

Comparative Assessment of the Immunological Significance of Semaphorin 5A and Anti-Cyclic Citrullinated Peptide Antibodies in Iraqi Patients with Rheumatoid Arthritis

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Abstract

The objective of this research was to assess the predictive potential of Semaphorin 5A in comparison to Anti-Cyclic Citrullinated Peptide antibodies for forecasting disease progression and treatment responses among Iraqi patients with Rheumatoid Arthritis (RA). Conducted as a case-control study, the investigation encompassed a total of 150 participants, comprising 100 RA patients and 50 healthy individuals. The study took place at Baghdad Teaching Hospital over the period from November 2021 to February 2022. Enrolled participants were selected based on the 2010 criteria established by the American College of Rheumatology. The levels of biomarkers were assessed using the enzyme-linked immunosorbent assay (ELISA) method. The findings demonstrated a noteworthy elevation in the levels of both ACCP and Semaphorin 5A among RA patients compared to the control group ($p < 0.001$). Furthermore, these levels were found to be higher in individuals with active disease as opposed to those with inactive disease. In both active and inactive disease states, the levels of both ACCP and Semaphorin 5A remained considerably higher compared to the levels observed in the healthy control group ($p < 0.001$). In patients who had not undergone treatment, the levels of both ACCP and Semaphorin 5A exhibited considerably greater significance compared to those who received Methotrexate or etanercept ($p < 0.001$). A noteworthy and positive correlation was observed between ACCP and Semaphorin 5A, with a correlation coefficient of 0.476 ($p < 0.001$). The sensitivity for ACCP and Semaphorin 5A was determined to be 72% and 83% respectively, while the specificity values were 98% and 76%. The study's conclusion highlighted the promising prognostic potential of both ACCP and Semaphorin 5A, establishing them as potential biomarkers for distinguishing between Rheumatoid Arthritis patients and healthy individuals. Predict disease activity and response to methotrexate or etanercept. Active disease patients and without treatments patients ACCP and Semaphorin5A levels were higher than inactive and received treatments (methotrexate or etanercept).

Keywords: Peptide Antibody, Semaphorin5a, Methotrexate, Etanercept, RA.

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INTRODUCTION

Rheumatoid arthritis (RA) represents a chronic inflammatory autoimmune disorder arising from the interaction between immune cells and soluble mediators. It primarily targets small joints and can lead to extra-articular manifestations affecting various systems, such as skin, nerves, and eye conditions, alongside gastrointestinal, cardiovascular, pulmonary, and renal complications [1]. The underlying cause of RA remains unidentified, with a global prevalence of around 0.5-1.0%. Females experience 2 to 3 times

higher susceptibility than males, and individuals aged between 30 and 50 face an increased risk of developing RA [2]. The pathogenesis of RA involves auto-reactive B cells that contribute to the production of auto-antibodies, and T cells that release cytokines upon activation.

Autoantibodies targeting citrullinated peptides, including IgG, IgM, and IgA isotypes, are collectively known as Anti-cyclic Citrullinated Peptide antibodies (ACCP). These autoantibodies are linked to joint

deterioration and an elevated risk of disease progression [3]. The initial approach to treatment involves Disease-modifying anti-Rheumatic Drugs (DMARDs), aiming to mitigate the aggressiveness of Rheumatoid arthritis. If a patient does not respond to synthetic DMARDs, the next step is to initiate biological therapy, which targets various factors such as Interleukin-6 (IL-6) and Tumor Necrosis Factor (TNF- α) [4, 5].

Semaphorin 5A is belong to Semaphorins which are a family of proteins have a role in neuronal development, Airway development, cancer, autoimmune disorders, innate and adaptive immune response, Semaphorin 5A promotes angiogenesis by increase proliferation of endothelial cell and reducing apoptosis and acts as an immune semaphorin with increasing the production of tumor necrosis factor and Interleukin-8 genes in innate immune response [6]. T and Natural killer cells produce pro-inflammatory cytokines when are stimulated by Semaphorin 5A, which amplifies the effects of IL-2/IL-15 activation , Given the importance of Thelper1/T helper17 cells and high cytokine levels TNF, IL-1, IL-6, and IL-8 [7].

