Original Article

Galectin-3 and Its Binding Protein, Interleukin-6, and C-Reactive Protein in the Serum of Patients with Rheumatoid Arthritis in Iraq

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Abstract

Background: Rheumatoid arthritis is an autoimmune, systemic, chronic, and inflammatory disorder of obscure source that is differentiated by symmetric, polyarticular pain and tumefaction, stiffness in the first hours of the morning, and lassitude. **Objectives:** The aim of this study is to measure the galectin-3 (GAL-3), GAL-3 binding protein (GAL-3BP), and interleukin-6 (IL-6) levels in the serum of Iraqi patients who diagnosed with RA. **Materials and Methods:** The case-control group was made up of 45 additional, presumably healthy persons, whereas the ill group was made up of 45 rheumatic patients. A study was carried out in the Merjan Teaching Hospital in Hilla City and private clinics between February 2022 and January 2023. The serum concentrations of GAL-3, GAL-3BP, and IL-6 were measured using an enzyme-linked immunosorbent assay (ELISA), while C-reactive protein was measured using turbidimetry assay. SPSS software (version 26) was used to conduct the statistical analysis. **Results:** The levels of GAL-3, GAL-3BP, and IL-6 were increased in patients than the control group ($P < 0.001$). Furthermore, CRP is also elevated. The results also revealed that that there is no link between GAL-3 and GAL-3BP, CRP, and IL-6 concentrations in the control group.. **Conclusion:** Increased levels of GAL-3 and its binding protein with IL-6 have a role in the pathogenicity of RA.

Keywords: C-reactive protein, galectin-3, galectin-3 binding protein, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is an autoimmune, systemic, chronic, and inflammatory disorder of obscure source that is differentiated by symmetric, polyarticular pain and tumefaction, stiffness in the first hours of the morning, and lassitude. The course of RA is unclear; there are often periods in which it gets worse and, less commonly, sometimes occur apparent remissions.[1] The effects extend from seldom seen alleviating malady to severe malady that produces disability of movement and death prematurely for some patients.[2] It is the most widespread manifestation of chronic inflammatory arthritis and often results in damaged joints and physical incompetence. Because it is a systemic illness, RA may result in various extra-articular manifestations, including fatigue, skin nodules, pericarditis, lung involvement, peripheral neuropathy, vasculitis, and hematologic abnormalities.[3] Joint destruction progresses rapidly after onset, resulting in irreversible physical dysfunction and deformation of the affected joints. Thus, proper diagnosis and treatment are required in the early stages of the disease.[1,4]

"Risk factors for developing RA can be generically divided into host- and environment-related. Host factors that have been correlated with RA expansion may be classified additionally into genetic, epigenetic, also comorbid host

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factors reproductive and hormonal, and neuroendocrine." In turn, risk factors of the environment comprise smoking and other air-borne exposures, microbiota and infectious agents, food, and socioeconomic factors.[5]

C-reactive protein (CRP) is an acute-phase protein that pro-inflammatory cytokines during inflammatory/infectious processes induce its production and synthesis.[6] CRP plays an essential role in host defense mechanisms contra infectious agents and in the inflammatory response.[7] CRP is the prime inflammation biomarker utilized in modern healthcare. Abnormal CRP and/or Erythrocyte Sedimentation Rate (ESR)" is considered a distinct item along with joint involvement, the presence of autoantibodies, and the duration of symptoms in the most recent set of diagnostic criteria for RA. CRP values greater than 10 mg/L are often observed in untreated patients with recent on-set RA.[8]

Interleukin-6 (IL-6) is a cytokine pleiotropic function that intercedes many biological functions, including immune system regulation, regenerative processes, metabolism, bone homeostasis, and protection of neural and cardiovascular function. IL-6 plays a significant role in the innate and adaptive immune system development and activation.[9] Following the production of IL-6 in a localized lesion during the initial stage of inflammation, the bloodstream carries it to the liver, where it quickly induces the production of a broad range of acute-phase proteins, including serum amyloid A, fibrinogen, CRP, alpha 1-antichymotrypsin, and haptoglobin.[10] In RA, IL-6 signaling arouses a cascade of local and systemic events of inflammation, including inflammation of joints, activation of osteoclast cells and bone resorption, and chronic synovitis. One of the mechanisms entangled in joint devastation is the IL-6-mediated stimulation of endothelial cells to IL-8 and monocyte chemoattractant protein-1 production, which in turn triggers the adhesion molecules expression and leads to accumulation of leukocyte in affected joints.[11]

