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ORIGINAL ARTICLE

Correlation of Serum Galectin-3 Level with Disease Activity in Rheumatoid Arthritis Patients

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a multifactorial autoimmune illness that causes symmetric polyarthritis and can result in deformities in addition to several extra articular manifestations. Galectin-3 is a protein involved in modulating inflammation and apoptosis. The objective of this workis to measure the serum level of Galectin-3 in RA patients and to assess its correlation with disease activity.

Methods: A case control study was carried on 66 subjects, 33 RA patients (28 females and 5 males), and 33 healthy subjects, taken as control (23 females and 10 males). Disease Activity score (DAS28) was estimated for RA patients. Blood samples from patients and controls were tested for Galectin-3.

Results: Serum Galectin-3 levels were substantially greater (P<0.001) in RA patients than in controls as it ranged between (2.3-33.6 ng/ml vs 5.21-9.67 ng/ml P<0.001). Serum Galectin-3 correlated with DAS28 (r=0.62, p=<0.00),CRP(r=0.35,p=0.04) also with ESR(r=0.36,p=0.04). Galectin-3 showed a sensitivity of 96.97% and a specificity of 84.85% at a cutoff of 6.95 ng/ml to discriminate RA from controls.

Conclusions: Serum Galectin-3 levels were higher in RA patients than controlsand correlated with disease activity So, Galectin-3 can be used as a biomarker for RA disease activity.

Keywords: Rheumatoid arthritis, Disease activity, serum Galectin-3.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder that affects the synovial joints leading to destruction. The defining characteristic is persistent symmetric chronic synovitis that leads to deformities, functional disabilities in addition to several extra articular manifestations [1].

RA is considered a multifactorial disease. The precise pathogenesis of RAremains unclear but some studies attribute the cause of disease to environmental, genetic and hormonal factors or even combination of them [2]. It affects females more than males and peaks in prevalence in the fourth and fifth decades [3].

The pathogenesis of RA has been linked to numerous cells, proteins, and pathways; among these, galectins are proteins that play an important role in inflammation and immune activation [4].

Galectins are lectin family members. They are highly expressed in the immune system [5]. Evidences have been accumulating that many family members of galectins have significant roles in development of RA through their actions on cells of the myeloid lineage, B and T lymphocytes [6].
Galectin-3 is the only chimera type of the galectin family, it has a C-terminal carbohydrate recognition domain (CRD) connected to an extra N-terminal domain that is rich in glycine, proline and tyrosine [7]. Galectin-3 promotes proliferation, transformation of fibroblast and stimulates collagen production [8]. Through these effects it contributes significantly to cell differentiation, proliferation, adhesion, new blood vessels formation, and cell death [9]. According to studies, Galectin-3 activates

numerous cytokines and chemokines,so it plays a significant role in RA pathogenesis [10].
The aim of this study was to measure the serum level of Galectin-3 in RA patients and to detect its correlation with disease activity.

METHODS

Ethical consideration:

This case control study was done at Department of Rheumatology and Rehabilitation after gaining approval from the Institutional Review Board (IRB) (No.: 9671). Analysis of the samples was completed by the Clinical Pathology Department. This research was done between November 2022 and June 2023. Each participant gave an informed consent, indicating their agreement.

Subjects selection:

The sample size was calculated using open epi at 80% power and 95% CI, assuming the mean Galectin-3 were 14.1±9.6 and 9.2±3.7 in patients and controls respectively. The estimated sample size was 66 subjects(33 in each group). This case-control study was conducted on 66 subjects divided into two groups: Group I included 33 RA patients diagnosed according to the 2010 American College of Rheumatology/European League against Rheumatism classification criteria for RA [11] with age of onset more than 18 years, group II included 33 apparently healthy volunteers of age and sex matched with patients served as controls.

Exclusion criteria;

Subjects with any other autoimmune rheumatologic, cardiovascular, liver, or renal diseases were excluded. Patients who had any type of malignancies were also excluded.

Patients with RA underwent a detailed history taking, clinical examination, and DAS28-ESR was used to determine disease activity status [12]. The laboratory investigations included ESR, CRP, complete blood count (CBC), Rheumatoid factor (RF) and Anti-cyclic citrullinated peptide (Anti - CCP2).