The current study aimed to investigate the prognostic ability of Semaphorin5A and compare it with Anti-cyclic Citrullinated peptide antibody, also to predict disease activity and response to (RA) treatments in Iraqi patients.

PATIENTS AND METHODS

Design of the Study

Conducted at the Rheumatology Consultation Clinic of Baghdad Teaching Hospital, this case-control study involved participant recruitment spanning from November 2021 to February 2022.

Criteria for Inclusion

Rheumatoid Arthritis patients were selected based on the 2010 diagnostic criteria that was set by the American College of Rheumatology. This included individuals who had received Disease-Modifying Anti-Rheumatic Drugs (DMARDs) like methotrexate (2.5mg) or Biological DMARDs like Etanercept (50mg), as well as those who had not undergone any treatment for RA.

Exclusion criteria encompassed active infections, as well as additional rheumatologic autoimmune conditions apart from RA or systemic disorders. Furthermore, the study excluded RA patients who were undergoing treatments with DMARDs other than methotrexate or receiving a methotrexate (MTX) dosage exceeding 2.5mg. Patients who had received Biological DMARDs, excluding Etanercept 50mg, were also not considered for inclusion.

Patients Group

The study comprised a total of 100 patients, with 83 being female and 17 male, falling within an age

range of 19 to 65 years. These patients were diagnosed with Rheumatoid Arthritis by a specialist rheumatologist through clinical assessment, and the diagnosis was further substantiated through laboratory investigations and radiological examinations.

The patient cohort was divided into three distinct subgroups:

- A. The initial subgroup consisted of 37 patients who were administered DMARDs (methotrexate 2.5mg).
- B. The subsequent subgroup comprised 42 patients who were receiving bDMARDs (etanercept 50mg).
- C. The final subgroup encompassed 21 patients who had not undergone any treatment for duration of 2 months or more.

The Control Group

The control group of healthy individuals comprised 50 participants (43 female and 7 male), with ages ranging from 22 to 72 years. These controls were carefully selected to match the patients' group in terms of both age and gender.

Collection of Blood Samples

Venous blood, amounting to five milliliters, was obtained from both the patients and the control group.

Separation of Serum

Serum was extracted from the blood samples and allowed to stand at room temperature for 30 minutes. Subsequently, it was centrifuged at 3000 rpm for duration of ten minutes. The resulting serum from each sample was carefully collected into Eppendorf tubes and stored at a temperature of -20 °C until needed. Enzyme-linked immunosorbent assay (ELISA) was employed to measure the levels of ACCP (Aeskulisa - Germany) and Semaphorin 5A (Bioassay - USA).

COLLECTION OF DATA

- The patient questionnaire gathered information regarding name, age, disease duration, gender, family history, and the completion of the American College of Rheumatology criteria 2010 (ACR-2010).
- The clinical disease activity index (CDAI) was utilized to evaluate disease activity through clinical examination.
- Comprehensive medical history, encompassing current and past medications, along with details of diseases, was documented.
- Laboratory investigations involved assessments such as complete blood count (CBC) and erythrocyte sedimentation rate (ESR).

Statistical Analysis

The statistical analysis of all the data included in the study was conducted using IBM SPSS Statistics version 26 and Microsoft Excel 2010. Descriptive statistics including mean, standard deviation (SD), and range were calculated. Independent T-test was employed to assess statistical significance between groups, while the One-way ANOVA test was utilized to determine any statistically significant differences among the subgroups. A p-value of less than 0.05 indicated statistical significance, while a higher p-value of less than 0.001 was considered highly statistically significant. The p-value that is greater than 0.05 indicated lack of statistical significance. Graphs and tables were employed for data presentation. Pearson correlation and regression analysis were performed to determine the correlation coefficient between study variables. Receiver Operating Characteristic (ROC) curves were used to determine cutoff points, sensitivity, and specificity of the tests.

