Influence of *LGALS3* Gene Polymorphism as A Potential Predictor for Cardiopathy Complication of Type 2 Diabetes Mellitus

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Abstract

Galectin-3(Gal-3) belongs to lectins family binding β-galactoside that have at least one of the carbohydrate recognition domains (CRDs) in their own structure, which located on chromosome 14 (14q22.3).Gal-3Plays an important role in modulating cardiac inflammation and fibrosis. Therefore, LGALS3 gene, encoding galectin-3 protein, which considered promising candidate for the genetic study of cardiovascular diseases (CVDs),and evaluating the association between LGALS3 gene polymorphisms and the susceptibility and prognosis of myocardial infarction(MI) .Gal-is a pleiotropic molecule expressed on the cell membrane, within the cytoplasm or the nucleus. It has been proposed to function as a mediator of inflammation, fibrosis, cell adhesion, apoptosis, and chemotaxis. It is highly expressed in macrophages within and atherosclerotic plaques.galectin-3 can mediate production of profibrotic factors and collagen accumulation in a variety of diseases. The role of galectIn-3 in regulating macrophage invasion and consequent modulation of atherosclerotic plaque phenotype. Finally, the current study concluded that the amount of LGALS-3 gene expression is related to rs4652 SNP variant in patients with MI due toT2D complications.

Design and Methods: *The current study included 100 samples that divided into two groups the first involved 70 individuals, their age ranged between 30-66 years with MI who underwent to elective Percutaneous coronary intervention (PCI) while the second group included 30 samples with the age range 30-55 years were enrolled in the present study as a control group. , (5mL)of blood was gathered from both groups The blood samples were divided into two parts. the first (3 mL) were lived in yhe blank tubes until clotted. Using sandwich ELISA method to determine galectin-3 levels for both groups. Patients were drawn from Al-Sadder Medical City in Al-Najaf Governorate. All participants underwent to medical examinations to ensure they were suffering from T2DM, genotyping Study included extraction of DNA where blood sample of the study groups which were collected in EDTA tubes and frozen, was subjected to Quick-DNA™ Blood MiniPrep Catalog numbers D3024 and D3025. Electrophoresis of DNA. The*

Real-time-PCR method was used to analyze the genotyping of the LGALS3 gene (rs4652)SNP in the LGALS3 Gene, the PCR products were separated on a 2% agarose gel electrophoresis and visualized by exposure to UV light (302 nm) after ethidium bromide staining.

Results: *In order to determine the efficiency of the DNA extraction process, the purity and concentration of the extracted DNA were estimated. The current study recorded that allelic and genotypic distributions of LGALS3 polymorphism at position (rs4652A/C) were not significantly different between control subjects and PCI patients is showing OR=0.641for AA, OR=1.437 for AC and OR=1 for CC, therefore, it was found that AC genotype more affected by MI infection, while CC genotype is less likely to be affected by MI infection, but AA genotype is not affected. Whenchi-square value was 2.947(p=0.229) for comparison of the two main groups, while allele was (OR=0.833 at p=0.556) for A and C.While the results obtained between healthy control and PCI patients in recessive genotype results for the two study groups, it was found that OR=1 at p=1 for AA+AC and CC, while dominant genotype result was OR=0.641 at p=0.352 for AA and CC+AC.*

Key Words: T2D, CVDs, PCI, Gal-3, MI, SNP, HF, PCR

Introduction

Gal-3 is a structurally distinct glycoprotein that belongs to the lectin family and has been widely researched in a variety of disorders. The *LGALS3* gene, which has 6 exons and 5 introns and is located at 14q22.3, also has a unique regulatory element termed galig (gal-3 internal gene) [1]. Numerous single nucleotides in *LGALS-3* are found in exon 3 of chromosome 14 and influence its gene expression, such as; rs2274273, rs4644, and rs4652 [2].Gal-3 expression is regulated by promoter methylation status of *LGALS3* also has been widely researched in several diseases due to its involvement in a variety of biological functions [3].Gal-3, is monomeric strong inflammation protein, goes through physicochemical alterations that broaden its spectrum of biological functioning, especially extracellular activity [4].It is a potent inflammatory protein at the onset of the inflammatory response and it associated to many diseases, including organ cirrhosis, infections, cancers, atherosclerosis and cardiovascular disease (CVDS) [5] .This led to the use of Gal-3 as a predictive marker for heart failure [6],Recently Gal-3 has been suggested in several studies as a good tool for identifying and understanding there

