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# CORRELATION OF SERUM SEMAPHORIN 3A WITH DISEASE ACTIVITY IN ANKYLOSING SPONDYLITIS PATIENTS

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**ORIGINAL ARTICLE**

# CORRELATION OF SERUM SEMAPHORIN 3A WITH DISEASE ACTIVITY IN ANKYLOSING SPONDYLITIS PATIENTS.

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## ABSTRACT

**Objective:** To assess the serum level of Semaphorin 3A in ankylosing spondylitis (AS) patients and to assess its correlation with disease activity.

**Subjects and Methods:** The study was carried on 40 subjects, 20 AS patients (12 males and 8 females) fulfilled the 1984 Modified New York criteria for diagnosis of ankylosing spondylitis, and 20 healthy subjects, taken as control group (12 females and 8 males). A consent was taken from all subjects to share in the study. Disease Activity Score (ASDAS) was calculated for AS patients and functional assessment was done using the Bath Ankylosing Spondylitis Functional Index (BASFI).

**Results:** Serum levels of Semaphorin 3A were significantly higher($P<0.05$) in AS patients more than controls as it ranged between 0.22-31.48 years with a median of 2.93 in AS patients and (0.04-3.91) with the median of 1.83in controls. Also, there was a significant difference($P<0.05$) of Serum Semaphorin 3A level in ankylosing spondylitis patients regarding ASDAS-CRP score being higher in active AS (ASDAS-CRP $\geq 1.3$) and ranged between (2.17-31.48) vs (0.22- 2.23) in the active vs the inactive AS patients respectively. Serum Semaphorin 3A level (ng/ml) in ankylosing spondylitis patients were significantly correlated with ASDAS- CRP ($r=0.82$), ASDAS-ESR ($r=0.76$) as $p<0.05$.

**Conclusion:** Serum levels of Semaphorin 3A were increased in patients with ankylosing sponndylitis and correlated with disease activity. So, Semaphorin 3A can be used as a biomarker for AS disease activity.

**Keyword: Ankylosing spondylitis- Semaphorin 3A- biomarker.**

## INTRODUCTION

Ankylosing spondylitis (AS) is an auto-immune disease which is characterized by inflammatory back pain with inactivity stiffness, enthesitis, sacroiliitis, peripheral arthritis, and anterior uveitis. AS may have significant mental and economical burdens in the patients and the society as a whole [(1)]. AS burden results from back pain due to acute inflammation and limitation of spinal mobility due to new bone formation [(2)] .

Regarding the acute inflammation occurring in ankylosing spondylitis, both interleukin (IL)-23/IL-17 axis and T helper-17 (Th17) cells are major players[(3)]. When stimulated by IL-23 cytokine, Th17 cells produce proinflammatory cytokines including tumor necrosis factor (TNF)-alpha, interferon (IFN)-gamma, interleukin (IL-6), and IL-17. IL-17 was showed to stimulate T cells, macrophages, other cells to produce cytokines

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which promote inflammation including TNF-alpha, IL-1 and IL-6[(2)].

Moreover, AS is characterized by formation of new bone in the form of enthesophytes originating from the entheses where ligaments and tendons are inserted in bones, syndesmophytes joining adjacent intervertebral bodies and bony changes of the sacroiliac joints leading to their ankylosis [(4)].

Semaphorins are formed of 'Sema' part containing amino acids and c-terminal part. They include eight subclasses. Classes 1 and class 2 are present in the invertebrate while Classes 3-7 are found in vertebrates. However, class 8 is virus encoded [(5 )]. Class 1 and 4–7 semaphorins are membrane-bound but classes 2, 3 and 8 are secreted [(6)].

Semaphorins are secreted by many cells including neuronal cells, cells of cardiovascular, gastrointestinal, hepatic, renal and reproductive systems [(7)], cancer and immune cells [(10)]. In addition, they are secreted by all bone cells including osteoclasts, osteoblasts, and osteocytes [(5)]. They have different functions as bone remodeling, bone diseases pathology, immune regulation, angiogenesis, cardiac development and cancer growth[(5)].

Semaphorins bind to both neuropilins and plexins receptors. The plexins function is signal transduction while neurophilins are the sites where Semaphorins bind. The neurophilins include NP-1 and NP-2 subtypes (8).

It was found that Semaphorin 3A (Sema 3A) and its receptors, plexins, NP-1 and NP-2, Sema 3A was expressed on multiple immune cells including activated T-cells and differentiating macrophages. So, it was suggested that Sema 3A may play a vital role in inflammatory diseases including ankylosing spondylitis disease [(9)].

In addition, Sema 3A and its receptors were found to be expressed by osteoblasts, osteoclasts and osteoclast precursors. It was suggested that Sema 3A could stimulate the process of bone formation through induction of osteoblast differentiation and inhibit the process of bone resorption through suppression of osteoclast differentiation [(10)].

