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Research Article

Galectin-3 Gene Polymorphism Role in Rheumatoid Arthritis in Iraqi Patients

[1]Ahmed Jabbar Abbas Alsaidi, [2]Maha Fadhil Smaism and [3]Ali Mohammed Hussain Alkazzaz
[1]*Merjan Teaching Hospital, Ministry of Health, Iraq*
[2]*Department of Biochemistry, College of Medicine, University of Babylon, Iraq*
[3]*Department of medicine, College of Medicine, University of Babylon, Iraq*

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Abstract: Autoimmune, systemic, chronic and inflammatory rheumatoid arthritis is characterized by symmetric, polyarticular pain and tumefaction, morning stiffness and lethargy. The aim of this study is to evaluate of galectin-3 and galectin-3 binding protein and their role in rheumatoid arthritis pathogenesis. Also evaluation of gene level of galectin-3 gene polymorphism in rheumatic patients to predict its relationship with rheumatoid arthritis in future. 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 to January 2023. An ELISA was used to calculate the serum concentrations of galectin-3, galectin-3 binding protein (enzyme-linked immunosorbent assay), galectin-3 genotyping is measured by tetra ARMS -PCR. SPSS software 26 was used to conduct the statistical analysis. Increased levels of galectin-3, galectin-3 binding protein in patients than the control group ($p<0.001$), The results demonstrated that LGALS3 rs4652 AC genotype increased the risk of RA (OR = 10.57, 95% CI = 2.78-40.14, p = 0.001) when compared with the AA genotype and CC genotype is not associated with RA. The LGALS3 rs4652 may has a role in pathogenesis of RA in Iraqi patients. And further studies must be done in large population to confirm its role in RA pathogenesis.

Corresponding Author:
Ahmed Jabbar Abbas Alsaidi,
Merjan Teaching Hospital, Ministry of Health, Iraq Ahmedalsaidi675@gmail.com

INTRODUCTION

Autoimmune, systemic, chronic and inflammatory rheumatoid arthritis (RA) is characterized by symmetric, polyarticular pain and tumefaction, morning stiffness and lethargy. In patients with RA, the disease tends to progress in unpredictable ways, with flare-ups and, less frequently, remissions[1]. The effects can range from helping with a rare illness to incapacitating one so severely that it causes premature mortality and limited mobility. It is the most common form of chronic inflammatory arthritis and is characterized by joint destruction and functional impairment. Fatigue, skin nodules, pericarditis, lung involvement, peripheral neuropathy, vasculitis and hematologic abnormalities are only some of the extra-articular symptoms that can occur

with RA[2]. Cartilage and bone erosion occur with chronic joint inflammation. When the duration of RA symptoms is less than six months, it is considered early RA but when it is longer than six months, it is considered established RA[3].

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 factors reproductive and hormonal and neuroendocrine[4]. In turn, risk factors of environment comprise smoking and other air-borne exposures; microbiota and infectious agents; food; and socioeconomic factors[5].

Galectin-3 (gal-3) is a ß-galactoside-binding protein that controls cell proliferation, motility, adhesion, differentiation and death through mediating interactions between cells and the extracellular matrix[6]. Increased monocyte chemotaxis and macrophage activation, as well as neutrophil activation, degranulation and superoxide generation, suggest a role for Gal-3, a chimera-type member of the galectin family, in the maturation of innate immune responses[7]. Patients with RA have elevated levels of galectin-3 expression and release from inflamed synovium[8].

The gene LGALS3 that codes for GAL-3 has been mapped to q21-q22 on chromosome 14. There are a total of 6 exons and 5 introns in the human LGALS3 gene[9]. SNPs rs4644, LGALS3+191 and rs4652, +292, both affect GAL-3, with the former causing a shift from histidine to proline at residue 64 and the latter from threonine to proline at residue 98. Since the proline at GAL-3 residue 98 is positioned in a crucial protein transport determining area, the LGALS3 +292 C allele was related with reduced serum GAL-3 levels in RA. The polymorphism LGALS3 +292C, located in the gene encoding Gal-3, is more prevalent in RA patients[10].

The innate immunological activity of galectin-3 binding protein (GAL-3BP), a ubiquitous multifunctional secretory glycoprotein, was first discovered in humans in response to viral and bacterial infections[11]. In addition to controlling Nuclear Factor of Activated T Cells activation in macrophages, GAL-3BP has been shown to regulate the activation of cyclophilin C, a protein that controls phagocytosis[12].