Autoantibody production is promoted by IL-6 and causes an imponderable between 2 types of T cells: type 17 T helper (T17H) and T regulatory cells. While T17H is embroiled in the RA pathophysiology and other inflammatory diseases through inflammatory cytokines production, for example, tumor necrosis factor, IL-17, IL-21, IL-22, and IL-26, the role of T regulatory cells is to inhibit activation of T-cells and suppress the production of pro-inflammatory cytokine.[12]

Galectin-3 (GAL-3) is a binding protein of the ß-galactoside family, which adjusts cell–cell and cell–extracellular matrix communications, affecting cell propagation, immigration, differentiation, adherence, and programmed cell death. GAL-3 is synthesized by macrophages, monocytes, dendritic cells, eosinophils, mast cells, natural killer cells, and activated T and B cells.[13] The observation that GAL-3 is raised in RA patients' synovial fluid—significantly greater than in individuals with osteoarthritis—provides more support for GAL-3's pro-inflammatory involvement in RA.[14] In RA, additionally, GAL-3 levels are elevated in both synovial fluid and peripheral blood compartments, in correlation with CRP.[15]

GAL-3 binding protein (GAL-3BP) is a ubiquitous multifunctional secretory glycoprotein, which was, at first, recognized as having innate immune function in humans following viral and bacterial infections.[16] There are several targets for GAL-3BP, such as GAL-1, GAL-3, GAL-7, and GAL-9. And GAL-3BP interacts with proteins of extracellular matrix and receptors on the cell surface, such as b1-integrins, calcineurin, and nuclear factor of activated T cells 1, thus regulating cell–cell and cell–matrix interactions.[17] GAL-3BP has binding sites not only for GAL-3, but also for type V and VI collagens, integrins, and fibronectin. Therefore, a cell membrane–bound GAL-3BP could be participatory both in liaison to the extracellular matrix and in the devastating process in RA.[17]

Materials and Methods

A total of 45 patients with RA aged between 35 and 55 years old participated in this study. Their mean age [mean ± standard deviation (SD)] was 46.15 ± 5.216 years.

The control group is apparently healthy. The age of this group was ranged between 35 and 50 years, with a mean ± SD of 45.08 ± 5.749 years.

Persons who smoke, have chronic illness such as diabetes or high blood pressure, or who use illicit drugs were excluded from both groups.

Measurement of GAL-3, GAL-3BP, and IL-6 was done by enzyme-linked immunosorbent assay technique (bioassay lab kit), while CRP measurement was done by immunoturbidimetric method.

Inclusion and exclusion criteria

Inclusion criteria include patients who have RA.

Statistical analysis

The results of phenotypes data were expressed as mean ± SD. Student *t* test and the linear regression analysis were used for the evaluation of data. The output data are expressed as odd ratio, 95% confidence interval (CI), and *P* value. Statistical analyses were performed with SPSS (version 26, IBM statistics). A P value < 0.05 was considered to be statistically significant.

Ethical approval

The scientific committee of Babylon Medical College's Biochemistry Department granted ethical permission for this study. The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptance. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number 5 on November 23, 2021 to get this approval.

Table 1: Biochemical characteristics of the control and rheumatoid populations

Variables	Group	No.	Mean ± SD	95% Confidence interval for mean		Significant value
				Min	Max	
GAL-3 (pg/mL)	Patients	45	357.9 ± 78.23	196.25	537	<0.001
	Control	45	275.5 ± 41.72	203	357.5	
GAL-3BP (ng/mL)	Patients	45	33.52 ± 8.21	20.63	52.0	<0.001
	Control	45	19.57 ± 5.68	14.3	44.0	
CRP (mg/mL)	Patients	45	26.95 ± 8.32	15.88	46.0	<0.001
	Control	45	3.6 ± 1.18	0.97	5.82	
IL-6	Patients	45	84 ± 29.6	75.13	92.95	<0.001
	Control	45	43.2 ± 14.13	39.0	47.4	

Significant = $P < 0.05$, SD = standard deviation, GAL-3, galectin-3, GAL-3BP = galectin-3 binding protein, IL-6 = interleukin-6

Results

There is a significant increase in parameters in the patients group in comparison with the control group as shown in Tables 1 and 2 and Figures 1–3, all reveal that the GAL-3 concentration is positively correlated with the GAL-3PB and CRP concentrations while no correlation with IL-6 in the patient group. Table 3 and Figures 4–6 demonstrate that there is no link between GAL-3 and GAL-3BP, CRP, and IL-6 concentrations in the control group.

