrease of mean value of serum Semaphorin 3A in SLE group ( $0.483 \pm 0.109$ ) when compared with control group ( $2.76 \pm 0.94$ ) and the difference was statistically significant ( $p < 0.001^*$ )

Our results were in agreement with Vadas et al. studied serum level of semaphorin 3A in 32 SLE patients and 40 healthy controls. Their results showed that serum level of Sema 3A was significantly lower in SLE patients when compared with control group. They found that Sema 3A plays a role in the pathogenesis of SLE, this role in humoral responses, as well as a role in modulating the autoimmune properties of B cells in SLEm [17].

Sema 3A protein is produced by several immune cells such as B and T cells and activated monocytes. Sema3A expression is mainly present on CD19, CD25 NP-1 expression, along with decreased Sema3A expression on B cells in SLE further suggests that this defect may play an important role in the pathogenesis of SLE. As Sema3A and NP-1 are essential for the regulatory function of B cells, one would expect to see that when their expression on B cells is decreased, B cells may shift, to become more pro-inflammatory rather than regulatory, and contribute to the development of SLE. When Sema3A expression is diminished, NP-1 down-regulation on B cells also contribute to development of B cell auto reactivity and auto-immunity in SLE. B cell may lose their regulatory signal with lose of self-tolerance becoming auto-reactive and autoantibody producers cell. Also Sema 3A important in the induction of the apoptosis of many immune cells, such as monocytes and macrophages, when these were found to be resistant to Fas-induced apoptosis. Therefore, when sema 3A expression is altered auto-reactive B cells escape apoptosis and survive to produce auto-antibodies, thus contributing to autoimmunity in SLE [21,22].

Vadas et al. used pharmaceutical composition comprising isolated Semaphorin 3A for use in treating Systemic Lupus Erythematosus (SLE). They found that treatment improvement in at least one clinical parameters; renal function, a decrease in anti-dsDNA antibody concentration in the serum, a decrease in anti-Cardiolipin antibody concentration in the serum, an increase in serum concentration of complement factor C3 and C4 [23].

There was significant negative correlation between Semaphorin 3A and SLEDAI score, ANA, anti-dsDNA and renal affection (proteinuria) in SLE patients. Vadas et al. agreed with our results as regards the negative correlation of serum level Semaphorin 3A with SLEDAI, renal affection, ANA and anti-dsDNA in SLE patients. They also found negative correlation between serum level of semaphorin 3A and C3, C4 and anti cardiolipin they found that 73% of SLE patients with a Sema 3A level below 50 ng/ml had kidney involvement. Whereas, 5% of SLE patients with serum level of Sema 3A above 50 ng/ml had kidney involvement [17].

Vadas et al. found the increase of second Sema3A measurement compared with first measurement is indicative of decrease of disease activity in SLE patients [23].

Our results showed that there was significant decrease of Semaphorin 3A in RA and SLE group when compared with controls.

Also there was significant reduction of Semaphorin 3A level in SLE group when compared with RA patients.

## Conclusions

The present results indicated that there was significant decrease of serum level of semaphorin 3A in SLE and RA patients when compared with controls with the serum level of semaphorin 3A in SLE patients was significantly lower than RA patients.

In our RA patients semaphorin 3A was negatively correlated with duration of the disease and disease activity (DAS28 score ESR and CRP). In SLE patients semaphorin 3A was negatively correlated with SLEDAI score, ANA, Anti dsDNA and renal affection (proteinuria).

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