GAL-3BP is involved in a wide variety of physiological and pathological processes, including cell proliferation, cellular adhesion, inflammation and visceral fat accumulation, thanks to its interactions with a large number of target molecules via its many functional domains. As a member of the family of scavenger receptor cysteine-rich domain proteins, the secreted glycoprotein G3BP has been hypothesized to play a role in both host defense and tumor invasion[13].

G3BP was initially characterized as a macrophage marker. Not only does G3BP have binding sites for galectin 3 but it also has binding sites for collagen types V and VI, integrins and fibronectin. G3BP may play a role in both the attachment to the extracellular matrix and the damaging process in RA, as it is localized on the cell membrane[12].

MATERIALS AND METHODS

The patients group who subjected to this study were 45 persons of rheumatoid arthritis with their age ranging between 45-60 years old, the Mean±SD was (48.15±3.216 years).

The control group apparently healthy. The age of this group was ranged between 40-60 years with Mean±SD was (49.08±3.749 years).

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

All individuals (patients and controls) have a body mass index (BMI) that is used to exclude obesity. Measurement of both galectin-3, galectin-3 binding protein were done by ELISA technique (PT lab kit), while galectin-3 genotyping was done by ARMS- PCR technique.

The LGALS3 polymorphism was determined by a tetra-primer amplification refractory mutation system-PCR (tetra-ARMS PCR). Two external primers[14]. Forward outer, 5'-GGCTTATCCTGGACAGGC ACCTC-3' and Reverse outer, 5'-TTTTTGACTCTACCAA CATACACCCAT-3' as common primer for control of PCR reaction and the two internal primers Forward inner, 5'-CATCTTCTGGA CAGCCAAGTGTCA-3' specific for A allele Reverse inner, 5'-AGTGGCAGGGTAGGCTCC AGG-3' specific for C allele Product sizes were 203 bp for the A allele, 157 bp for the C allele and 314 bp for the control outer band.

Template DNA (1 µL ~100 ng μL^{-1}), 0.6 µL of each inner forward and reverse primer (10 µM) and 1.5 µL of each outer forward and reverse primer (12 µM) and 14.8 µL DNase-free water were added into a 0.2-ml PCR tube. After determination of the optimum annealing temperature, the following program was set in the thermocycler to amplify the target DNA fragments as shown in the Table 1.

PCR products were visualized on 2% agarose gel containing 0.5 µ mL^{-1} SYBR and images were obtained by UV transilluminator.

Inclusion and exclusion criteria: Inclusion criteria include patients who have rheumatoid arthritis and exclusion criteria include Any subject with chronic liver disease, Any subject with thyroid problems, Heart disease, Smoking, Subjects with renal failure, Subjects with other autoimmune disease, Subjects with glucocorticoid medications, Diabetic patient, Obese patients.

Statistical analysis: The data was analysed using Software Package for Social Science (SPSS-22.0 version). The data was presented as a mean and Standard deviation (SD). Continuous variables were tested for normality according to the t- test and linear regression analysis that have been used to determine the significant difference between the groups[15]. Genetic analysis was performed using Chi-square ($\chi 2$) test. p-values less than (0.05) was considered significant and less than (0.001) considered highly significant[16].

Ethical approval: Approval of scientific committee in Biochemistry Department of Babylon Medical College. The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptance. 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 from were reviewed and approved by a local ethics committee according to the document number 1129 in 23/8/2022 to get this approval.

RESULTS

In this study, the gal-3, G3BP were significantly increased in patients in comparison with control groups (Table 2).

Galectin-3 genotyping: Polymerase chain reaction (PCR) was used to analyze the GAL-3 gene for the LGALS3 polymorphism. The A/C polymorphism site was defined by performing a PCR amplification using site-specific primers and the result was seen as a single band on an agarose gel electrophoresis. Figure 1 shows that the control outer band was 314 bp, the A allele was 203 bp and the C allele was 157 bp in the LGALS3 gene. Table 3 displays the frequency of alleles for the LGALS3 gene polymorphism.

The significance of these findings was determined by comparing the genotypic distribution of the control and diabetes groups using the Chi-square test, as shown in table 4 and calculating the odds ratio (O.R).

