Galectin-3 As A Potential Serological Marker In Iraqi Rheumatoid Arthritis Patients

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Abstract

Rheumatoid arthritis (RA) is a systemic autoimmune disease with an unknown etiology that affects 0.5–1% of people worldwide. It is characterized by hyperplasia, an overgrowth of synoviocytes, and chronic inflammation of the synovial joints. During the inflammatory phase of arthritis, many pro-inflammatory cytokines and mediators are secreted by infiltrating immune and resident joint cells, which are responsible for cartilage degradation and excessive bone remodelling. Amongst them, a β-galactoside-binding lectin, galectin-3 (Gal-3), has been reported to be highly expressed and secreted by inflamed synovium of rheumatoid arthritis. The study findings show that serum gal-3 level in patients has a high discriminating power in the diagnosis of RA patients with high sensitivity (86.70) and specificity (85.30). In addition, it has a significant correlation with Anti CCP and PLT with P values 0.017 and <0.001 respectively. However, there is no correlation with the DAS28 (CRP) level with a P value of 0.06. In conclusion, Gal-3 serum levels have a potential role as diagnostic markers in patients with RA.

Objective: The present study aims to assess the level of serum galectin-3 in rheumatoid arthritis (RA) patients as a potential serologic marker and to study their association with some disease parameters (DAS28, Anti CCP, PLT) in Aldewaniya Province.

Keywords: Galectin-3 (Gal-3), Rheumatoid Arthritis (RA), Disease Activity Score (DAS28), and Platelets (PLT).

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by a dysfunctional immune system that mostly affects several peripheral joints (1). This autoimmune condition affects 1% of the population, with women twice as likely to be affected as males (2).

The autoantigen-modified ACPA antibody is produced by deaminating arginine to citrulline, a process known as citrullination. About two-thirds of RA patients have ACPA, whereas fewer than 2% of healthy people have it (3). Most frequently, anti-citrullinated peptide/protein antibodies (ACPA, typically tested by anti-CCP ELISA) are found (4) and due to high predictive score is a part of the classification criteria for RA (5).

The most used assays for determining acute phase response are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) because of their affordability, cost-effectiveness, and consistency (6).

For platelets in RA, the inflammatory environment of rheumatoid plasma offers enough agonistic stimulation. There is a large increase in both their quantity and level of activity in the disease. A significant percentage of RA patients had thrombocytosis, which was primarily related to their worse survival and higher turnover rates (7).

The unique characteristic of the ancient lectin family called galectins is their capacity to bind β-galactosides via the evolutionarily conserved sequence elements (CRD) of the carbohydrate-recognition domain.) (8). The only chimeric variety in the galectin family is called galectin 3 (gal-3). It was once known as Mac-2 in macrophages, but its current nomenclature is more appropriate. It was shown to have a broad distribution in several tissues, including the kidneys, brain, digestive tract, and bones (9).

Numerous intracellular processes, such as cytokine release, RNA splicing, differentiation, apoptosis, and protein trafficking, are mediated by Gal-3. It travels back and forth between the cytoplasm and nucleus to do this. For instance, intracellular gal-3 can stop T lymphocytes, macrophages, and breast cancer cells from undergoing apoptosis (10, 11).

Several inflammatory cells that express galectins include stromal cells, innate and adaptive immune cells, endothelial cells, and synovial fibroblasts. Several cellular pathways that control the escalation and subsidence of inflammatory responses are impacted by certain glycan-binding proteins (12).

Gal-3 mainly serves as a pro-inflammatory molecule in autoimmune and chronic inflammatory diseases including rheumatoid arthritis (13, 14).

Materials and methods :

A total of 5 ml of blood was collected and divided into two tubes. 2ml was collected in an EDTA-k2 blood collection tube for hematologic assessment, while 3ml was collected in a gel tube for immunological and serological examination. The serum was separated and divided into two sections; one for CRP measurement and the other for Gal-3 and anti-CCP evaluation. The second part of the serum was labelled and stored at -20°C in a deep freeze until all specimens were obtained.

This study involved a total of 150 individuals, out of which 75 were identified as established RA patients based on the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) diagnostic criteria for rheumatoid arthritis (15). The patient group received medical care at Al-Dewaniya Teaching Hospital's rheumatology outpatient clinic. The results of this group were then compared to those of a healthy control group that was matched for age and other relevant factors.

Gal-3 and Anti-CCP serum levels were measured using enzyme-linked immunosorbent assay (ELISA) kits from Sunlong and Wuhan Feiyue Inc. China. C-reactive protein (CRP) was also measured by fluorescence immunoassay (FIA) following the manufacturer's instructions from Boditech Med Inc. Republic of Korea. The immunological determinations were performed in our college laboratory, while blood routine tests (CBC) were evaluated using the Beckman automatic blood instrument from Beckman Inc. Germany.

