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Effect of LGALS3 Gene Polymorphism on MI Recurrence in Clopidogrel-Responsive Patients Undergoing Elective PCI

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Abstract

Background: Coronary heart disease (CHD) so known coronary artery disease (CAD) where is result from hindrance of blood transit to muscle of heart cause to collect the lipid and cholesterol on the inner wall of the heart. Because of atherosclerosis. smoking, obesity, dyslipidemia, hypertension and diabetes mellitus. Percutaneous Coronary Intervention (PCI) is a non-surgical operation performed using a catheter (tube is flexible and thin) to cram either inflate a balloon in the narrowing place or stent (a very small and fin grid tube is made of either plastic or metal of stainless steel). Galectin-3, the chimaera category in the family of galectins, Galectin-3 among the galectins family contains glycin, tyrosine, and proline, which are necessary for the galectin-3 biological activity, which are rich N-end addition to the domain of carbohydrate-recognition where they can formula oligomers. LGALS3 is the coding gene of galectin-3, which is sited on chromosome 14 (arm q between 21-22). A current study has shown that LGALS3 gene single nucleotide polymorphisms (SNPs) (rs1009977), SNP can occur in the genome coding (gene) and so single nucleotide variation (SNV) may be associated with changes in protein levels or affect response to a drug.

Subjects: A hundred individuals in the present work were divided into two main groups, the first included 70 patients their age ranged between 30-66 years with MI who underwent to elective PCI (30 of them underwent for the firstly time elective PCI, and the remained number underwent for more than one). The second group involved 30 healthy individuals with the age range 30-55 years were enrolled in the present study as a control group. Sandwich-ELISA technique was applied for measurement Gal-3 concentration in the sera samples. 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, Performing Reverse Transcription Polymerase Chain Reaction, Study the Sequencing of SNP (rs1009977) in the LGALS3 Gene, Sequencing and Sequence Alignment, 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: After the DNA extraction process, the efficiency of the extraction process was evaluated by estimating the purity and concentration of the DNA, on the basis of which the subsequent operations were continued in order to continue the sequencing process. Also showed that TT genotype is less likely to be affected for treatment by clopidogrel (OR=0.814 at p=0.683) while TG genotype revealed the most affected by treatment with clopidogrel (OR=1.166 at p=0.725) and GG is not affected (OR=1 at p=1), when chi-square value was 0.571 (p=0.751) for comparison of the two main groups, while allele frequency% was (OR=0.926 at p=0.804) for T and G, while shows the recessive genotype results for two study groups, it was found that OR=1 at p=1, while dominant genotype results was OR=0.814 at p=0.638, in addition to that, the co-dominant genotype result show OR=0.814 at p=0.683 for TT genotype, OR=1.166 at p=0.725 for GT genotype and OR=1 at p=1 to GG genotype. The additive genotype result illustrated the value of OR was 0.964 at p=0.945 for

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2TT+TG and OR=1.214 at p=0.690 to 2GG+TG genotypes; respectively. After alignment of product amplification of the LGALS3 gene, the results are five samples with 100% concordance of two healthy subjects, two patients with one PCI and one patient with multiple PCI, so five samples have a 99% concordance of two healthy subjects, two patients with one PCI; and one patient with multiple PCI, three samples containing 98% for patients with. As 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 of rs1009977 SNP. In addition to that, its observed that the highest level of galectin-3 was recorded in TG genotype for patients.

Keywords: Coronary Heart Disease CHD, Percutaneous Coronary Intervention PCI, Lectins, Galectin-3, **Clopidogrel** and Single Nucleotide Polymorphisms (SNPs)