mechanism of CVD.Gal-3 increased expression is associated with the development and progressing of cardio/cerebrovascular diseases [6,7]. Gal-3 normally is expressed at low levels in the cytoplasm, serum and tissue fluids in healthy individuals in the other side Gal-3 is seemed to be expressed in a high levels when lesion accrue, Nearly Gal-3 concentrations have been confirmed to be increased in CVDs [8].A cardiovascular risk assessment is critical for such patients because it determines how long they should be followed and what type of testing they should undergo [9,10]. Genetic tests are useful for detecting early CVD in DM patients and establishing predictive factors [11].

Participants and Methods

During an extended period from August 2021 to February 2022, 100 participants were enrolled in the present project. These instances were split into two categories, with the first group consisting of 70 Arabic Iraqi T2 diabetic patients suffered MI (30-65 years old) who underwent to elective coronary Percutaneous Intervention (PCI) in Najaf Center for Cardiac Surgery and Interventional Catheterization, Al-Saddar Medical City, Iraq. Specialist physicians made the initial diagnosis based on clinical and laboratory examinations of MI patients. The second group included 30 healthy individuals (55-30 years). Sandwich ELISA method was applied to assess Gal-3 in which Patient and control serum samples were analyzed. Genotyping Study included Extraction of DNA was subjected to Quick-DNA™ Blood MiniPrep Catalog numbers D3024 and D3025 [Psifidi et al., 2015]. Electrophoresis of DNA, is carrying to determine DNA pieces after the process of extraction or to detect the result of the interaction of polymer chain reaction (PCR) The Primer Used in The Interaction, The preparation of the primer stock was done by following manufacturer simple instruction. The PCR products were separated on a 2% agarose gel electrophoresis and visualized by exposure to UV light (302 nm) after ethidium bromide staining. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program. The statistical analysis of results was done using the Statistical Package for the Social Science (SPSS) software for Windows, version 23.0, p-values less than 5% ($p<0.05$) was considered statistically significant. The statistical analysis system (SAS) program was used to analyze the data of genetic parameter in the study. Hardy–Weinberg equilibrium (HWE) test, to predict the frequencies regarding allele from mentioned equation, thereafter, the anticipate frequencies (genotype) were verified and then evaluation for the deviation of the population from the HWE by chi-square test for the simile of observed values and expected genotypes. LSD test was used to

significant compare between means of genotypes. The probability of deflection than controls is considered statistically significant if p-value is below 0.05.

Results and Discussion

Identification of Concentration and Purity of DNA in the Samples of The Study Groups

Identification of DNA concentration and purity in the study group samples by estimating DNA purity and concentration after DNA extraction, the efficiency of the extraction process was evaluated.DNA concentration was estimated by recording the absorbance of the sample at 260 nm wavelength, while the purity is measured by dividing the absorbance product at wavelengths 260 to 280 (260/280). The degree of purity is considered good when it ranges between 1.80 - 2.00.

The DNA concentration was estimated by recording the absorbance of the sample at 260 nm wavelength. Evaluating the ratio of A260/A280, which can be calculated after correcting of turbidity (absorbance at 320nm)**Table1**.

