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Serum Level of Galactin-1 Association with Disease Activity of Rheumatoid Arthritis Patients under Conventional Treatment

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ABSTRACT

Background: The small joints of feet, hands and wrists are particularly vulnerable to impacts of Rheumatoid Arthritis (RA), the most prevalent auto-inflammatory disorder. The pathogenesis of RA is still unknown which leads to the on-going studies for further understands the pathogenesis and find new treatment targets. Galectin family includes Galectin-1 (Gal-1). It could be found in a large number of tissues with pro-inflammatory as well as anti-inflammatory effects relying on the cellular microenvironment. **Objectives:** The aim of the current study is to evaluate the relation between the disease activity of active rheumatoid cases under conventional medical treatment, and the level of Gal-1 in serum. **Patient and methods:** A total of 50 healthy controls and 50 RA patients; at least 18 years old using conventional medical treatment, were included in this case control study. Clinical evaluation, laboratory testing, serum Gal-1 levels, and evaluation of disease activity, was performed to all participants. **Results:** At a cutoff of >15 (ng/ml), the serum Gal-1 level in the RA group was significantly higher compared to the healthy control group. Serum Gal-1 was not significantly correlated with the activity of RA. **Conclusion:** Our results demonstrated the usefulness of measuring the serum levels of Galectin-1 in patients with RA with high validity. This proves the pro-inflammatory effect of Galectin-1 in RA patients.

Keywords: Galectin-1, Rheumatoid arthritis, Disease activity, Conventional treatment.

INTRODUCTION

Most cases of rheumatic autoimmunity are due to rheumatoid arthritis (RA). The exact cause of RA is still unknown, but it is may be a result of multiple variables, including genetics and environmental factors. This results in the continuous infiltration of the synovial membrane by different immune cells [(1)]. Joint degeneration resulted from cartilage and bone erosion is caused by the infiltrating inflammatory cells and persistent production of proinflammatory cytokines (such as, IL-6, IL-1as well as TNF-α) [(2)].

Carbohydrate recognition domains (CRDs) in galectins allow them to bind β-galactoside [(3)]. Up to 15 mammalian galectins have been discovered and characterized so far; each one has 1 or 2 CRDs totaling roughly 130 amino acids. Galectins are involved in many different biological processes, including as immunological control, because to their broad dispersion and many binding partners [(4)]. The protein galectin-1 (Gal-1) can self-assemble into homodimers. Extremely abundant in immune cells, its expression changes with differentiation as well as cell activity [(4)]. There is a correlation between Gal-1 pro-apoptotic activity in activated lymphocytes and its anti-inflammatory and immunosuppressive properties [(5)].

It has an enhancing effect to shift Th1 responses to the regulatory T cell (Treg) and Th17 responses away from the Th1 cell [(6)], as well as preventing the production of inflammatory cytokines (like TNF-α, IFN-γ as well as IL-2) in vitro [(7)]. The Gal-1 serum levels of RA patients and healthy controls have been reported to be similar in various research [(8)].

Incorporating disease-modifying antirheumatic medications into RA treatment over the past two decades has led to a more sophisticated approach to disease management (DMARDs). Methotrexate, the most often prescribed DMARD, has revolutionized the treatment of this disease by blocking enzymes necessary for the production of purines and pyrimidines. Many people feel better after using this medication [(9)]. However, a deeper understanding of the function of each immune system component can lead to more effective treatment plans, including when to administer them and in what doses. In spite of the fact that there are those who advocate for aggressive treatment of arthritis in order to reduce inflammation [(10)], It seems sense to not over-treat those who aren't at high risk for developing a serious illness.

Aside from the effects of Gal-1 on T cells, very little work has been conducted on the role of Gal-1 on disease activity and its serum level usefulness in differentiating RA patients from normal healthy population. Therefore, the current study aimed to evaluate the relation between the disease activity of active rheumatoid cases under conventional medical treatment, and the level of Gal-1 in serum.

PATIENTS AND METHODS

A total of 50 patients with RA (according to the 2010 ACR/EULAR criteria) and 50 healthy controls took part in this study. After being apprised of the study's procedures, all participants voluntarily supplied written consent.

Inclusion criteria: 1. Patients diagnosed as RA; by American College of Rheumatology and European League Against Rheumatism (ACR/EULAR) in 2010, 2. Above 18 years old, and 3. Under conventional

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medical treatment of RA with conventional Disease-Modifying Antirheumatic Drugs (cDMARDs).
Patients suffered from other rheumatological diseases. previous history of malignant disease, uncontrolled medical illness. pregnant patients or younger than 18 years old were excluded.