1. Introduction

Coronary heart disease (CHD) so known coronary artery disease (CAD) where is result from hindrance of blood transit to muscle of heart cause to collect the lipid and cholesterol on the inner wall of the heart. Because of atherosclerosis [Dayana, 2021; Virani *et al.*, 2021]. Mortality rates from cardiovascular diseases (of all deaths) range from 13% in Somalia to 49% in Oman. The increase in cardiovascular disease is caused by inactive routines and collective risk factors, such as hypertension (ranging from 28% in the UAE to 41% in Libya and Morocco); diabetes (ranging from 4% in Iran to 19 % in Sudan) and increase of blood cholesterol (ranging from 14% in Lebanon to 52% in Iran). [World Health Organization, 2022]. Risk factors of the heart disease are include heredity factor, stress, smoking, obesity, dyslipidemia, hypertension and diabetes mellitus. [César *et al.*, 2021]. **Percutaneous Coronary Intervention** (PCI) is a non-surgical operation performed using a catheter (tube is flexible and thin) to cram either inflate a balloon in the narrowing place or stent (a very small and fin grid tube is made of either plastic or metal of stainless steel) [Mansoor *et al.*, 2021]. **Galectin-3**, the chimaera category in the family of galectins, Galectin-3 among the galectins family contains glycin, tyrosine, and proline, which are necessary for the galectin-3 biological activity, which are rich N-end addition to the domain of carbohydrate-recognition where they can formula oligomers [Gao *et al.*, 2020]. The major functions of galectin-3 can be described as macrophage migration promotion, proliferation of fibroblasts and synthesis of collagen [Tan *et al.*, 2021]. While The most important function of galectin-3 is its role in the acute and chronic inflammations [Sygitowicz *et al.*, 2022]. **Single Nucleotide Polymorphisms** (SNPs) are a basis for alteration in the genome. SNP ("snip") is a lone base change in DNA. SNPs are the simplest and most public cause of genetic polymorphism in the genome of humans (90% of individuals have polymorphisms of DNA) [Eva *et al.*, 2022]. There are more than ten million single SNPs that have been identified and are in the database; this number is constantly increasing to reach the potential number depending on the current db-SNP release. There are 84.7 million SNPs in the genome of human [Robert & Pelletier, 2018; Chauhan *et al.*, 2022; Yang *et al.*, 2022]. ***LGALS3*** is the coding gene of galectin-3, which is sited on chromosome 14 (arm q between 21-22). A current study has shown that *LGALS3* gene single nucleotide polymorphisms (SNPs) (rs1009977), SNP can occur in the genome coding (gene) and so single nucleotide variation (SNV) may be associated with changes in protein levels or affect response to a drug. Variation ID of the present study SNP is rs1009977, the variation type of this SNP is SNV. The target alleles of the goal SNP are T and G. Genomic locations of rs1009977 SNP are: GCF-000001405.39: NC-000014.9@ 55136284 and GCF-000001405.25: NC-000014.8@ 55603002. SNP may be linked to alterations in levels of protein, and may change the blood galectin-3 content, but less is known about these genetic variations in relation to MF patients. In the present study, the prospective investigated relationship between galectin-3 coding gene polymorphism and circulating plasma galectin-3, and its relationship to CAD, the number of catheter operations that the patient undergoes, as well as the relationship of the SNP with other supporting parameters to prove this disease [Fu *et al.*, 2020].

2. Subjects and Methods

A hundred individuals in the present work were divided into two main groups, the first included 70 patients their age ranged between 30-66 years with MI who underwent to elective PCI (30 of them underwent for the firstly time elective PCI, and the remained number underwent for more than one). The second group involved 30 healthy individuals

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with the age range 30-55 years were enrolled in the present study as a control group. After subjecting to PCI procedure, sera samples of patients were collected from The Heart Center in Al-Diwaniyah Teaching Hospital, Al-Diwaniyah Governorate, Iraq. Complete information about the current study individuals was provided through oral meeting with patients and in support with the supervising physicians. The participants (patients and controls) subjects were fasting for 8-12 hours before blood collection. Sandwich-ELISA technique was applied for measurement Gal–3 concentration in the sera samples. **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 applied PCR primers sequences for amplification of ***LGALS3*** gene SNP (**rs1009977**) was elucidated. **Performing Reverse Transcription Polymerase Chain Reaction,** called RT-PCR, instead of DNA, uses mRNA as the starting template. **Study the Sequencing of SNP (rs1009977) in the *LGALS3* Gene,** before beginning the sequencing protocol, absolute ethanol was added to the wash buffer prior to initial use. This protocol is initiation with the step of gel extraction DNA [Vogelstein, 1979]. **Sequencing and Sequence Alignment,** 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 are considered statistically significant if *p*-value is below 0.05.

3. Results and Discussion

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

After the DNA extraction process, the efficiency of the extraction process was evaluated by estimating the purity and concentration of the DNA, on the basis of which the subsequent operations were continued in order to continue the sequencing process. It was used to measure purity and concentration using Nanodrop technology, a modern technology that has unique properties as it deals with a DNA sample (RNA and DNA) and determines its purity and concentrations of pollutants present in a record time not exceeding ten seconds using a very small amount of sample. Purity is measured by dividing the absorbance product at wavelengths 260 and 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 320 nm). **Table 1** shows the mean ± S.D. levels of concentration and purity of the nucleic acids extracted from the study samples; respectively.