Results :In this study, a group of 75 patients with rheumatoid arthritis (53 females (71%) and 22 males (29%)) were compared to a group of 75 healthy individuals (53 females and 22 males). None of the controls or their first-degree relatives had a history of rheumatoid arthritis or any other autoimmune diseases.

Table 1 indicates no significant difference in gal-3 levels between patients and controls, on a sex basis, among study groups.

Table 1. Serum concentration of gal-3 according to gender among study groups

	Group	Sex	N	Mean	Std. Deviation	P value
Gal-3	Patient	Male	22	4.2245	0.40937	0.929*
		Female	53	4.2379	0.65387	0.915
	Control	Male	22	3.6200	0.61524	0.410
		Female	53	3.5223	0.38868	0.496

*Significant at P value < 0.05

Patients in the study group had higher Gal-3 levels compared to healthy controls, with mean values of 4.23±0.59 and 3.55±0.46, respectively (P = < 0.001), in addition to other study characteristics presented in Table 2.

Table 2. Serum concentration of gal-3 and other study characteristics

	Group	N	Mean	Std. Deviation	P value
Gal-3	Patient	75	4.2340	.58994	< 0.001
	Control	75	3.5509	.46431	
Anti-CCP	Patient	75	68.1177	41.56968	< 0.001
	Control	75	7.5389	3.65637	
DAS28(CRP)	Patient	75	47.2748	57.69686	< 0.001
	Control	75	3.7487	3.12698	
PLT	Patient	75	326.4267	89.04071	< 0.001
	Control	75	262.6133	62.09950	

*Significant at P value < 0.05

About other patient characteristics, gal-3 has a significant correlation with Anti CCP and PLT (with P values of 0.01 and <0.001, respectively). However, there is no correlation with DAS28 (with a P value of 0.06). It is worth noting that in the control group, gal-3 does not show any significant correlation with any characteristic (table 3).

Table 3: The correlation between gal-3 and other study characteristics for study groups

		Anti CCP	DAS28	PLT
Gal-3 Patient	R	0.275	0.216	0.416
	P value	0.017*	0.062	<0.001**
	N	75	75	75
Gal-3 Control	**R**	0.066	0.150	0.129
	P value	0.576	0.200	0.272
	N	75	75	75

*Sig at P value < 0.05. No Sig at P > 0.05. **Highly Sig at P <0.001

According to this study, Gal-3 can diagnose RA patients with good sensitivity and specificity with cutoff 3.80 ng/ml, sensitivity 86.70, specificity 85.30, confidence interval 0.799 – 0.928, and Area Under the Curve AUC 0.864 this can be found in the Figure (1) and table (4).

Table 4: ROC values of study groups and Gal-3.

GALECTIN3	Value
CUT OFF	3.80 ng/ml
Sensitivity	86.70
Specificity	85.30
Confidence interval	0.799 - 0.928
AUC*	0.864

*AUC (Area under the curve)

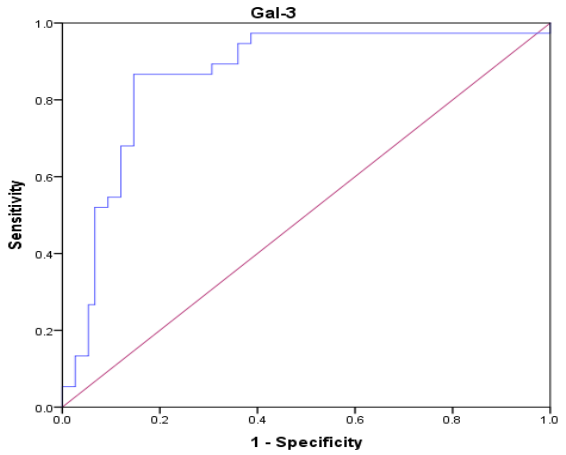

Figure 1. Finding the best gal-3 cutoff value to diagnose and distinguish RA from other inflammatory arthritis using Receiver-Operator Characteristic (ROC) Curve Analysis

Discussion:

The study found a significant correlation between serum Gal-3 and anti-CCP), which is a conventional risk aspect for rheumatoid arthritis (RA) disease. Moreover, the study found a notable correlation between Gal-3 and PLT. However, the inflammatory marker (CRP) did not show any correlation. Gal-3 did not show any significant differences between males and females in any study group, which is in line with previous research (16).