6 ml whole blood specimens were collected from blood by venipuncture in plain, ESR, and EDTA tubes (Becton Dickinson Company, NJ, and USA). Sample in plain tubes was left to coagulate for 10-20 minutes at room temperature, centrifugation at the speed of 1200 x g, and supernatant serum was separated in 2 aliquots one utilized for routine tests and the other stored at -20° C until estimation of Galectin-3 by enzyme-linked immunosorbent assay (ELISA) analysis.

The CBC was performed using a Sysmex XN 2000 Hematology analyzer (Sysmex, Kobe, Japan). The ESR was assessed using a Vision-B analyzer (YHLO Biotech Co., Ltd., Shenzhen, China). CRP and RF were assessed using the Cobas 6000-c501 Modular Analyzer (Roche, Germany). Anti-CCP2 antibodies were detected using the Elecsys anti-CCP kit on Cobas E411 (Roche, Germany).

Serum Galectin-3 level:

Human ELISA Kit was used to measure its level (Catalogue number: 201-12-1952), according to the instructions of the manufacturer (SunRed, Shanghai, China). After applying the stop solution, the optical density (OD) was measured at 450 nm after 15 minutes. The OD was calculated using a sunrise absorbance reader (Tecan Trading AG, Männedorf, Switzerland). The standard curve was developed based on the standards' concentration and the related OD values. To determine the relevant sample's concentration, the OD values of the sample were applied to the curve. The Galectin-3 results were presented in ng/mL units.

Statistical analysis

The data were collected then analyzed using IBM_SPSS_23.0 software (SPSS Inc., Chicago, IL, USA) for data analysis & presentation. Qualitative data: is represented in the form of number and percentages (N. %) while quantitative data after testing of normality using Shapiro-Wilk test: the data that was normally distributed presented as mean± standard deviation and skewed data as median and range.

Infererntial statistics:

At the level of significance value where P-value> 0.05 (considered as non-significant), while P-value ≤ 0.05 (considered significant). Qualitative data: we used Chi-square (X_2) test while Quantitative data that was normally distributed: t-test & ANOVA test were used. Skewed data: Mann-Whitney test & Kruskal-Wallis test were utilized.

Pearson and Spearman's rank tests for correlation were utilized to evaluate correlations between two continuous variables followed by multiple linear regression analysis.Receiver operator curve (ROC) analysis was applied to detect optimal cut-off point of Galectin-3 to discriminate between healthy and diseased individuals.

RESULTS

All together 66 subjects were recruited anddivided in 2 groups. The first group included 33 patients suffering from Rheumatoid arthritis, 28of them were females (84.8%) and 5 were males (15.2%), with range of age from 28 to 50 years with a mean±SD of 43.1 years±7.1. Duration of the disease ranged from 1 to 20 years with mean ± SD of 7.79 years ±5.21. The second group included 33 apparently healthy volunteers served as controls, 23 (69.7%) of them were females and 10 (30.3%) were males. Their ages ranged from 25 to 50 years with a mean±SD of 38.9 years±7.6.

Upon clinical assessment of our RA patients various clinical findings were detected, we illustrated them in (Table 1). The most frequently encountered extra-articular manifestation in the studied RA patients was dry eye (30.3%) followed by dry mouth (27.3%) and subcutaneous nodules (27.3%). (45.5%) of our RA patients had a high disease activity followed by moderate activity (36.4%).

Also ESR and CRP levels were higher among RA patients compared tohealthy subjects and this difference was highly statistically significant (p<0.001). As regard serum Galectin-3 levels difference between the tested groups wedetected a highly significant difference between the 2 groups (P<0.001) (Table 2).

We didn't find statistically significant relation between Galectin-3 and clinical data among RA patients (P>0.05) except for dry eye where (P<0.05) (Table 3).

Our results showed that serum Galectin-3 levels correlated positively with acute phase reactants(ESR, CRP),also there was statistically positive correlation with pain (VAS) in addition to the clinical parameters of the patients such as morning stiffness (MS), NTJ, NSJ, and DAS28 score all correlated significantly with Galectin-3 levels (P<0.05) (Table 4).

To identify factors that independently affect Galectin-3 levelsmultiple linear regression analysis was done using age, sex, disease duration, ESR, CRP and DAS28 as independent variables and Galectin-3 as dependent variable. Galectin-3 levels were positively associated with DAS28 (($\boldsymbol{\beta}$=3.12, P< 0.001) (Table 5).