Table 1: Amplification conditions of LGALS3 genotyping

Stage	Temperature (°C)	Time	Function	Cycles
1	95	5 min	Initial denaturation	
2	95	58 sec	DNA denaturation	30
	58	30 sec	Primer annealing	
	72	45 sec	Template elongation	
3	72	10 min	Final elongation	
4	8	-	Incubation	Hold

Table 2: Biochemical characteristics of the control and rheumatoid populations

Variables	Groups	No.	Mean±SD	Sig. values
Gal-3 pg mL^{-1}	Patients	45	368.0±144	p<0.001
	Control	45	282.0±46.9	
G3BP ng mL^{-1}	Patients	45	24.5±5.6	p<0.001
	Control	45	18.0±2.49	

*Significant: p<0.05

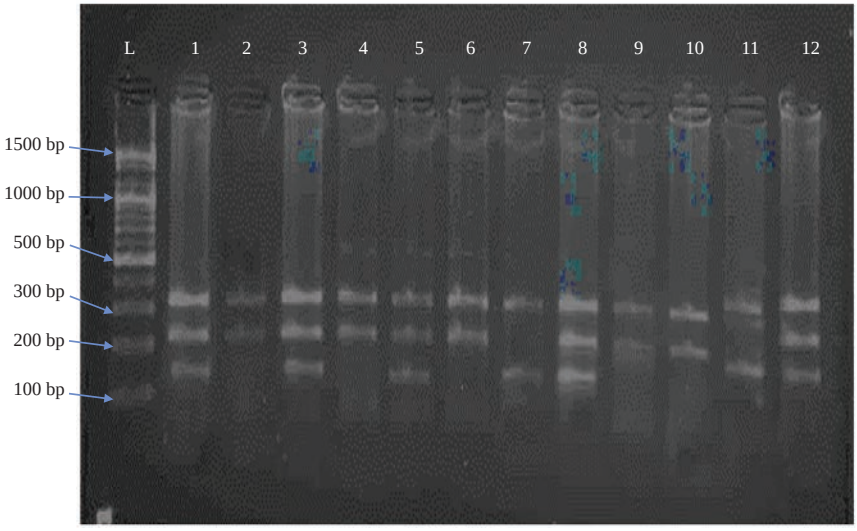

Fig. 1: The genotype and allele frequencies of LGALS3 gene variant in the study subjects. L: ladder, AC: 1,3,5,8,12. CC: 7,11. AA: 2, 4, 6, 9 and 10

Table 3: Genotyping of LGALS3 gene polymorphism with allele frequency

	Genotype				Allele frequency	
Groups	No.	AA	AC	CC	A allele	C allele
Patients	45	3 (6%)	37 (83%)	5 (11%)	47.7%	52.3%
Control	45	18 (40)	21 (46.7%)	6 (13.3%)	63.3%	36.7%

Table 4: LGALS3 gene polymorphism characterization in patient groups and control group

Genotype	Patients	Controls	Odds ratio	CI (95%)	p-value
AC	37	21	10.57	2.78-40.14	<0.001
CC	5	6	5.0	0.91-27.47	0.064
AA	3	18	1 reference		

DISCUSSIONS

RA is an inveterate, systemic autoimmune illness related to synovial tissue propagation, formation of pannus, cartilage demolition and systemic complexity.

In this study found that concentration of galectin-3 binding protein in patients group is higher than that found in control group, while Shiro *et al*.[17] found that Galectin 3 was elevated in RA sera and synovial fluids, whereas G3BP was increased in RA synovial fluids only and concluded galectin 3 and G3BP represent novel markers of disease activity in RA. So the binding of G3BP to Gal-3 can induces multi- processes in immunopathology of RA, especially in elevated levels in serum and synovial fluid of patients with RA.

Gal-3BP is a serum marker of inflammation in patients with autoimmune hepatitis, rheumatoid arthritis, juvenile idiopathic arthritis, asthma. It can also be a marker of liver fibrosis in viral hepatitis, non-alcoholic fatty liver disease, autoimmune hepatitis, primary biliary cholangitis, idiopathic pulmonary fibrosis and chronic pancreatitis[18]. Through its carbohydrate-mediated interaction with Gal-3 at the cell surface, Gal-3BP stimulates the expression and release of pro-inflammatory cytokines such as IL-6 in a wide variety of cell types[19,20]. Eta The high degree of glycosylation on Gal-3BP suggests that it may be recognized by other galectins. It is possible that a single glycoprotein is recognized by more than one galectin, through either the same or different oligosaccharide side chains, despite the fact that each member of the galectin family displays high specificity for b-galactoside-containing oligosaccharides and appears to recognize a distinct set of glycoproteins[21].