Table 1: Concentration and Purity of DNA for The Study Participants

Participant Groups	DNA Concentration (µg/mL) Mean±S.D.	DNA Purity Mean±S.D
PCI Patients	**160.31±67.27**	**1.82±0.99**
Controls	**118.36±47.42**	**1.81±0.12**

Outcomes of Reverse Transcription Polymerase Chain Reaction

In the present study, the expressions of rs4652 SNP in the *LGALS3*gene was examined in the whole blood of MI patients underwent to elective PCI and healthy control group.MedCalc of odd ratio (Online) software and SPSS software were used for multi-name logistic regression analysis to examine the results of both genotype and allele frequencies under recessive, dominant models with respect to the SNP studied for the *LGALS3* gene which is rs4652 in the current study to both (disease group with PCI and healthy individuals).

Table 2 is showing OR=0.641for AA, OR=1.437 for AC and OR=1 for CC, therefore, it was found that AC genotype more affected by MI infection, while CC genotype is less likely to be affected by MI infection, but AA genotype is not affected. When chi-square

value was 2.947(p=0.229) for comparison of the two main groups, while allele was (OR=0.833 at p=0.556) for A and C

Table 2: Results of Hardy–Weinberg Equilibrium Analysis for rs4652 in The Patients and Controls Groups

Genotype	Patients (70)	Controls(30)	p-value	Odds Ratio	95% C.I.
AA	17	10	0.352	0.641	0.251 to 1.634
AC	39	14	0.407	1.437	0.609 to 3.392
CC	14	6	1	1	0.343to 2.913
Chi-square	2.947		p-value		0.229
Allele	Patients	Controls	p-value	Odds Ratio	95% C.I.
A	73	34	0.556	0.833	0.453 to 1.531
C	67	26			

Table 3 shows the recessive genotype results for the two study groups, it was found that OR=1 to AA+AC and CC, while dominant genotype result was OR=0.641 to AA and CC+AC.

rs4652 SNP	Patients (70)	Controls (30)	p-value	Odds Ratio	95% C.I.
Recessive Genotype					
AA+AC	56	24	1	1	0.343 to 2.913
CC	14	6			
Dominant Genotype					
AA	17	10	0.352	0.641	0.251 to 1.634
CC+AC	53	20			

Table 3: Genotype of *LGALS3* Gene SNP rs4652 (A/C) in The Studied Groups

Table 4 revealed the comparative results between PCI patients after classified according to their gender. Outcomes showed there is no significant variations between males and females patients (p=0.856; OR=1.111 to AA, OR=1.127 at p=0.808 to AC and OR=0.740 at p=0.621 to CC, Chi-square OR=0.600, p=0.741 for genotyping, while allele frequencymodel OR=1.146 at p=0.696 to A and C.

Table 4: Results of Hardy–Weinberg Equilibrium Analysis for rs4652 in The Patients Underwent PCI Group

Genotype	Male Patients	Female Patients	p-value	Odds Ratio	95% C.I.
AA	11	6	0.856	1.111	0.355 to 3.471
AC	25	14	0.808	1.127	0.425 to 2.989
CC	8	6	0.621	0.740	0.225 to 2.438
Chi-square	0.600		p-value		0.741
Allele frequency (%)					
Allele	Male Patients	Female Patients	p-value	Odds Ratio	95% C.I.
A	47	26	0.696	1.146	0.577 to 2.276
C	41	26			

Also, there is no clear differences between the two genders of T2D patients for recessive genotypes (OR=1.350, p=0.621) as well as for dominant genotypes (OR=1.111, p=0.856), as illustrated in **Table 5**.

Table 5: Hardy–Weinberg Equilibrium Analysis for rs4652 SNP in The Elective PCI Patients Group

rs4652 SNP	Male Patients	FemalePatients	p-value	Odds Ratio	95% CI.
Recessive Genotype					
AA+AC	36	20	0.621	1.350	0.410 to 4.443
CC	8	6			
DominantGenotype					
AA	11	6	0.856	1.111	0.355 to 3.471
CC+AC	33	20			

Table 6 illustrates that no significant differences for genotypes and allele frequencies model between healthy subjects (p>0.05) in receive and dominated genotypes, alternately OR=0.200 at p=0.168for AA+AC and CC genotype, OR=1.227 at p=0.794 for AA and CC+AC genotype.