To test robustness of our results a ROC curve analysis (Receiver operation Curve) was conducted to detect the optimal cutoff value of Galectin-3 level that is able to discriminate RA patients from healthy subjects. At cut-off point (6.95 ng/ml) AUC was (89%) with sensitivity (96.97%) and specificity (84.85%)(Figure1).

Also to detect the ability of Galectin-3 to detect activity among RA patients ROC curve analysis showed AUC (82%), sensitivity (82.76%) and specificity (75%)(Figure 1).

TABLES

Table 1: Epidemiological characteristics, Clinical data and laboratory findings in RA patients.

	RA group (n=33)
Smokers *n.%*	5 (15.2%)
MS (min) *median (range)*	30 (0-120)
Pain VAS *median (range)*	6 (0-10)
NTJ *median (range)*	10 (0-28)
NSJ *median (range)*	2 (0-10)
Deformity *n.%*	9 (27.3%)
Subcutaneous nodules *n.%*	9 (27.3%)

Dry eye *n.%*	10(30.3%)
Dry mouth *n.%*	9 (27.3%)
Fever *n.%*	5 (15.2%)
DAS28 *mean ± SD*	4.95±1.56
TLC *mean ± SD* **Hb** *mean ± SD* **Platelets** *mean ± SD*	7.97±2.74 12.23±1.06 307.67±82
RF *median (range)* positive **Anti-CCP** *median (range)* positive	60 (18.2-512) 30 (90.9%) 108 (6-493) 23 (69.7%)

*: significant as P value ≤0.05

RA; rheumatoid arthritis**, MS;** Morning stiffness**, VAS;** visual analogue scale, **NTJ;** number of tender joint, **NSJ:** number of swollen joint, **TLC:** total leucocytic count, **Hb:** hemoglobin, **RF:** rheumatoid factor, **Anti-CCP**; Anti-cyclic citrullinated peptide, **DAS28;**disease activity score 28.

Table 2: Serum Galectin-3 level and acute phase reactants among studied groups;

variable	**RA group (n=33)**	**Control (n=33)**	***P-value***
ESR (mm/hr) *median (range)*	16 (4.5-62)	7.2(5.2-9.67)	**<0.001** *
CRP (mg/l) *median (range)*	5 (1.29-46)	1.1 (0.89-7.2)	**<0.001** *
Galectin-3 level (ng/ml) *median (range)*	10.5 (2.3-33.6)	7.25 (5.21-9.67)	**<0.001** *

*: significant as P value ≤0.05

ESR: erythrocyte sedimentation rate **, CRP;** C-reactive protein.

Table 3: Relation between Galectin-3 levels and clinical data among RA group.

Variable	**Clinical presentation (N=33)**		**P-value**
	Absent	**Present**	
Smoking *median(range)*	N=28 10.3 (2.3-33.6)	N=5 15.2 (9.4-30.2)	0.17
Deformity *median(range)*	N=24 10.5 (6.95-33.6)	N=9 10.5 (2.3-31.2)	0.71
Subcutaneous nodules *median(range)*	N=24 10.52 (2.3-33.6)	N=9 9.62 (8.6-31.2)	0.56
Dry eye *median(range)*	N=23 9.4 (2.3-33.6)	N=10 16.9 (8.7-31.2)	**0.03** *
Dry mouth *median(range)*	N=24 10.5 (2.3-33.6)	N=9 12.6 (8.6-31.2)	0.35
Fever *median(range)*	N=28 10.52 (2.3-33.6)	N=5 9.44 (7.8-30.2)	0.64
Sex *median(range)*	**Female** N=28 10.3 (2.3-33.6)	**Male** N=5 15.2(9.4-30.2)	0.29

*: significant as P value ≤0.05

Table 4: Correlation between Galectin-3 level, serological and clinical parameters of RA patients.

