

Serum Level of Wingless Integration 5a Protein in Rheumatoid Arthritis

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ABSTRACT

Background: Genesis and molecular etiology of rheumatoid arthritis (RA) remain important in spite novel achievements of disease modifying anti-rheumatic drugs (DMARDs). Wnt5a is up regulated in synovial fibroblasts among RA patients, suggesting it could contribute to the development of illness.

Objective: To measure Wnt5a protein levels among RA patients' serum and to find its relationship with disease activity.

Patients and Methods: Twenty patients with RA and twenty people without the disease who were matched for age and sex served as the study's control group. The patients were all examined thoroughly and had taken their histories. The Disease Activity Score-28 was used to measure disease severity (DAS-28). All participants gave their serum Wnt5a levels assessed using an enzyme-linked immunosorbent test (ELISA). Patients' complete blood cell count, anti-cyclic citrullinated peptide (anti-CCP) titer, rheumatoid factor titer, C-reactive protein, as well as erythrocyte sedimentation rate were determined.

Results: Serum Wnt5a levels of RA patients were significantly higher than those of the control group. Wnt5a levels correlated positively with several other measures of inflammation and illness severity (DAS-28, ESR, CRP, RF, and disease duration). Serum Wnt5a was not correlated with age, sex, or Anti-CCP. With an area under the curve of 0.891, a specificity of 75%, a sensitivity of 80%, NPV of 65.2%, PPV of 86.5% and an accuracy of 78.3%, a cutoff of being equal or higher than 2.06 ng/ml for serum Wnt5a protein in the diagnosis of RA was optimal.

Conclusion: Results from the current investigation revealed a correlation between Wnt5a and RA.

Keywords: Wnt5a, Rheumatoid arthritis, Disease activity.

INTRODUCTION

Significant morbidity and death are associated with rheumatoid arthritis (RA), a prevalent chronic autoimmune disease. In the United States, the prevalence of this condition is around 1%, with a higher chance of occurrence among women ⁽¹⁾.

There is still much mystery around the precise cause of RA, however many factors have been implicated ⁽²⁾.

The evolution of the disease, without adequate treatment, implies the formation of pannus, in which the synovial membrane behaves like a mass of growing tissue composed by macrophages, osteoclasts and fibroblast-like synoviocytes (FLS). In this sense the FLS play an important role, their activation and proliferation contribute to processes of recruitment, retention and activation of inflammatory cells through the creation of adhesion molecules, chemokines, and cytokines, with formation of new vessels through regulators of angiogenesis and destruction of articular cartilage and bone as a result of the production of collagenases and metalloproteases ⁽³⁾.

Several studies are focusing on identifying new genetic clues that can be involved in the pathogenetic processes, leading to the development of RA. A sub group of the non-canonical wingless integration (Wnt) molecule, named Wnt5a, has been recently identified. This molecule is able to modulate cellular differentiation, migration and inflammation. In particular, FLS of RA patients has overexpressed of Wnt5a, implying for Wnt5a role in disease development ⁽⁴⁾.

To initiate intracellular signalling pathways, wntless integration (Wnt) proteins serve as ligands by binding with frizzled (FZD) receptors on the cell surface ⁽⁵⁾. Planar cell polarity (PCP) and the WNT/Ca²⁺ route are two examples of non-canonical WNT pathways (catenin-independent pathways) ⁽⁶⁾.

Wnt5a has been considered non-canonical Wnt ligand. Human pathological problems like fibrosis, cancer, inflammatory illnesses, and metabolic abnormalities have all been linked to Wnt5a signalling dysregulation, often caused by Wnt5a overexpression ⁽⁷⁾. Evidence suggests that Wnt5a has a significant role in the development of RA, and further research is warranted ⁽⁸⁾. The chronic inflammatory chemokines/cytokines IL-15, IL-8 as well as IL-6 are mostly induced by Wnt5a-mediated signalling in RA-FLS ⁽⁹⁾.

THE STUDY AIMS

It was to measure Wnt5a protein levels among RA patients' serum and to find its relation with disease activity.

PATIENTS AND METHODS

Institutional review board (IRB) Committee, at Faculty of Medicine, Zagazig University, reviewed and approved this study before it could be conducted at Physical Medicine, and the Rheumatology, Rehabilitation Departments of the Zagazig University Hospitals. Between June 2020 and June 2022, 20 RA patients participated in this trial. Rheumatoid arthritis patients met the 2010 ACR and EULAR (European

League Against Rheumatism) diagnostic criteria⁽¹⁰⁾. Patients with systemic lupus erythematosus, hypertension, cardiovascular illness, diabetes mellitus, chronic kidney or liver disease, as well as other autoimmune diseases were not included.