Previous studies have shown that patients with chronic RA have higher blood levels of Gal-3 (17)

and in early, undifferentiated arthritis (18), This study demonstrates that Gal-3 tends to be higher, which is consistent with previous studies.

Numerous studies have revealed higher concentrations of Gal-3 in RA patients' blood and synovial fluid as compared to healthy controls, indicating that this protein may serve as a biomarker for the severity and activity of the disease. (19-21)

These studies looked at different RA factors and the connection between Gal-3 and anti-CCP. Increased Gal-3 levels have been linked to anti-CCP antibodies and other factors, suggesting that these biomarkers may interact with the pathophysiology of RA, as mentioned in (22).

This study confirmed the favorable association between anti-CCP and Gal-3 levels. In contrast to previous research, this study found no correlation between Gal-3 and DAS28 (23).

Gruszewska et al (Gruszewska et al., 2020) found that there was no significant correlation between galectin-3 concentrations and DAS28. This suggests that the existence of substantial metabolic, hormonal, and/or inflammatory problems interferes with the relationship between DAS28 and circulating Gal-3.

Serum Gal-3 concentration and platelet count show a robust positive connection (24, 25)

The current study indicates that Gal-3 plays a role in the RA disease pathway, specifically in joint destructive processes, as it is linked to well-established risk factors for erosive progression represented by Anti-CCP and thrombosis represented by PLT but not by an inflammatory marker like DAS28.

The current findings demonstrated the high diagnostic sensitivity and specificity of serum Gal-3 for RA. Similarly, Gal-3 was reported to be a valuable diagnostic marker in RA by Baki et al and Gruszewska et al (26, 27) due to its excellent diagnostic specificity, sensitivity, and discriminating capacity. Research has shown that serum galectin-3 is a useful metric for differentiating between individuals with RA and those in good health.

Conclusions:

In patients with rheumatoid arthritis, the blood levels of galectin-3 had a positive association with anti-CCP and PLT levels, indicating that these proteins may function as pro-inflammatory agents in this condition.

Serum level Gal-3 is a useful indicator for discriminating RA patients from healthy people.

Anti-CCP and PLT significantly correlate with the serum gal-3 level in patients. But there is no relationship between it and the DAS28.

Due to high sensitivity and specificity, Gal-3 may act as a potential diagnostic markers in patients with RA.

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الجيلاكتين-٣ كعلامة سيرولوجية محتملة في مرضى الروماتويد العراقيين

الخلاصة: التهاب المفاصل الرثوي هو مرض مناعي ذاتي جهازي مجهول السبب يصيب ٠,٥-١٪ من الناس في جميع أنحاء العالم. يتميز بفرط تنسج الخلايا الزليلية، ونمو مفرط للخلايا الزليلية، والتهاب مزمن في المفاصل الزليلية. أثناء المرحلة الالتهابية من التهاب المفاصل، يتم إفراز العديد من السيتوكينات والوسطاء المؤيدين للالتهابات عن طريق الخلايا المناعية المتسللة والخلايا المقيمة في المفاصل، والتي تكون مسؤولة عن تهتك الغضاريف وإعادة تشكيل العظام المفرطة. من بينها، تم الإبلاغ عن أن الليكتين المرتبط بـ β-galactoside، الجالكتين ٣ (Gal-3)، يتم التعبير عنه وإفرازه بشكل كبير بواسطة الغشاء الزليلي لالتهاب المفاصل الرثوي.

النتائج:تشير نتائج الدراسة إلى أن مستوى الجالكتين ٣ في المصل لدى المرضى يتمتع بقوة تمييز عالية في تشخيص مرضى التهاب المفاصل الروماتويدي بحساسية عالية (٨٦,٧٠) وخصوصية (٨٥,٣٠). بالإضافة إلى ذلك، فإن له ارتباطًا مهمًا مع مضاد ببتيد السترولنيت الحلقي والصفيحات الدموية بقيمة P 0.017 و<٠,٠٠١ على التوالي. ومع ذلك، لا يوجد ارتباط مع مستوى درجة فعالية المرض (البروتين المتحفز C) بقيمة P 0.06 وفي الختام، فإن مستويات مصل الجالكتين ٣ لها دور محتمل كعلامات تشخيصية في المرضى المصابين بالتهاب المفاصل الرثوي.

الهدف: تهدف الدراسة الحالية إلى تقييم مستوى مصل الجالكتين ٣ في مرضى التهاب المفاصل الرثوي كعلامة مصلية محتملة ودراسة ارتباطهم ببعض معايير المرض (مضاد ببتيد السترولنيت الحلقي ودرجة فعالية المرض والصفيحات الدموية) في محافظة الديوانية.