Table 6: Genotype of *LGALS3*Gene SNP rs4652 (A/C) in The Healthy individuals

rs4652 SNP	Healthy Males	Healthy Females	p-value	Odds Ratio	95% C.I.
Recessive Genotype					
AA+AC	12	12	0.168	0.200	0.202 to 1.977
CC	5	1			
Dominant Genotype					
AA	6	4	0.794	1.227	0.262 to 5.734
CC+AC	11	9			

Table 7 shows the comparison between MImales patients who underwent elective PCI procedure and healthy males individuals. The results illustrated that no significant differences for genotype and allele frequencies % model alternately between these groups, when OR was 0.611 at p=0.423 for AA genotype, OR was 2.412 at p=0.136 for ACgenotype, while OR was 0.533 at p=0.341 for CC genotype,in spite of, it was noted that Chi-square was 8.983at p=0.011 so significant variations between the two male subgroups were recorded; moreover, analysis of A and C allele frequency % showed there is no specific link between T2D and these alleles when OR was 1.019 at p=0.963.

Table 7: Hardy–Weinberg Equilibrium Analysis for rs4652 in Patients and Controls Males In The Study Groups

Genotype	Patients Males	Healthy Males	p-value	Odds Ratio	95% C.I.
AA	11	6	0.423	0.611	0.182 to 2.041
AC	25	6	0.136	2.412	0.756 to 7.694
CC	8	5	0.341	0.533	0.146 to 1.945
Chi-square	8.983		p-value		0.011
Allele Frequency (%)					
Allele	Male Patients	Healthy Males	p-value	Odds Ratio	95% C.I.
A	47	18	0.963	1.019	0.461 to 2.252
C	41	16			

Table 8 showed that no significant linked between patients with PCI and healthy males were compared together, where in recessive genotypes (OR=1.875, p=0.341) and dominant genotypes (OR=0.611, p=0.423) were evaluated.

Table 8: Genotype of *LGALS3*Gene SNP rs4652 (A/C) in The Healthy and PCI Patient Males Groups

LGALS3 SNP	Patients Male	Healthy Male	p-value	Odds Ratio	95% CI.
Recessive Genotype					
AA+AC	36	12	0.341	1.875	0.513 to 6.841
CC	8	5			
Dominant Genotype					
AA	11	6	0.423	0.611	0.182 to 2.041
CC+AC	33	11			

In **Table 9**, for comparison between patients and healthy females, the results didn't recorded significant differences for genotypes and allele frequencies model; alternately, (OR=0.675 at p=0.605 for AA, OR=0.729 at p=0.648 for AC and OR=3.600 at p=0.261 for CC; Chi-Square=8.750 at p=0.013), as well as for A and C alleles (OR=0.625 at p=0.336).

Table 9: Hardy–Weinberg Equilibrium Analysis for rs4652 SNP in The Female Patients Underwent PCI and Healthy Females Subgroups

Genotype	Female Patients	Healthy Females	p-value	Odds Ratio	95% C.I.
AA	6	4	0.605	0.675	0.152 to 2.994
AC	14	8	0.648	0.729	0.187 to 2.834
CC	6	1	0.261	3.600	0.385 to33.638
Chi-square	8.750		p-value		0.013
Allele Frequency (%)					
Allele	Female Patients	Healthy Females	p-value	Odds Ratio	95% C.I.
A	26	16	0.336	0.625	0.239 to 1.630
C	26	10			

Table 10 shows that all of recessive and dominant genotypes had no significant differences (P>0.05) between females in both study groups.