The receiver operating characteristic curve was used for diagnosing RA using GAL-3 (pg/mL) showed an area under curve of 0.84 (P < 0.001, 95% CI 0.754–0.925). At a cut-off point of ≥307 pg/mL, the sensitivity was 82%, specificity was 75.6%, positive predictive value was 74.05%, and negative predictive value was 80.94%, as shown in Figure 7.

Table 2: A correlation (*r*) between the measured parameters sequence variables in patients group

Sequence	Variables against each other	Correlation (*r*)	Significant value
1	GAL-3 vs. GAL-3BP	0.554	<0.001
2	GAL-3 vs. CRP	0.441	0.002
6	GAL-3 vs. IL-6	0.289	0.054

Significant = $P < 0.05$, GAL-3 = galectin-3, GAL-3BP = galectin-3 binding protein, IL-6 = interleukin-6, CRP = C-reactive protein

Discussion

RA is an inveterate, systemic autoimmune illness related to synovial tissue propagation, formation of pannus, cartilage demolition, and systemic complexity.[18] In this study, the CRP was significantly increased in patients in comparison with control groups. This finding is consistent with many studies. Pope and Choy's study, for example,[19] found that CRP concentrations are frequently consistently elevated in RA patients, with baseline levels of >20 mg/L frequently reported in randomised clinical trials investigating RA treatments. Kim *et al.*[20] concluded CRP could play an essential function in the process of bony destructive in RA out of the recruitment of receptor activator of nuclear Kappa-B ligand expression and direct osteoclast precursors differentiation into mature osteoclasts. In the treatment of RA, Reducing the level of CRP is an important factor in both preventing bone loss and reducing disease activity.[20] CRP is the prime inflammation biomarker utilized in modern healthcare. Abnormal CRP and/or ESR" is considered a distinct factor along with joint involvement, the presence of autoantibodies, and the duration of symptoms in the most recent collection of categorization criteria for RA. In untreated patients with recent-onset RA, the CRP levels >10 mg/L are frequently seen.[8]

The exact cause of this sickness is still unknown, however cytokines, such as IL-6, are raised in the blood, synovium, and arthritic joints of those who have it..[21] After IL-6 is produced in a local lesion in the foremost stage of inflammation, it gets about to the liver through the bloodstream, followed by the quick induction of a comprehensive range of acute-phase proteins as an example of CRP, fibrinogen, haptoglobin, serum amyloid A, and a1-antichymotrypsin.[10] IL-6 might also be involved in a subtler way in the development of RA. It has been observed that IL-6 is entangled with cytokine release syndrome complicated by T-cell therapy. Blockage of IL-6 in these situations grants good outcomes, confirming the central part that it plays in inflammatory syndromes.[22] IL-6 appears to be accountable for else systemic symptoms linked with RA, particularly in the nervous and cardiovascular systems.[23] Overall, Santos Savio *et al.*[24] mentioned that in patients with RA, high concentrations of both serum IL-6 and serum IL-6R are established in the serum and synovial fluid of affected joints.

A ubiquitous molecule, GAL-3, is found in both extra- and intracellular compartments but is also regarded as a membrane molecule. It is particularly expressed in epithelial, endothelial, and cells of the

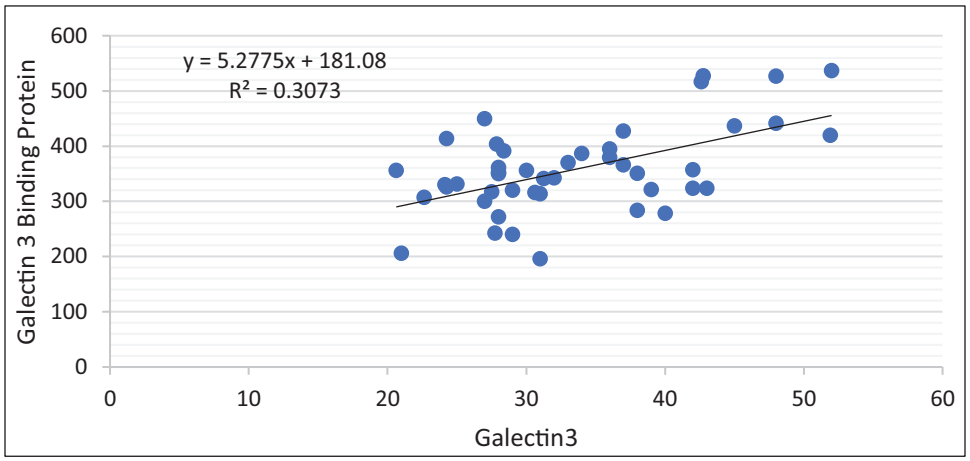

Figure 1: The correlation between galectin-3 and galectin-3 binding protein in patients group