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

4. Outcomes of Reverse Transcription Polymerase Chain Reaction

In the present study, the expressions of rs1009977 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

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examine the results of both genotype and allele frequencies under recessive, dominant, co-dominant and additive models with respect to the SNP studied for the *LGALS3* gene which is rs1009977 in the current study to both (disease group with PCI and healthy individuals).All genotypes and allele frequencies model revealed that no significant association with clopidogrel resistance in the patients as in the healthy, where the results in genotypes T/G when compared the two main groups (MI patients and controls) together. **Table 2** showed that TT genotype is less likely to be affected for treatment by clopidogrel (OR=0.814 at p=0.683) while TG genotype revealed the most affected by treatment with clopidogrel (OR=1.166 at p=0.725) and GG is not affected (OR=1 at p=1), when chi-square value was 0.571 (p=0.751) for comparison of the two main groups, while allele frequency% was (OR=0.926 at p=0.804) for T and G.

Table 2: Hardy–Weinberg Equilibrium Analysis for rs1009977 in The Patients and Controls Groups

T>G Genotype	Patients (70)	Controls (30)	p-value	Odds Ratio	95% C.I.
TT	16	8	0.683	0.8148	0.3049 to 2.1773
GT	40	16	0.7252	1.1667	0.4940 to 2.7554
GG	14	6	1	1	0.3433 to 2.9132
Chi-square	0.571		p value		0.751
Allele Frequency (%)					
Allele	Patients	Controls	p-value	Odds Ratio	95% C.I.
T	72	32	0.8049	0.9265	0.5055 to 1.6981
G	68	28			

Table 3 shows the recessive genotype results for two study groups, it was found that OR=1 at p=1, while dominant genotype results was OR=0.814 at p=0.638, in addition to that, the co-dominant genotype result show OR=0.814 at p=0.683 for TT genotype, OR=1.166 at p=0.725 for GT genotype and OR=1 at p=1 to GG genotype. The additive genotype result illustrated the value of OR was 0.964 at p=0.945 for 2TT+TG and OR=1.214 at p=0.690 to 2GG+TG genotypes; respectively.

Table 3: Genotypes of rs1009977 SNP in The *LGALS3* Gene of The Studied Groups

LGALS3 SNP	Patients (70)	Controls (30)	p-value	Odds Ratio	95% C.I.
Recessive Genotype					
TT+GT	56	24	1.0000	1	0.3433 to 2.9132
GG	14	6			
Dominant Genotype					
TT	16	8	0.6830	0.8148	0.3049 to 2.1773
GG+GT	54	22			
Co-Dominant Genotype					
TT	16	8	0.683	0.8148	0.3049 to 2.1773
TG	40	16	0.7252	1.1667	0.4940 to 2.7554
GG	14	6	1	1	0.3433 to 2.9132
Additive Genotype					
2TT+TG	72	32	0.945	0.964	0.339 to 2.736
GG+TG2	68	28	0.690	1.214	0.466 to 3.158

In **Table 4**, the comparative results between the patients who underwent to one time PCI and controls groups, illustrated that there are no significant variations between them (OR=1.18 at p=0.77 for TT, OR=1 at p=1 for TG and OR=0.8 for p=0.73 to GG genotypes, when chi-square=0.292 at p=0.864), moreover; T and G allele frequencies % recorded OR=1.14 at p=0.71, Which indicated the fact that clopidogrel treatment during the preparation period for elective PCI does not affect the nuclear structure of the studied gene (*LGALS3*) at rs1009977.

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Table 4: Hardy–Weinberg Equilibrium Analysis for rs1009977 in The Patients Underwent to One PCI and Controls Groups

T/G Genotype	One PCI Patients (30)	Controls (30)	p-value	Odds Ratio	95% C.I.
TT	9	8	0.77	1.18	0.38 to 3.62
TG	16	16	1.00	1.00	0.36 to 2.75
GG	5	6	0.73	0.80	0.22 to 2.91
Chi-square	0.292		p value		0.864
Allele Frequency (%)					
Allele	One PCI Patients (30)	Controls (30)	p-value	Odds Ratio	95% C.I.
T	34	32	0.71	1.14	0.55 to 2.35
G	26	28			

Also, there is no clear significant differences ($p>0.05$) in the recorded genotypes, as shown in **Table 5**, when OR of recessive genotype was 1.25, while it was 1.178 for dominant genotype, in addition to OR=1.18 at p=0.77 to TT genotype, OR=1 at p=1 to TG genotype and OR=0.80 at p=0.73 for GG genotype. With the same manner, for co-dominant genotype OR=1.275 at p=0.710 for 2TT+TG, also it was 0.825 at p=0.730 to 2GG+TG (additive genotype).