This study aimed to find out the correlation of Sema 3A with disease activity in ankylosing spondylitis patients. This study selected class 3 Semaphorins as they were shown to play a role in the pathogenesis of autoimmune diseases as rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus, so, it may play a role in AS pathogenesis [(11)]

**Subjects and methods:**

- This study was performed in Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University on 40 subjects divided into two groups: Group I (AS group) and group II (control group). Group I included 20 AS patients diagnosed according to 1984 Modified New York criteria for ankylosing spondylitis [(12)] but group II included 20 apparently healthy control subjects, age and sex matched with patients.
- Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

**Exclusion criteria:**

- Patients with osteoporosis or bony fractures or osteoarthritis or chronic kidney disease.
- Patients who are on anticoagulants.
- Patients with other autoimmune diseases as rheumatoid arthritis, scleroderma, Systemic lupus erythematosus, dermatomyositis, mixed connective tissue disease, psoriatic arthritis were excluded.

**We subjected the AS patients to:**

- Full history taking.
- Full clinical examination (general and musculoskeletal system examination).
- Calculation of disease activity scores: Ankylosing Spondylitis Disease Activity Score (ASDAS**)** [(13)].

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- Functional assessment by the Bath Ankylosing Spondylitis Functional Index (BASFI) [14].
- Laboratory investigations: Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

**C-reactive protein (CRP):**

It was measured by immunoturbidimetric assay on Cobas 6000 analyzer.

**Serum Semaphorin 3A level:**

It was measured in both patients and controls serum samples by enzyme-linked immunosorbent assay (ELISA) using Human Semaphorin 3A (Sema 3A) ELISA Kit according to the instruction of the manufacturer **(SunRed, Shanghi).** Venous blood samples were taken from each subject under aseptic condition, left for 20-30 minutes for spontaneous clotting then centrifuged at 4000 rounds per minute for 10 minutes then serums were collected and stored at -20° C until Semaphorin determination. All the work was carried out in the laboratory of the Clinical Pathology Department.

**Statistical Methods:**

- All data were statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA 2011). Quantitative data were expressed as the mean ± SD & minimum to maximum range but qualitative data were expressed as percentage.
- To compare between two groups of normally distributed variables, independent samples Student's t-test was used. On the other hand, we used Mann Whitney U test to compare between two groups of non-normally distributed variables.
- P-values < 0.05 were considered statistically significant and p-values ≥ 0.05 were considered statistically non-significant.

**Results:**

All AS patients included in the study were diagnosed according to 1984 Modified New York criteria for ankylosing spondylitis and had radiographic sacroiliitis grade ≥ 2 bilaterally or grade 3-4 unilaterally [12].

The mean age of AS patients was 32.8± 7.7 years, the mean of disease duration was 8.7±8.5 years. They were 12 males and 8 females. There was non-significant difference between ankylosing spondylitis patients and controls regarding age and sex (P>0.05) (Table1).

Table.2 demonstrates characteristics of AS patients.14 patients had peripheral arthritis in addition to axial arthritis (70%) while 6 AS patients had isolated axial arthritis (20%), only one patient had uveitis (5%), lateral flexion ranged from (2-22) cm and the median was 8.5 cm, Schober test ranged between (11-16) cm and the median was 12.75 cm, Chest Expansion ranged between (2-6) cm and the median was 4 cm, ESR ranged from (4-100) mm/h and the median was 30 mm/hr., CRP ranged between (1.2-48) mg/dl and the median was 9.2 mg/dl, ASDAS-CRP ranged between (1.04-4.8) with median of 2.95, ASDAS-ESR ranged between(1.03-5.23) with median of 2.84, BASFI ranged between (0-9) and the median was 4.5, Serum level Sema 3A ranged between (0.22-31.48) ng/ml and the median was 2.93 .

Serum Sema 3A level showed a statistically significant difference (p <0.05) in AS patients' group than in the control group, with a median value of 2.93 and 1.83 respectively (Table 3).

Using ASDAS-CRP, 85% of AS patients were active, while using ASDAS- ESR, 95% of patients were active (Table 4).

Table.5 shows statistically significant difference of Serum Sema 3A level (ng/ml) in ankylosing spondylitis patients regarding ASDAS-CRP as the median value of serum level of Sema 3A was 3.9 and 2 in active and inactive AS patients. It also shows that serum Sema 3A levels were significantly higher in AS patients with peripheral arthritis than in AS patients without peripheral arthritis with statistically significant difference p<0.05 as serum level of Sema 3A ranged between (2.13 - 31.48) in AS patients with peripheral arthritis and between (0.22-2.64) in AS patients without peripheral arthritis

Table.6 shows that a statistically significant correlation between serum

level (ng/ml) and number of peripheral

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joints affected (r =0.58), ASDAS- CRP (r=0.82), ASDAS-ESR (r=0.76) and BASFI.