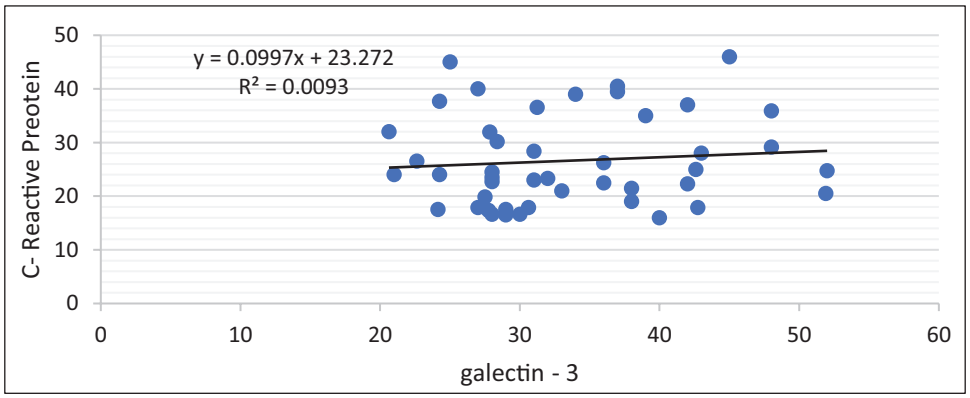

Figure 2: The correlation between galectin-3 and C-reactive protein in patients group

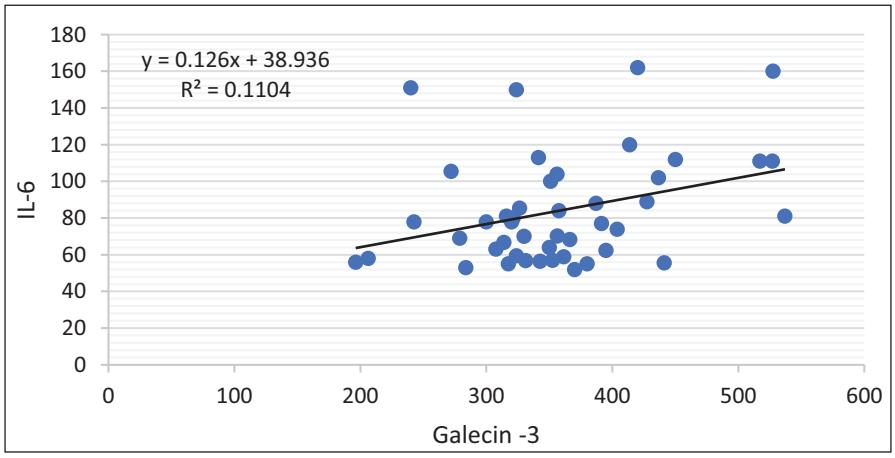

Figure 3: The correlation between galectin-3 and interleukin-6 in patients group

immune system. Inside the cells, GAL-3 is present in the cytosol, nucleus, or mitochondria and its allocation is affected by the kind of the cell and the cell cycle phase. Galectins act on each other with a set of cytosolic and nuclear ligands and thus organize signaling pathways.[25] According to reports, RA and osteoarthritis patients' inflammatory synovium expresses and excretes GAL-3.[26] It was also reported by Issa *et al*.[27] that concentrations of GAL-3 serum remained persistently high in early RA. They observed a positive relationship between serum levels of GAL-3 and some pathophysiology association, such as smoking, autoimmunity, and joint ruin. Rheumatoid fibrocyte-like synoviocytes are triggered by GAL-3 to release pro-inflammatory cytokines and chemokines, such as granulocyte–macrophage colony-stimulating factor, tumor necrosis factor, chemokine ligand-8, IL-6, and chemokine ligands 2, 3, and 5.[28]