Variable	Galectin-3 (n=33)	
	r	P
Age	0.14	0.45
Duration	0.07	0.67
Pain VAS	0.54	**0.001** *
MS	0.35	**0.04** *
NTJ	0.53	**0.004** *
NSJ	0.68	**0.001** *
DAS28	0.62	**<0.001** *
ESR	0.36	**0.04** *
CRP	0.35	**0.04** *
RF	-0.08	0.68
Anti-CCP	0.31	0.15

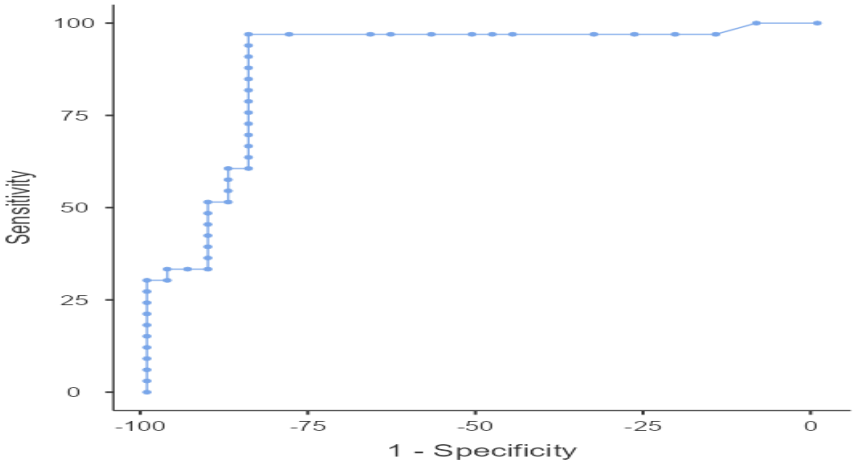
(r): correlation coefficient*****: significant as P value ≤0.05

Table 5: The multiple linear regression analysis for serum Galectin-3 and some independent variables in RA patients.

Model Fit Measures	R=0.715	R^2=0.512	
Model Coefficients	**Estimate**	**t**	**p-value**
Intercept	7.7075	0.636	0.530
Age	-0.2003	-1.337	0.193
Duration	0.2738	1.194	0.243
ESR	0.0747	1.030	0.313
CRP	-0.0384	-0.270	0.790
DAS28	3.1087	3.819	< 0.001 *

*: significant as P value ≤0.05

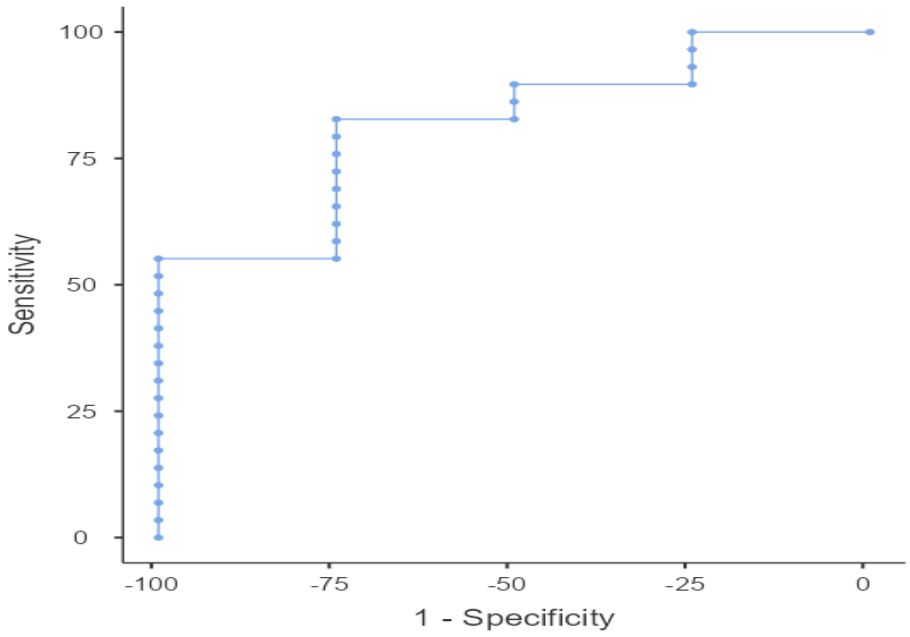
Figure 1: ROC curve analysis of Galectin-3 level in



RA group

a. Discriminate RA patients from control

Cut-off	Sensitivity	Specificity	AUC
6.95	96.97%	84.85%	89%



a. Detect disease activity

Cut-off	Sensitivity	Specificity	AUC
8.79	82.76%	75%	82%

AUC: area under the curve, **ROC;** Receiver operation Curve

DISCUSSION

RA is an autoimmune condition marked by the production of autoantibodies, chronic synovial membrane inflammation, cartilage erosion, and progressive joint degeneration [13]. There are many theories for explaining the pathophysiology of RA, but till now there has been no obvious cause [14]**.** Through its impact on different cells of the immune system, Galectin-3 plays a significant role in the development of RA [8].