Clinical evaluation:

Full medical histories and physical examinations were performed on all individuals. Disease Activity Score-28 (DAS-28) was used to evaluate the activity of RA patients' disease. Joint tenderness and swelling, as well as the erythrocyte sedimentation rate and patient's overall rating of health, are all components of the DAS⁽¹¹⁾.

Laboratory investigations:

- 1- All individuals had their serum Wnt5a levels tested using an enzyme linked immunosorbent assay (ELISA) calibrated per the manufacturer's instructions (SunRed, Shanghai).
- 2- Erythrocyte sedimentation rate (ESR), Complete blood count (CBC), rheumatoid factor (RF) titre, anti-cyclic citrullinated peptide (Anti-CCP) antibody titre, C-reactive protein (CRP), liver and kidney function tests were also performed on RA patients.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

In order to analyze the data acquired, Statistical Package of Social Sciences (SPSS), version 28 was used to execute it on a computer. In order to convey the findings, tables and graphs were employed. The quantitative data was presented in the form of the mean, median, standard deviation, and confidence intervals. The information was presented using qualitative statistics such as frequency and percentage. The student's t test (T) is used to assess the data while dealing with quantitative independent variables. Pearson Chi-Square and Chi-Square for Linear Trend (X²) were used to assess qualitatively independent data. The significance of a P value of 0.05 or less was determined.

RESULTS

Demographics are showed in (Table 1); mean age was 40.35±6.91 in RA group while it was 39.65±4.25 years among control group, females were 19 (95%) among RA group while they were 16 (80%)

among control group with no significant difference in gender or age.

Table (1): demographic data comparison:

Parameter	RA group (N=20)	Control group (N=20)	P
Age (year): Mean ± SD	40.35±6.91	39.65± 4.25	0.702
Sex:			
Female	19 (95%)	16 (80%)	0.342
Male	1 (5%)	4 (20%)	

Table (2) showed laboratory as well as clinical characteristics of RA group patients; Mean Disease duration was 9 (3 – 35) years, mean Morning stiffness duration was 30 (10 – 120) minutes, mean DAS score was 4.89 ± 0.67.

Table (2): Clinical and laboratory characteristics of RA group patients:

Parameter	RA group (N=20)
Disease duration (years)	9 (3 – 35)
Morning stiffness (min)	30 (10 – 120)
DAS-28	4.89 ± 0.67
CRP (mg/l)	16.01 ± 3.21
ESR(mm/hr)	25 ± 5.31
Anti-CCP(u/ml)	67 ± 13.5
RF (IU/ml)	33.72 ± 7.11
NSAID	6 (30%)
GC	10 (50%)

Table (3) demonstrated a statistically significant difference in Wnt5a protein serum concentration between the groups tested. Wnt5a protein levels in the blood were significantly higher in RA group.

Table (3): Serum Wnt5a protein levels were compared between the groups:

Parameter	Groups		P
	RA group	Control group	
	N=20 (%)	N=20 (%)	
Wnt5a (ng/mL): Mean ± SD	2.414±0.543	1.783±0.41	<0.001**

Table (4) showed there were significant positive correlation between serum level of Wnt5a protein and all of DAS-28, CRP, ESR, disease duration and rheumatoid factor. Serum levels of Wnt5a protein were not correlated with age, sex, or anti-CCP levels.

Wnt5a protein levels in the blood were positively correlated with disease activity indicators as DAS-28, CRP, ESR, illness duration, and rheumatoid factor. Serum Wnt5a protein levels were not

significantly correlated with age, sex, or anti-CCP levels.

Table (4): Serum levels of Wnt5a protein are correlated with initial findings in rheumatoid arthritis patients:

	r	P
Age	-0.19	0.146
Sex	0.26	0.05
DAS 28	0.326	0.002*
Disease duration	0.473	<0.001**
CRP	0.53	<0.001**
ESR	0.604	<0.001**
Anti-CCP	-0.064	0.694
Rheumatoid factor	0.539	<0.001**

Table (5) and Figure (1) showed that with an area under the curve of 0.891, a specificity of 75%, a sensitivity of 80%, NPV of 65.2%, PPV of 86.5% and an accuracy of 78.3%, a cutoff of being equal or higher than 2.06ng/ml for serum Wnt5a protein in the diagnosis of RA is optimal (p<0.001).