RESULTS

The study encompassed a total of 150 participants, with both genders represented. Among these, there were 100 Rheumatoid arthritis patients (17% males, 83% females), and 50 healthy controls (14% males, 86% females). The age distribution in both groups exhibited no statistically significant differences between RA patients and the healthy control group ($p < 0.05$). Age was further categorized into four groups: ≤ 35 years (18%), 36 – 45 years (22%), 46 – 55 years (35%), and ≥ 56 years (25%). Among RA patients, 87% were smokers, while 13% were non-smokers. The average disease duration was 9.13 ± 8.25 years, ranging from 6 months to 40 years. Additionally, patients were assessed for family history, as well as symptoms such as fever, joint redness, joint tenderness, joint swelling, and Visual Analogue Scale scores. The demographic and clinical characteristics, along with relevant features of the RA condition, are detailed in Table 1.

Table 1: Demographics and Clinical characteristics of RA

RA variables		Values
Family history RA patients	With family history	18 (18 %)
	Without family history	82 (82 %)
Fever	With fever	64 (64 %)
	Without fever	36 (36 %)
Redness	With Redness	37 (37 %)
	Without Redness	63 (63 %)
Tender joints	mean \pm SD	8.92 ± 5.53
Swollen joints	mean \pm SD	1.13 ± 1.13
Visual analogue scale (VAS 0-10)	mean \pm SD	5.44 ± 1.73
<i>SD: Standard Deviation</i>		

In the study, the Clinical Disease Activity Index (CDAI) for the included RA patients was determined to be 20.34 ± 8.89 , with a range from 1 to 45. Among these patients, 12% (12 individuals) demonstrated low disease activity, with a CDAI of 5.92 ± 3.20 and a range of 1 to 9. Moderate disease activity was observed in 46% (46 patients) of the RA cases,

with a CDAI of 16.59 ± 3.84 and a range of 10 to 22. Additionally, 42% (42 patients) displayed high disease activity, resulting in a CDAI of 28.57 ± 5.11 and a range spanning from 23 to 45. The differences among these groups were statistically significant with a p-value of less than 0.001 (Figure 1).

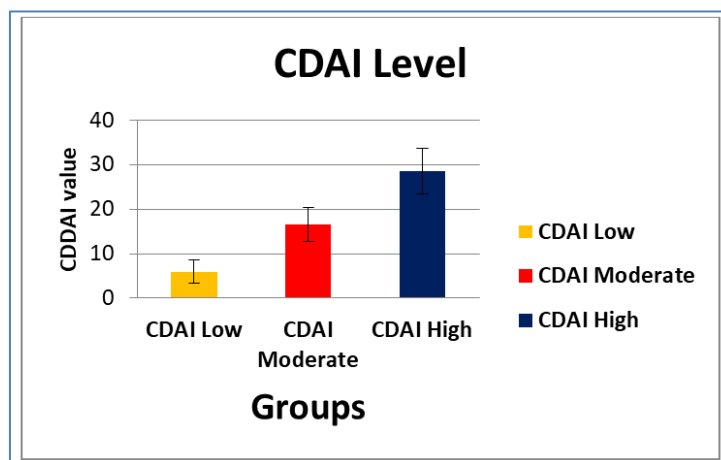


Figure 1: Difference in means of CDAI in RA patients

In terms of RA subgroup medication distribution, 37 patients (37%) were on DMARDs methotrexate (MTX), 42 patients (42%) were undergoing biological bDMARDs treatment (etanercept), and 21 patients (21%) remained without any treatment for a duration of 2 months or more.

Analysis of investigations for both RA patients and the healthy control group revealed notable differences in the means and levels of ESR, ACCP, and Semaphorin 5A. These differences in RA patients were highly significant ($P < 0.001$) compared to the health control group, as indicated in Table 2.

Table 2: The investigations of RA patients and healthy control

Investigations	Groups	Mean ± SD	P – value
ESR mm / hr	RA patients	39.85 ± 25.47	<0.001
	Control	11.60 ± 8.9	
ACCP U /ml	RA patients	58.98 ± 13.04	<0.001
	Control	5.05 ± 1.70	
Semaphorin 5A ng/ml	RA patients	1.79 ± 0.34	<0.001
	Control	0.67 ± 0.81	

SD: Standard deviation, ESR : Erythrocytes sedimentation rate, ACCP: Anti-cyclic Citrullinated peptide antibody, significance

The levels of ACCP and Semaphorin 5A among RA patients were stratified based on disease activity using the Clinical Disease Activity Index (CDAI). Notably, the levels in patients with active disease were significantly higher than those in RA

patients with moderate and low disease activity, as determined by CDAI. Moreover, all three categories displayed higher levels compared to the healthy control group, as detailed in Table 3.