Table 10: Genotype of rs4652 SNP for Females in Both Studied Groups

Genotype	Female Patients	HealthyFemales	p-value	Odds Ratio	95% C.I.
Recessive Genotype					
AA+AC	20	12	0.261	0.277	0.029 to 2.595
CC	6	1			
Dominant Genotype					
AA	6	4	0.605	0.675	0.152 to 2.994
CC+AC	20	9			

Comparison of Galectin-3 Levels with The Genotype rs4652 SNP in The *LGALS3* Gene Under The Combined Control Model

In order to compare the results of galectin-3 levels in different genotypes between the two main study groups. **Table 11**shows that there are significant (p=0.000) increases in the galectin-3 levels in the MI patients group comparison to controls, for each genotypes ofrs4652 SNP. In addition to that, it's observed that the highest level of galectin-3 was recorded in (AA)genotype for patients.

Table 11: Comparison of Galectin-3 Levels of MI Patients and Controls to The Genotype of rs4652 SNP in The *LGALS3* Gene Under The Combined Control Model

Parameters	Subjects		*p-value*
	Patients 70	Controls 30	
	CC(14) CA(39) AA(17) Mean ± S.D.	CC(6) CA(14) AA(10) Mean ± S.D.	
Galectin-3	10.35±2.46	3.88±1.48	*0.000 for CC,CA and AA*
	9.6±2.06	7.64±2.24	
	10.37±1.6	6.37±2.02	

Table 12 shows the results of comparison galectin-3 of patients subgroups and healthy controls for the genotypes in the rs4652 SNP of the *LGALS3* gene. Highly significant

(p=0.000) elevations in the galectin-3 levels of two patients subgroups comparison to controls according to the same gender, while the current work failed to find significant differences in the galectin-3 levels between the patients and healthy subgroups. The greatest galectin-3 levels were recorded in (CC) genotype (Mean ± S.D.=11.17±2.28) for females patients.

Table 12 Comparison of Galectin-3 Levels of Patients with PCI Patients as well as Controls to The Genotype of rs4652 SNP in The *LGALS3* Gene Under The Combined Control Model

Parameters	Subjects				*p-value*
	PCI Patients 70		Controls 30		
	(8) CC (6) (25) CA (14) (11) AA (6) Mean ± S.D.		(5) CC (1) (6) CA (8) (6) AA (4) Mean ± S.D.		
	Male	Female	Male	Female	
Galectin-3	10.04±2.57	11.17±2.28	3.69±1.57	4.81±0.0	<0.05 for: 2 and 3
	9.3±2.07	10.13±2	7.3±2.18	7.89±2.39	<0.05 for:2 and 3
	9.76±1.64	11.12±1.55	5.7±2.17	7.38±1.49	<0.05 for:2 and 3

1: Male vs Female Patients; 2: Male Patients vs Healthy Males; 3:Female Patients vs Healthy Females; 4: Healthy Males vs Healthy Females

Gale-3 is crucial in controlling cardiac fibrosis and inflammation, Additionally, it participates in the pathways that support heart remodeling [7,12].Consequently, the *LGALS3* gene, which codes for the protein galectin-3, is a good candidate for the genetic research of cardiomyopathy .The expression of Gal-3 in the normal human heart is low , but it has been identified as the best predictor of HF among 48 genes [3,13,14] .The most common cardiovascular complication is coronary heart disease in those with T2DM. Genetic variants were also a new area in an epidemiological study to determine the genetic component underlying these risk factors and to combine T2DM with CVD. These mechanisms were implicated in cardiovascular diseases among T2DM patients [2,15,16,17]. All of that trigger inflammation which is the main factor of atherosclerosis in T2DM patients.Gal-3 has been widely recognized as a biomarker of heart failure (HF). Previous studies concluded that genetic variants at Gal-3 gene single-nucleotide polymorphism (SNP) sites are able to change the protein levels of Gal-3, In patients with

acute HF. The possible relationship between Gal-3 (*LGALS-3* rs4652) gene variant and its expression with cardiac atrial diseases CAD risk in T2D [18,19]. The results have shown that the frequency of AA genotype of LGALS-3 rs4652 gene variant was most common among the control group than controls and T2DM with MI, and the opposite was found in Gal-3 CC genotyping which were higher among T2DM with MI than the control group, which means that the rs4652 CC genotype could be a MI risk factor in the current study.

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