Figure.1 shows that the optimum cut-off for serum Sema 3A level to detect AS cases from healthy one was (2.294 ng/ml) with 80% sensitivity, 80% specificity and 80% accuracy. It also shows that the optimum cut-off for serum Sema 3A level to detect active and inactive AS patients was (3.46 ng/ml) with 94% sensitivity, 100% specificity and 95% accuracy.

**Table (1): Demographic characteristics of both groups:**

| Items | Ankylosing spondylitis (n=20) | Healthy control (n=20) | Sig Test | p |
|---|---|---|---|---|
| **Age** | | | t=0.04 | 0.97 |
| **Mean ±SD** | 32.8± 7.7 | 32.7±7.8 | | |
| **Minimum- maximum** | 19-50 | 18-50 | | |
| **Sex** | 12/8(1.33) | 12/8(1.33) | $\chi^2$ =0 | 1 |
| **Males/females ratio** | | | | |
| **Disease Duration (years)** | 8.7±8.5 | - | - | - |
| **Mean ± SD** | 1-36 | | | |
| **Minimum-maximum** | | | | |

**$\chi$ 2 = Chi-square test.**

**Table (2): Characteristics of patients with ankylosing spondylitis:**

| | Median (minimum-maximum) | |
|---|---|---|
| Lateral flexion (cm) | 8.5(2-22) | |
| Schober test(cm) | 12.75(11-16) | |
| Chest Expansion (Cm) | 4.(2-6) | |
| ESR (mm/h) | 30(4-100) | |
| CRP (mg/dl) | 9.2(1.2-48) | |
| ASDAS CRP | 2.95(1.04-4.8) | |
| ASDAS ESR | 2.84(1.03-5.23) | |
| Serum level Semaphorin 3A (ng/ml) | 2.93(0.22-31.48) | |
| BASFI | 4.5(0-9) | |
| peripheral arthritis | No | % |
| present | 14 | 70 |
| absent | 6 | 30 |
| Uveitis | 1 | 5 |
| Yes | 19 | 95 |
| no | | |

**ESR:** erythrocyte sedimentation rate**, CRP:** C-reactive protein, **BASFI:** Bath Ankylosing spondylitis Functional Index, **ASDAS-CRP:** ankylosing spondylitis disease activity score using CRP, **ASDAS ESR:** ankylosing spondylitis disease activity score using ESR.

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**Table (3): Comparison between ankylosing spondylitis patients and healthy controls regarding serum Semaphorin 3A level :**

| | Ankylosing spondylitis (n=20) | Healthy control (n=20) | MW | P |
|---|---|---|---|---|
| **Serum level Semaphorin 3A (ng/ml)** Median(minimum-maximum) | 2.93(0.22-31.48) | 1.83(0.04-3.91) | 4.1 | 0.0005(*) |

**MW:** Mann-Whitney test ***** significance at $p < 0.05$.

**Table (4): Disease activity in AS patients' group:**

| | No | % |
|---|---|---|
| **ASDAS-CRP score** | | |
| inactive (<1.3) | 3 | 15 |
| active (≥ 1.3) | 17 | 85 |
| **ASDAS- ESR score** | 1 | 5.0 |
| inactive (<1.3) | 19 | 95 |
| active (≥1.3) | | |

**ASDAS-CRP score:** ankylosing spondylitis disease activity score using CRP**, ASDAS -ESR:** ankylosing spondylitis disease activity score using ESR**.**

**Table (5): Comparison serum Semaphorin 3A level of ankylosing spondylitis patients' group regarding ASDAS disease activity and peripheral arthritis:**

| Variables | Number of AS patients | Serum Semaphorin 3A level (ng/ml): Median(min-max) | MW | p |
|---|---|---|---|---|
| **ASDAS-CRP** | | | | |
| Active (≥1.3) | 17 | 3.9(2.17-31.48) | 2.59 | 0.01(*) |
| Inactive (<1.3) | 3 | 2 (0.22- 2.23) | | |
| **Peripheral arthritis** | 14 | 5.94 (2.13 -31.48) | | |
| Present | 6 | 2.4(0.22-2.64) | | |
| absent | | | 2.3 | 0.02(*) |

**MW:** Mann-Whitney test**. *** significance at $p < 0.05$. **ASDAS-CRP**: ankylosing spondylitis disease activity score using CRP