The GAL-3 is a good marker in prediction, diagnosis, and prognosis of RA due to its good diagnostic power according to ROC curve mentioned above. This result is inconsistent with the results of Abdel Baki *et al*. [28], who found that serum GAL-3 had an AUC of 0.962 (95% CI: 0.927–0.99, $P < 0.001$), 84.8% sensitivity, and 100% specificity.. This suggests that GAL-3 serves as a diagnostic marker for RA. Gruszewska *et al*.[29] demonstrated that GAL-3 exhibited excellent diagnostic power in RA (AUC = 0.0911) with a sensitivity of 71% and specificity of 100%. They suggested that GAL-3 on account of the high diagnostic power can be a worthy standing by the marker for the diagnosis of rheumatic diseases, especially RA.[29]

Table 3: A correlation (*r*) between the measured parameters sequence variables in control group

Sequence	Variables against each other	Correlation (*r*)	Significatnt value
1	GAL-3 vs. GAL-3BP	0.269	0.078
2	GAL-3 vs. CRP	0.084	0.585
6	GAL-3 vs. IL-6	0.088	0.567

Significant = $P < 0.05$, GAL-3 = galectin-3, GAL-3BP = galectin-3 binding protein, IL-6 = interleukin-6, CRP = C-reactive protein

GAL-3BP is a regarded as serum marker of inflammation in patients with RA, juvenile idiopathic arthritis, autoimmune hepatitis, and asthma.[30] GAL-3BP induces the secretion and expression of pro-inflammatory cytokines, including IL-6, in various cell kinds, through carbohydrate-mediated interaction with GAL-3 at the cell surface.[31,32] GAL-3BP, primarily qualified as a macrophage marker, is a member of the scavenger receptor cysteine-rich domain proteins family. It is a secreted glycoprotein proposed to play a role in host defense and invasion of tumor.[33] GAL-3BP contains binding sites not only for GAL-3, but also for type V and

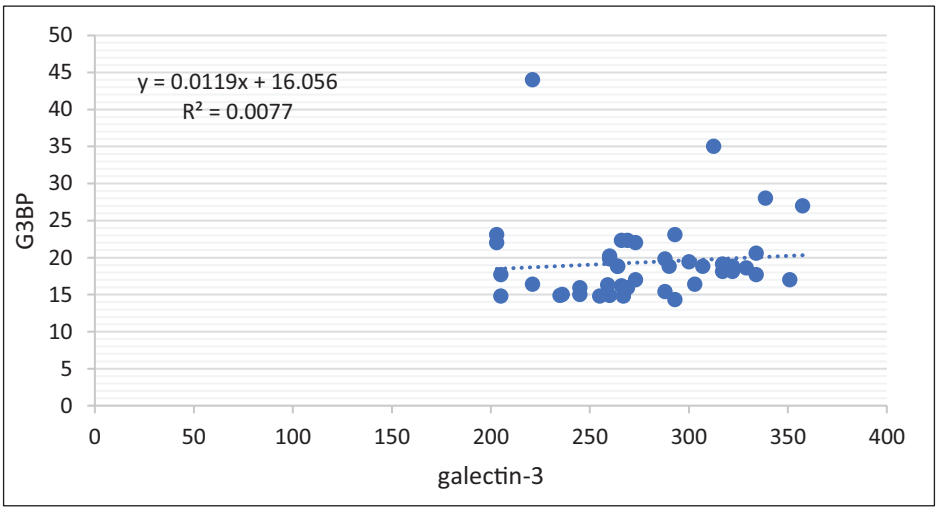

Figure 4: The correlation between galectin-3 and galectin-3 binding protein in control group