The following were performed for all patients:

- Complete patient history and physical examination.
- Evaluation of disease activity score using DAS28 (Disease activity score in 28 joints): DAS28-CRP as well as DAS28-ESR.
- Laboratory investigations: 1. CBC (complete blood count) using coulter counter, 2. ESR (Erythrocyte Sedimentation Rate) as measured by the Westergren technique, 3. Latex agglutination techniques for measuring CRP (C-reactive protein), 4. Measurement of Rheumatoid Factor (RF) with ELISA, 5. Anti-CCP (anti-cyclic citrullinated peptide antibodies) by ELISA, 6. Liver function tests (LFT) using synchron CX5, 7. kidney function tests (KFT) using synchron CX5, 8. Quantitative Assessment of serum Galectin-1 (Gal-1) level using a sandwich enzyme-linked immunosorbent test with two different antibodies.

Ethical consent:

An ethical approval was obtained from the Research Ethical Committee at Ain Shams University (FWA 00017585, FMASU R 170/2022). Each person who agreed to take part in the study did so after signing an informed written consent form. Confidentiality was guaranteed on handling the data according to revised Helsinki deceleration of biomedical ethics.

Statistical analysis

After data was collected, edited, coded, and double-checked for accuracy, it was entered into IBM's Statistical Package for Social Sciences (SPSS) version 23. When the numbers were determined to be parametric, we showed them as means and standard deviations; when they were found to be non-parametric, we showed the median and the IQR. In addition, numerical and percentage presentations of qualitative characteristics were provided. Chi-square test was utilized to evaluate the similarities and differences of qualitative data between each group. Due to the parametric nature of the quantitative data, an independent t-test was utilized to draw comparisons between the two groups. With non-parametric quantitative data, the Mann-Whitney test was used to compare two groups. The Spearman correlation coefficient was used to determine the strength of the relationship between two numerical variables among the same set of people. The serum galectin-1 cut off point that most reliably distinguished the patient group from the control group was established by calculating the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and area under the curve (AUC) of a receiver operating characteristic (ROC) curve. The 95% confidence interval included a margin of error of 5%. The p-value was then interpreted using the following cutoffs: a value higher than 0.05 indicated that the result was not significant, while a value of 0.05 or less indicated that the result was significant, and a value of 0.01 or less indicated that the result was high significant.

RESULTS

In our case-control research comparing 50 RA patients against 50 healthy controls of the same age and gender. All the people diagnosed with RA were women, and their ages varied from 19 to 65 (with a mean of 46.36±11.64). Median disease duration was 24 months (range, 8-190 months), and IQR, 12 months (IQR, 24-48 months). All of the participants in the control group were women, with their ages ranging from 32 to 64 (with a mean of 40.36±9.28).

Joint swelling was found to have a wide range, from 2 to 22 joints, with a median of 9 and an IQR of 4.5 to 13 joints, while joint tenderness was found also to have a wide range, from 3 to 21 joints, with a median of 3.5 and an IQR of 1 to 7.5 joints. Rheumatoid nodules and neuropathy are examples of extra-articular symptoms that have been found in 33 cases (66%). Thirteen cases have articular abnormalities, or 34%.

Thirty-three cases (66%) were on methotrexate (MTX), 17 cases (34%) were on leflunomide (LEF), 35 cases (70%) on Hydroquine, 20 cases (40%) on Glucocorticoides (GC) less than 7.5 mg and 3 Cases (6%) on GC more than 7.5 mg (**Table 1**).

Table (1): Types of conservative medical treatment used by RA patients.

Drugs	RA Cases (No.= 50)
MTX	33 (66.0%)
LEF	17 (34.0%)
HYDROQUINE	35 (70.0%)
GC< 7.5 MG	20 (40.0%)
GC >7.5 MG	3 (6.0%)

Among the studied patients 30 cases (60%) were RF positive, 43 cases (86%) were Anti- CCP positive, The ESR varied from 12 to 85 mm/hour, with a median of 32.5 and an IQR of 15 to 70 mm/hour; the CRP ranged from 3.5 to 25 mg/L, with a median of 5.5 and an IQR of 4.5 to 10 mg/L; and the median CRP was 5.5.
The activity scores among the RA Cases were measured using DAS28-ESR and DAS28-CRP scores. According to DAS28-ESR score; the disease activity ranged from 2.6 to 7.5 with a mean of 3.11±0.61; 28 cases (56%) with low activity, 14 cases (28%) with moderate activity and 8 cases (16%) with high activity. According to DAS28-CRP score; the disease activity ranged from 2.5 to 6.8 with a mean of 3.22±0.42; 4 cases (8%) in remission, 25 cases (50%) with low activity, 13 cases (26%) with moderate activity and 8 cases (16%) with high activity (**Table 2**).