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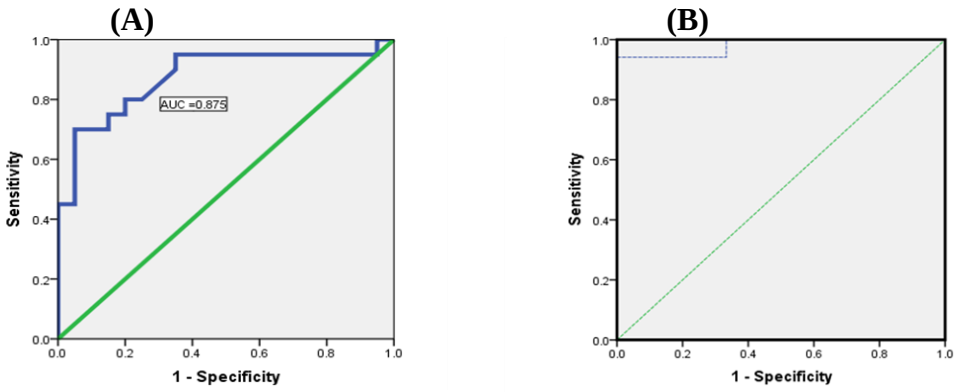
**Table (6): Correlation of serum Semaphorin 3A level with age, clinical parameters and disease activity in ankylosing spondylitis patients:**

| parameters | Serum level Semaphorin 3A (ng/ml) in Ankylosing spondylitis patients: | |
|---|---|---|
| | (r) | p |
| Age | 0.23 | 0.34 |
| Disease duration per years | 0.073 | 0.76 |
| Number of peripheral joints affected | 0.58* | 0.007 |
| Occiput to wall (cm) | 0.01 | 0.97 |
| Lateral flexion (cm) | 0.107 | 0.65 |
| Schober test(cm) | -0.18 | 0.44 |
| Chest Expansion (Cm) | 0.19 | 0.43 |
| ESR | 0.33 | 0.16 |
| CRP | 0.55* | 0.01 |
| ASDAS CRP | 0.82* | 0.0001 |
| ASDAS ESR | 0.76* | 0.0001 |
| BASFI | 0.69* | 0.001 |

**(r):** correlation coefficient ***** Statistically significant **p<0.05**




*ROC: Receiver operating characteristic curve.

**Figure (1). In figure A: ROC curve detects optimal cut off of serum Semaphorin level 3A between ankylosing spondylitis patients and controls. In figure B: ROC curve detects optimal cut off of serum Semaphorin 3A level between active and inactive AS patients according to ASDAS-CRP score.**

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## DISCUSSION

Ankylosing spondylitis (AS) is a chronic disease that mostly affects patients aged 20- 30 years old. AS affects skeletal sites include the spine, the sacroiliac joints and the entheses. AS also affects extra-skeletal sites including the eye, the heart and the gastro-intestinal tract [(15)].

In the present study, serum levels of Semaphorin 3A (Sema 3A) were significantly higher in AS patients than in normal subjects. In addition, serum Sema 3A levels were higher in active AS patients than in inactive patients.

In addition, serum level of Sema 3A was compared between active and inactive AS patients and it was observed that serum Sema 3A levels were significantly higher in active AS patients than in inactive patients with the range of serum Sema 3A levels was (2.2-31.477) in the active AS patients versus (0.22- 2.32)in the inactive patients (P<0.001).

This is consistent with the results of Liao et al.,[(16)] and Qian et al., [(3)] who compared between Sema 3A serum levels in AS patients and Sema 3A serum levels in healthy controls and found there was a significant difference of between Sema 3A serum level in both groups with higher levels in AS group.

However, these results are inconsistent with the findings of Perrotta et al., [(17)] that there was no difference between AS patients and healthy controls as regarding serum Sema 3A . This conflict may be explained by exclusion of subjects with condition affecting serum level of Sema 3A as taking anticoagulants, having osteoporosis or renal failure or bony fracture in this study and in the study of Liao et al., [(1)]

This study showed that serum level of Sema 3A significantly correlated with BASFI and AS Disease Activity measured by ASDAS-CRP and ASDAS-ESR.

There is a conflict between our results and results of Liao et al.,[(1)] who found non-significant correlation between serum levels of Sema 3A, BASFI and AS disease activity and Perrotta et al., (17) who didn't find any correlation between serum levels of Sema 3A with ASDAS-CRP. This may be due to small sample size in our study.

## CONCLUSION

This study showed that Semaphorin 3A serum levels were elevated in AS patients and correlated with AS disease activity measured by ASDAS-CRP, ASDAS-ESR, so, Sema 3A is a potential a bio-marker for AS disease activity. Semaphorin 3A serum levels were also correlated with functional ability of AS patients measured by BASFI.

We recommend further studies with larger sample size for more evaluation of the correlation of Semaphorin 3A with AS disease activity as 19 out of 20 AS patients are active according to ASDAS-ESR.

**Conflict of interest:** - No Conflict of interest
**Financial disclosure:** - No funding or grants

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