In this study,we noticed a higher level of Galectin-3 in RA patients than controls, as the median was (10.5ng/ml vs 7.25 ng/ml) with P-value <0.001. In agreement with our study, **Baki et al.,** [15] found a higher level of Galectin-3 in RA patients**.**

Also,thediagnostic power of Galectin-3 in autoimmune diseases was tested by **Gruszewska et al.** [16] and they discovered that, compared to controls, individuals with RA, SLE, and SSc had considerably higher serum levels of Galectin-3. **Kaur et al.** [17] also reported the same findings.

Issa et al. [18] conducted a study on newly diagnosed RA patients who are not on treatment and found that Serum Galectin-3 levels were higher in patients than in controls.

In a study by **Nielsen et al.** [19]**,** they studied both early and chronic RA patients and found that both groups had persistently higher plasma level of Galectin-3 compared to the control. Moreover, they followed up their patients for two years and found that the initial low level of Galectin-3 will predict a low disease activity status.

Also,**Ohshima et al.** [20] found that serum and synovial Galectin-3 levels were increased in RA

patients compared to healthy controls and osteoarthritis patients.

But in contrast to our work, **Mendez-Huergo et al.** [21]detected lower levels of Galectin-3 in RA patients than controls. Despite these negative results, they found that Galectin-3 levels tended to be higher in RA patients with higher disease activity. They explained the contradiction in their results most likely as a result of immunosuppressive treatment as they included only patients under treatment and supported their assumption by the fact that glucocorticoid treatment inhibits lipopolysaccharides, thereby decreasing the level of Galectin-3 in T-helper 1 cells [22].

On the contrary, **Hu et al.** [24] found no difference between patients and controls regarding Galectin-3 levels. This might be due to different disease status and different methodologies used.

Regarding the relation of Galectin-3 to clinical and lab parameters, this study showed that Galectin-3 was correlated with MS duration, VAS, NTJ, NSJ, ESR, CRP, DAS28 and dry eye but not correlated with RF or Anti-CCP **.** These findings agreed with **Baki et al.** [15] who found that serum Galectin-3 was significantly correlated with age, NTJ, NSJ, DAS28, ESR, and fever but not correlated with the RF, Anti-CCP, or erosions and they stated that it can be used as promising marker correlated with disease activity.

However **Issa et al.** [18] found a positiveassociation with Anti-CCP level, CRP and DAS-ESR, but not with NSJ and NTJ. It is also correlated with bone marrow edema, synovitis, and erosions detected by MRI.

Mendez-Huergo et al. [21], found that Serum Galectin-3 levels were strongly correlated with HAQ and age of patients, but there was no significant correlation with ESR, VAS, or DAS-ESR**.** Also**Kaur et al.** [17] reported no correlation with CRP levels in RA patients.

Gruszewska et al. [16] did not report a significant correlation between Galectin-3 concentration and CRP values, also there was no correlation with DAS28. But, they noted a positive correlation with ESR also with age of RA patients.

In the current study Galectin-3 exhibited a very good diagnostic capacity for RA(AUC above 0.89) with sensitivity (96.97%) and specificity (84.8%), according to ROC curve analysis. Similarly **Baki et al.** [15] discovered that Galectin-3 was an effective diagnostic marker for RA**.** Also **Gruszewska et al.** [16] stated that ithad a great diagnostic power for RA (AUC over 0.9)**.**

In the present work, our ROC curve analysis detected a cutoff value of 8.79 ng/mL, so this value will be able to estimate the status of disease activity in RA. As far as our knowledge is concerned, this is the first study that sheds light on the disease activity cutoff value for Galectin-3 in RA patients.

CONCLUSIONS

Galectin-3 can be employed as a biomarker for the activity of RA since serum levels were significantly greater in RA patients than controls and correlated with disease activity.

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