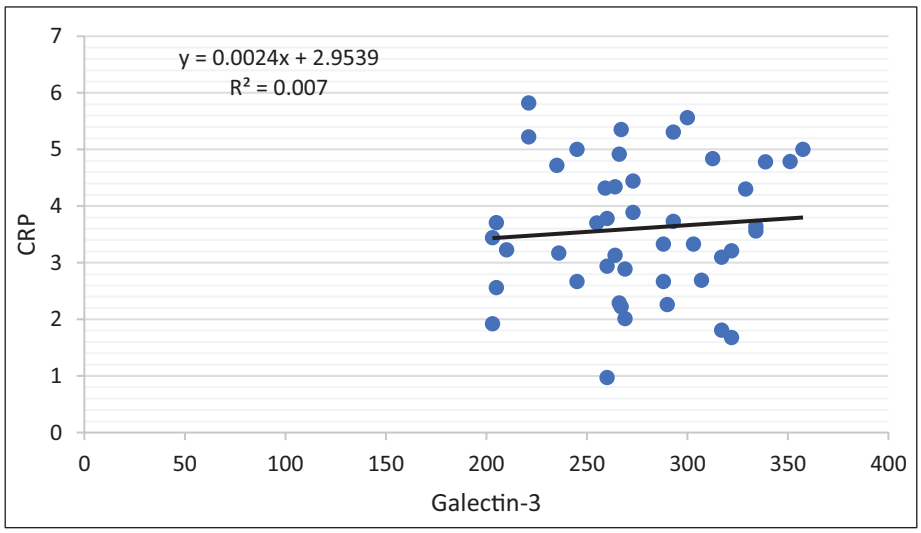

Figure 5: The correlation between galectin-3 and C-reactive protein in control group

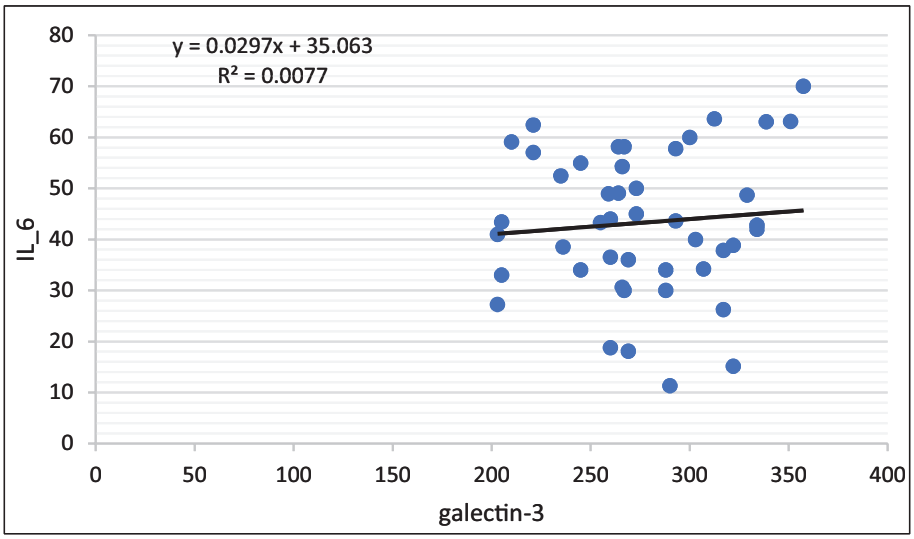

Figure 6: The correlation between galectin-3 and interleukin-6 binding protein in control group

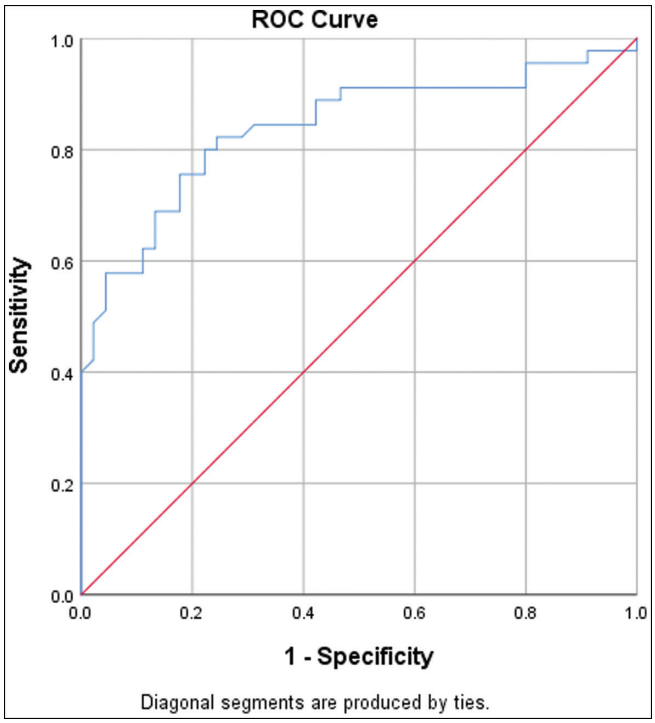

Figure 7: ROC curve of galectin-3

VI collagens, integrins, and fibronectin. Therefore, a cell membrane–bound GAL-3BP could play a role in both attaching to the extracellular matrix and contributing to the destructive process in RA.[17]

Conclusion

The investigation's findings clearly point to GAL-3 and its binding protein's role in the RA pathogenesis. Moreover, GAL-3 can be utilized to diagnose RA.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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