As a member of the family of scavenger receptor cysteine-rich domain proteins, the secreted glycoprotein G3BP has been hypothesized to play a role in both host defense and tumor invasion. G3BP was initially characterized as a macrophage marker. Galectin 3, type V and VI collagen, integrins and fibronectin are only some of the proteins that G3BP can bind to. G3BP may play a role in both the attachment to the extracellular matrix and the damaging process in RA, as it is localized on the cell membrane[21].

Extracellularly, intracellularly and as a membrane molecule, galectin-3 is a really ubiquitous molecule. Epithelial, endothelial and immunological cells are the primary cell types that express it. Galectin-3 can be found anywhere from the nucleus to the mitochondria to the cytoplasm, depending on the type of cell and where it is in the cell cycle. Signaling pathways are controlled by galectins because of their interactions with structural proteins found in both the cytosol and the nucleus[22]. Galectins have a crucial role in modulating the immune system and inflammatory response. Macrophages are just one type of inflammatory cell that express galectins. Galectins induce either pro-inflammatory or anti-inflammatory responses, depending on the inflammatory context. Inflamed synovium in people with rheumatoid arthritis and osteoarthritis has been shown to produce and secrete Gal-3[8]. There is a positive link between Gal-3 serum levels and several related pathologies, including autoimmune, smoking and joint damage, as documented by Issa and colleagues[23].

Pro-inflammatory cytokines and chemokines, such as granulocyte-macrophage colony-stimulating factor, chemokine ligand-8, interleukin-6, tumor necrosis factor, chemokine ligand 2, 3 and 5, can be released by rheumatoid fibrocyte-like synoviocytes in response to galectin3[7]. During the inflammatory phase of arthritis, invading immune and resident joint cells secrete a number of pro-inflammatory cytokines and mediators that degrade cartilage and cause excessive bone remodeling. Inflamed synovium from people with rheumatoid arthritis and osteoarthritis has been shown to produce and secrete a galectin-3[23]. So the elevated levels of galectin-3 can cause RA by enhancing and recruitment of immune cells to release cytokines and chemokines[8,20], this finding in corroborate with Mendez-Huergo *et al*.[24] who found elevated levels of galectin-3 in rheumatoid arthritis patients but they contradict those of Mendez-Huergo *et al*., who discovered lower levels of Gal-3 in RA patients compared to controls.

These findings corroborate those of Mahdi Atabaki and colleagues, who found that the LGALS3 rs4652 AC genotype significantly increased the risk of RA in the Iranian population, showing that approximately 37% of rheumatoid patients have this polymorphism[14]. This supported the findings of Hu *et al*.[25] who found that the LGALS3 +292C allele was a dominant risk factor for

RA. Subjects with the LGALS3 +292C allele (odds ratio = 1.8, 95% confidence interval = 1.2-2.8, p = 0.009) were more likely to develop RA than those with the +292AA genotype.

Gal-3 stands apart from the other galectins as a chimaera variant. LGALS3 (human locus 14q21-22) encodes the Gal-3 protein and consists of 17 kbp split between six exons and five introns. The second intron of LGALS3 contains a gene called Gal-3 internal gene. Since the proline at GAL-3 residue 98 is positioned in a crucial protein transport determining area, the LGALS3 +292 C allele was related with reduced serum GAL-3 levels in RA[26].

Among those with RA, the LGALS3 +292C polymorphism in the gene encoding Gal-3 is more prevalent. The gal-3 gene polymorphism leads to changed gal-3 levels, specially increasing levels, this leads to susceptibility the diseases in particular autoimmune diseases like rheumatoid arthritis[10].

CONCLUSION

The LGALS3 polymorphism in rheumatic patients specially patients with LGALS3 polymorphism (A/C) may has a role in causing and progression of rheumatoid arthritis. Further studies with larger sample sizes and populations of different ethnicities are required to validate our findings.

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