Table (5): Performance of serum level of Wnt5a protein in diagnosing RA across the studied groups:

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≥2.06	0.891	80%	75%	86.5%	65.2%	78.3%	<0.001**

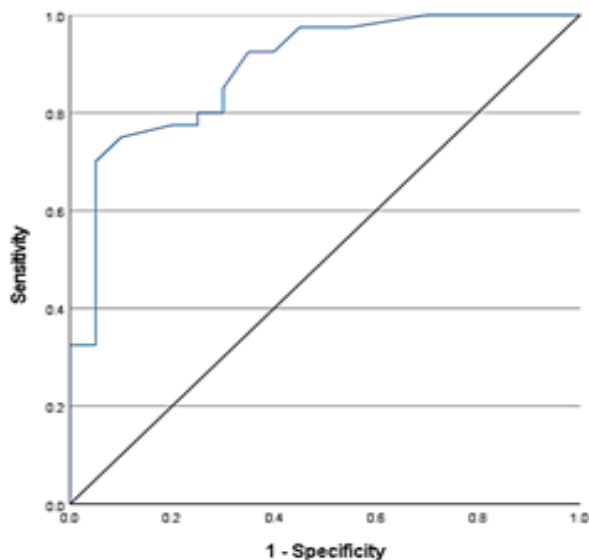


Figure (1): ROC curve illustrating Wnt5a's ability to predict RA.

DISCUSSION

Progressive disability, systemic complications, and high mortality are all features of rheumatoid arthritis (RA), a prevalent systemic autoimmune and inflammatory disease (12).

Although the exact cause of RA is still unknown, environmental and genetic factors are likely contributors (13).

During embryonic morphogenesis, Wnt5a, a ligand that promotes non-canonical Wnt signalling in the regulation of cell migration and polarity, causes inflammation by activating macrophages (14).

Evidence for the expression of Wnt5a protein and mRNA has been reported in a variety of inflammatory diseases and disorders, including rheumatoid arthritis, atherosclerosis, and TB (14). Recently, it has been discovered that the JAK-STAT3/NF-kB/TLR signalling cascade is a crucial activation mechanism for Wnt5a expression (15).

Pashirzad *et al.* (16) suggested that Wnt5a would be a unique diagnostic component and very important as post-treatment marker for RA, atherosclerosis, psoriasis vulgaris, and sepsis.

Wnt5a has been shown to play a part in systemic lupus erythematosus, and its potential as a biomarker for measuring SLE activity was recently proposed in a study by Shuhong *et al.* (17).

Another paper demonstrated Wnt5a role in psoriatic arthritis (PsA) as Lin *et al.* (18), found higher levels of Wnt5a mRNA and protein in MDOC (monocyte-derived osteoclasts) in PsA patients than in those from healthy control.

Our findings agreed with Sen (19), who discovered the importance of the Wnt pathway in the development of RA particularly synovial samples from people with RA have been discovered to have greater levels of Wnt5a and Fz5 than those from people with osteoarthritis (OA), whose joints are less inflamed.

In accordance with these results, Xiao *et al.* (20) observed that Wnt/β-catenin expression is increased in RA-FLS both in vitro and in vivo. Wnt/β-catenin signalling activity underlies the observed increase. Stable activation of RA-FLS involves activation of Wnt/β-catenin signalling.

There was statistically significant positive correlation between serum level of Wnt5a and all of DAS-28, CRP, ESR, disease duration and rheumatoid factor. This may indicate that Wnt5a can be used as a biomarker for RA disease.

These findings went ahead with Yu *et al.* (21), who found a positive correlation between the plasma Wnt5a protein and plasma RF and CRP.

Serum levels of Wnt5a protein were shown to be unrelated to demographic factors including age, sex, or anti-CCP status in our study.

With an area under the curve of 0.891, a specificity of 75%, a sensitivity of 80%, NPV of 65.2%, PPV of 86.5% and an accuracy of 78.3%, a cutoff of being equal or higher than 2.06ng/ml for serum Wnt5a protein in the diagnosis of RA is optimal.

There were some limitations in this research; small sample size and most of patients were stable with the treatment.

Further studies are required before being able to confirm its usefulness as a biomarker and therapeutic target in RA.

CONCLUSION

Our findings showed that Wnt5a may be an indicator helping in prediction of RA disease and correlated with disease activity in RA patients. It will be important to confirm these results in a broader sample of the population in the future.

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