Table (2): Disease activity scores using DAS28-CRP and DAS28 ESR.

Variable		RA Cases (No.= 50)
DAS28 ESR	Range	2.63 – 7.52
	Mean ± SD	3.11 ± 0.61
	Low	28 (56.0%)
	Moderate	14 (28.0%)
	High	8 (16.0%)
DAS28 CRP	Range	2.52 – 6.84
	Mean ± SD	3.22 ± 0.42
	Remission	4 (8.0%)
	Low	25 (50.0%)
	Moderate	13 (26.0%)
	High	8 (16.0%)

The levels of serum Gal-1 in the control group were 3-14 ng/ml with a median of 7 ng/ml; in the RA group, the levels were 20-55 ng/ml with a median of 33.5 ng/ml. In contrast to the control group, the Cases group had significantly higher serum Galectin-1 levels ($p<0.001$) (**Figure 1**).

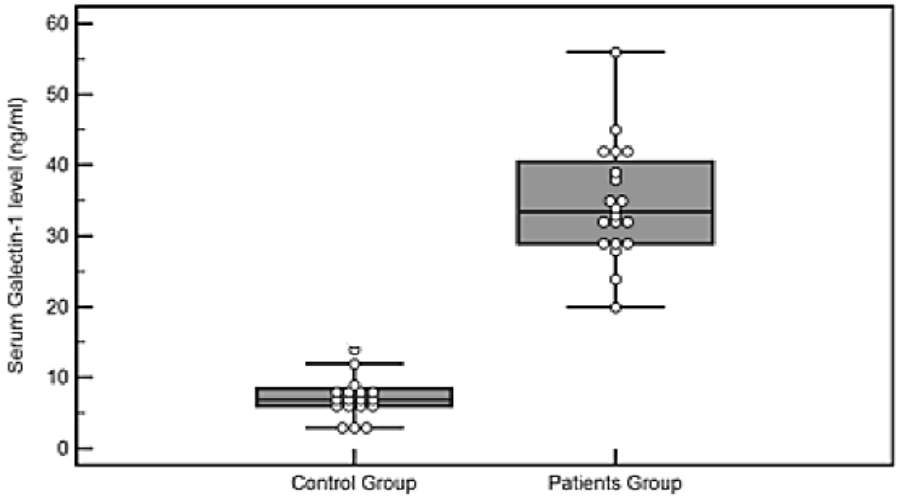

Figure (1): Comparison between control and RA groups regarding serum Galectin-1 level.

ROC curve demonstrates that the optimal cut off point between the control and the cases regarding serum galectin-1 level was discovered >15 (ng/ml) with 100% sensitivity, 95% specificity, and 99.9% AUC (**Table 3**).

Table (3): The cut-off point for serum galectin-1 level in RA cases.

Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
>15ng/mL	0.999	100.00	95.00	95.2	100

The Correlations studies

Illness activity scores; DAS28-ESR and DAS28-CRP were not correlated with the levels of serum Galectin-1 in RA patients, and neither were age, disease duration, or clinical symptoms. Neither was there a linkage between serum Gal-1 level and conventional therapy used in RA patients (**Table 4**).

Table (4): Correlation of serum Galectin-1 level and disease activity scores.

Variable		DAS28-ESR		
		Low	Moderate	High
Serum Galectin-1 level in ng/ml	Mean ± SD	32.8 ± 7.58	32.83 ± 7.78	37.22 ± 7.32
P value		P1: 0.995¥	P2: 0.364α	P3: 0.324 α
Variable		**DAS28-CRP**		
		Low	Moderate	High
rum Galectin-1 level in ng/ml	Mean ± SD	32.25 ± 7.81	33.55 ± 6.02	40.2 ± 9.59
P value		P1: 0.770¥	P2: 0.286¥	P3: 0.155¥

DISCUSSION

RA has a complex set of causes and is the most common autoimmune rheumatic illness. Damage to cartilage and bone as well as articular deformity are hallmarks of the condition, which is caused by the immune system's persistent infiltration of the synovial membrane [(11)]. We aimed in our study to evaluate the relation between the disease activity of active rheumatoid cases under conventional medical treatment, and the level of Gal-1 in serum.

In our study, serum Galectin-1 level mean levels in RA cases was 33.5 ng/ml (ranged from 20 to 55 ng/ml). In consistent with our study, **Mendez-Huergo and his colleagues** in 2019 assessed Gal-1 concentrations in RA patients and found significantly higher serum Gal-1 levels in RA cases than healthy controls [(12)]. Also, **Triguero-Martínez and his colleagues** in 2020 was agreed, RA patients reported statistically highly significant increase in the baseline serum Galectin-1 level in RA patients compared to control group. The elevated Gal-1 levels compared to healthy control samples persisted throughout the follow-up period included four visits (baseline $p=0.007$; 6 months $p<0.001$; 12 months $p=0.040$; and 24 months $p= 0.008$) [(1)]. All these studies which recorded the higher serum Galectin-1 level in RA cases indicate the pro-inflammatory role of Galectin-1 in rheumatoid arthritis pathogenesis.