Table 3: Differences in mean ± SD of biomarkers according CDAI

Biomarkers	Healthy Control	RA Patients			P- value
		Levels in Low disease activity CDAI ≤ 9	Level in moderate disease activity CDAI ≤ 22	Levels in High disease activity CDAI > 22	
		No= 12	No= 46	No= 42	
	No=50 Total = 50	Total = 100			
ACCP U /ml	5.05± 1.70	20.5 ± 5.12	38.29 ± 18.79	93.08 ± 38.93	<0.001
Semaphorin 5A mg/ml	0.67± 0.81	0.84 ± 0.12	1.46 ± 0.37	2.16 ± 0.36	<0.001

ACCP: Anti-cyclic Citrullinated peptide antibody

The impact of treatments on ACCP levels was evident, with patients who did not receive any treatment showing significantly higher levels compared to those who received treatments (Methotrexate or etanercept) ($p < 0.001$). However, there was no statistically significant difference observed between patients treated with Methotrexate and those treated with etanercept ($p > 0.05$). Furthermore, in terms of Semaphorin 5A

levels, patients without any treatments exhibited significantly higher levels compared to both Methotrexate and etanercept treatment groups ($p < 0.001$), but no statistically significant difference was found between patients treated with Methotrexate and those treated with etanercept ($p > 0.05$), as indicated in Table 4.

Table 4: Differences in (mean±SD) of biomarkers according treatments

Biomarkers	Without treatments	With treatments		P – value
		Methotrexate	Etanercept	
ACCP U /ml	82.8 ± 35.53	55.03 ± 14.31	51.46 ± 11.63	< 0.001
Semaphorin 5A mg/ml	2.28 ± 0.59	1.55 ± 0.46	1.50 ± 0.37	< 0.001

ACCP: Anti-cyclic Citrullinated peptide antibody

A significant positive correlation was observed between ACCP and ESR, with a correlation coefficient of $r = 0.258$ ($p \leq 0.01$). Furthermore, a significant positive correlation was found between ACCP and the Clinical Disease Activity Index (CDAI), with a correlation coefficient of $r = 0.459$ ($p \leq 0.001$). Additionally, there

was a notable positive correlation between Semaphorin 5A and CDAI, with a correlation coefficient of $r = 0.412$ ($p < 0.001$). Another significant positive correlation emerged between ACCP and Semaphorin 5A, yielding a correlation coefficient of $r = 0.476$ ($p < 0.001$), as depicted in Figure 2.

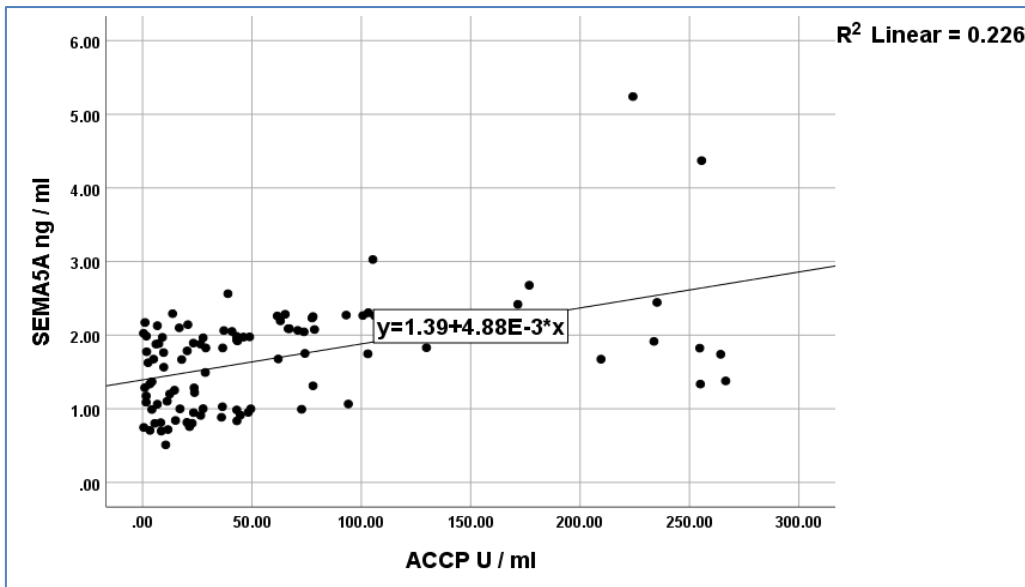


Figure 2: Scatter plot diagram show correlation between ACCP and Semaphorin 5A in patients group

The Receiver Operating Characteristic (ROC) curve was utilized to compare ACCP and Semaphorin 5A in terms of their capability for Rheumatoid Arthritis (RA) detection. The analysis also involved evaluating Sensitivity, Specificity, and determining cutoff values. The area under the curve for ACCP and Semaphorin 5A

were calculated as 0.87 and 0.88, respectively, with corresponding cutoff values of <13.13 U/ml and <0.98 ng/ml. The calculated Sensitivity values were 72% for ACCP and 83% for Semaphorin 5A, while the Specificity values were 98% for ACCP and 76% for Semaphorin 5A, as illustrated in Figure 3.

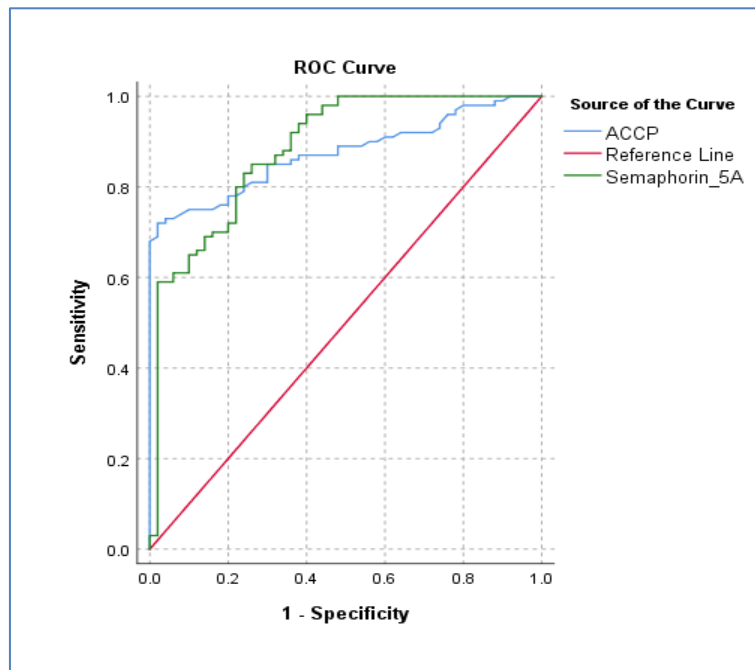


Figure 3: ROC curve of ACCP and Semaphorin 5A

DISCUSSION

The occurrence of RA in the study demonstrated a higher incidence among females compared to males, with a ratio of 4.8:1. This finding aligns with previous local and international studies that also reported a higher incidence of RA among females than males [8].

This trend can be attributed to environmental factors and the influence of female hormones, particularly estrogen, which have been linked to RA pathogenesis, inflammatory responses, and the generation of cytokines in the synovium, ultimately impacting cartilage directly [9]. Among the participants who were RA patients in the study, they were categorized into four age groups. The age range most

affected by RA was observed to be between 46 and 55 years. This observation corresponds with findings from previous local and international studies, which have consistently demonstrated that the majority of RA patients are above the age of 40 [10].

Advancing age is linked with a decline in humoral immunity and immune defense mechanisms, resulting in an elevated susceptibility to autoimmunity. One notable age-related change in B cell compartments is the buildup of auto-reactive B lymphocytes, commonly referred to as age-associated B cells. Additionally, there is a variation in the B cell receptor and gene expression. In the context of RA, smoking stands as a risk factor, primarily because it amplifies citrullination within the lung, leading to ACCP production. This, in turn, hampers the response to DMARDs [11].