In contrast to our study, **Xibillé-Friedmann and his colleagues** in 2012 reported that serum Galectin-1 levels didn't differ significantly between RA patients and controls [(8)]. In our study, there were statistically significant disparities between the case and control group in terms of age; the cases were older as their age mean was 45.1±10.3 years old versus 32.3± 8.6 years old for controls. Additionally, the cases showed high seropositivity to RF 85% and anti-CCP antibodies 68%. The mean activity score (DAS-28) of the cases was 4.9±1.2. The difference in these results suggests that the actual relationship of Galectin-1 with the inflammatory process in RA is quite complex.

The variable serum Galectin-1 level results between studies could be caused by variable disease duration, level of disease activity, sample size and study population.

In this study the cut off value of Galectin-1 level in RA was >15 ng/ml to detect RA cases (with an AUC of 99.9%, sensitivity of 100%, and specificity of 95%). This was close to that by **Triguero-Martínez and his colleagues** in 2020 who reported that with an AUC of 0.761, a Gal-1 serum concentration of 19.12 ng/ml or higher might distinguish rheumatoid from healthy persons (71%) and controls (79%) respectively [(1)]. However, **Mendez-Huergo and his colleagues** in 2019 evaluated whether or not Gal-1 serum levels can be used to distinguish between RA patients and healthy individuals. Serum Gal-1 levels exceeding 60.94 ng/ml were shown to successfully distinguish RA cases from controls (sensitivity = 80%, specificity = 73.3%) [(12)].

In our study, there was no significant relation between the different medical treatments used by RA cases in form of MTX, LEF, hydroquine, GC< 7.5 and >7.5 mg and the level of serum Gal-1 level with $p>0.05$. To our knowledge, the relation between these parameters and to serum Galectin-1 level were not explored in other studies.

Our findings showed that serum Gal-1 levels were not significantly correlated with ESR. In contrast, **Mendez-Huergo and his colleagues** have discovered a robust positive relationship between Gal-1 serum levels and ESR. They also discovered a favorable connection between Gal-1 serum levels and DAS28 ($r = 0.25$, $p = 0.029$) [(12)].

In Our study, the disease activity scores (DAS28-ESR and DAS28-CRP scores) showed no significant correlation with the serum levels of Gal-1 ($p>0.05$). In agreement with our study, **Triguero-Martínez and his colleagues** in 2020 also revealed that there was no connection between Gal-1 serum levels and disease activity in RA patients. Disease activity decreased over the course of the follow-up with treatment. However, Gal-1 serum levels stayed elevated and almost constant throughout the follow-up [(1)].

In contrast to our findings, **Vilar and his colleagues** in 2019 reported increased Gal-1 levels in patients with a moderate presentation compared to those in remission or low activity. In addition, Gal-1 serum levels were higher in cases with high activity than in those in remission or low activity [(13)]. The explanation of this variation in correlation of serum Galectin-1 with disease activity may be due to the variable disease activity of RA cases in these studies. Another explanation, that in our study we didn't track the course of the disease among the RA patients and the serum Gal-1 level could differ with disease remission.

In Our study, the duration of RA was not correlated with Gal-1 serum concentration and this was in consistent with **Mendez-Huergo and his colleagues** [(12)]. Likewise, a study conducted by **Triguero-Martínez and his colleagues** in 2020 also found no significant correlation between RA cases and controls groups regarding disease duration [(1)].

This study faced some limitations, for starters, the study began during the COVID-19 epidemic. Second, we did not do serial monitoring of serum Gal-1 to track its evolution as the disease progressed.

CONCLUSIONS

Our study demonstrated the usefulness of measuring serum Galectin-1 in patients with RA with high validity. This proves the pro-inflammatory effect of Galectin-1 in RA patients. We also concluded that serum Galectin-1 level of more than 15ng/ml was the best cut off point with sensitivity of 100%, specificity of 95% and AUC of 99.9%. There was no significant

relation noted between serum level of Galectin-1 and disease activity. In addition, there was no significant relation between the different medical treatments used by RA cases and Gal-1 level in serum. However, further studies considering serial monitor of the Gal-1 serum level through the course of the disease are highly required to obtain high level of evidence regarding routine use of serum galectin-1 in RA patients.

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