The RA patients participating in the study had disease duration of 9.13 ± 8.25 years, which corresponds with findings from a local study where the disease duration was reported as 9.5 ± 3.7 years [12]. The mean ESR value in RA patients was notably higher than in the healthy control group ($p < 0.001$), as presented in Table 2. This observation aligns with the outcomes of another previous local study that also indicated a significantly elevated ESR in RA patients compared to healthy controls [13]. In terms of ACCP and Semaphorin 5A levels in RA patients' serum, the study demonstrated highly significant differences compared to the healthy control group ($p < 0.001$), which is consistent with similar results reported in previous studies that highlighted the substantial elevation of ACCP and Semaphorin 5A levels in comparison to healthy controls [14].

Immune activation by soluble Semaphorin 5A resulted in a significant increase in T cells and Natural killer (NK) cells, as well as proinflammatory cytokine release by T cells and NK cells and include IL-17 production by Thelper-17 which play important role in RA pathogenesis [27]. In current study Levels of ACCP as well as Semaphorin 5A in patients with active disease $CDAI > 22$ were highly significant than the levels in RA patients with moderate and low disease activity $CDAI \leq 22$ $p < 0.001$ but in all disease categories ACCP and Semaphorin 5A levels remain higher than healthy control (Table 3). Serum ACCP level in RA patients is increase with increasing severity of disease, higher ACCP level in sever and moderate disease activity in comparison with mild severity but in all disease stages were higher than healthy control [15], moreover previous study observed the level of Semaphorin 5A was changed according disease activity of RA which higher in active disease in compassion with inactive [16].

ACCP and Semaphorin 5A levels of patients without treatments in the study was highly significant

than patients received methotrexate or etanercept $p < 0.001$ (Table 4), ACCP level was significantly decrease in serum of patients received etanercept and lower than other RA patients whom without treatments [17]. Sema5A is one immune pathway to induces the pro-inflammatory cytokines which play role in RA pathogenesis including TNF- α production by Natural killer (NK) and cytokines by T cells with induces proliferation of immune cells [18]. Increase TNF production in RA is associated with increasing proinflammatory mediators such as prostaglandins (PG) and metalloproteinases, the effect of etanercept (TNF inhibitor) is specific as anticytokine effect for RA treatment in early and established stages of disease [19].

Correlation between Semaphorin 5A level and disease activity based on CDAI value was significant positive correlation $r = 0.412$ and $p < 0.001$, other previous study shown significant positive correlation between Semaphorin 5A and disease activity (CDAI) [20]. The Correlation between Semaphorin 5A and ACCP was significant positive correlation $r = 0.476$ and $p < 0.001$ (Figure 2).

Similarly other study found significant positive correlation between ACCP and Semaphorin 5A, and higher Semaphorin 5A level associated with higher ACCP level in seropositive patients which (ACCP positive) in comparison with seronegative which (ACCP negative) therefore suggest Semaphorin 5A is complementary biomarker for RA including as prognosis of disease and monitoring of disease progression in association with other biomarkers. ACCP and Semaphorin 5A the area under curve were (0.87, 0.88), Cutoff values (> 13.13 U/ml, > 0.98 ng/ml) Sensitivity (72%, 83%) and Specificity (98%, 76%) respectively (Figure 3). Other study observed Sensitivity and Specificity of ACCP are (67%, 85-95%) respectively [21].

CONCLUSIONS

Both Anti-citrullinated peptide antibody and Semaphorin 5A display noteworthy prognostic potential when compared to each other, and they also serve as effective differentiators between RA patients and healthy individuals. Additionally, they offer insights into disease severity and the response to MTX and etanercept treatments. Notably, RA patients who did not receive treatments exhibited higher levels of both ACCP and semaphorin 5A in comparison to patients treated with MTX or etanercept. An interesting finding is the significant positive correlation between ACCP and SAA2, which amplifies the prognostic capability for RA.

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