Table 5: Genotype of rs1009977 SNP in the *LGALS3* Gene for Patients Underwent to The First Percutaneous Coronary Intervention and Controls Groups

LGALS3 SNP	One PCI Patients (30)	Controls (30)	p-value	Odds Ratio	95% C.I.
Recessive Genotype					
TT+TG	25	24	0.738	1.25	0.3365 to 4.6437
GG	5	6			
Dominant Genotype					
TT	9	8	0.774	1.178	0.3828 to 3.628
GG+GT	21	22			
Co-Dominant Genotype					
TT	9	8	0.77	1.18	0.38 to 3.62
TG	16	16	1.00	1.00	0.36 to 2.75
GG	5	6	0.73	0.80	0.22 to 2.91
Additive Genotype					
2TT+TG	34	32	0.710	1.275	0.354 to 4.591
GG+TG2	26	28	0.730	0.825	0.277 to 2.459

The results of **Tables 6** and **7** showed that there were no statistically significant differences for all studied comparisons of genotypes between patients undergoing PCI for the first time and healthy individuals, but OR values indicate an increased risk of myocardial infarction coinciding with the TT genotype, while the GG genotype showed a lower probability of association with Disease incidence The results of allele analysis confirmed these findings. **Table 6** shows the comparison between MI patients who underwent to multi PCI procedure and healthy individuals. The results illustrated that no significant differences for genotype and allele frequencies % model alternately between these groups, when OR was 0.58 at p=0.357 for TT genotype, OR was 1.31 at p=0.577 for TG genotype while OR was 1.16 at p=0.8 for GG genotype, in addition, Chi-square was 1.719 at p=0.423; moreover, analysis of Allele Frequency showed there is no statistical different for T and G allele when OR was 0.79 at p=0.5.

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Table 6: Results of Hardy–Weinberg Equilibrium Analysis for rs1009977 in The Patients Underwent to Multi PCI and Controls Groups

Genotype	Patients with Multi PCI	Controls	*p-value*	Odds Ratio	95% C.I.
TT	7	8	0.357	0.58	0.18 to 1.84
TG	24	16	0.577	1.31	0.51 to 3.41
GG	9	6	0.800	1.16	0.36 to 3.71
Chi-square	1.719		p value		0.423
Allele Frequency (%)					
Allele	Patients with Multi PCI	Controls	*p-value*	Odds Ratio	95% C.I.
T	38	32	0.50	0.79	0.40 to 1.54
G	42	28			

Table 7 revealed absence of significant linked when MI patients with multi PCI and healthy individuals were compared together, OR recessive genotype was 1.252 at p=0.687, while OR of dominant genotype was 0.848 at p=0.790; while that OR were 0.58, 1.31 and 1.16 for TT, TG and GG; respectively in the co-dominant genotypes. While, for the additive genotypes; OR values of "2TT + TG" and "2GG+ TG" were 0.791 and 1.714; respectively.

Table 7: Genotype of rs1009977 SNP for Patients Underwent to Multi PCI and Controls Groups

LGALS3 SNP	Patients with Multi PCI (40)	Controls (30)	*p-value*	Odds Ratio	95% C.I.
Recessive Genotype					
TT+GT	31	24	0.6877	1.2525	0.4178 to 3.7552
GG	9	6			
Dominant Genotype					
TT	7	8	0.7902	0.8485	0.2529 to 2.8471
GG+GT	33	22			
Co-Dominant Genotype					
TT	7	8	0.357	0.58	0.18 to 1.84
TG	24	16	0.577	1.31	0.51 to 3.41
GG	9	6	0.800	1.16	0.36 to 3.71
Additive Genotype					
2TT+TG	38	32	0.686	0.791	0.254 to 2.463
GG+TG2	42	28	0.346	1.714	0.558 to 5.261

When comparing patients subject to more than one PCI and the control group (**Tables 6** and **7**), despite the absence of statistical differences between the two groups, the OR values show that the frequency of the presence of GG and TG genotypes in the patients group, as well as the case when examining the allele. This finding may be due the reason for the long period during which clopidogrel treatment was taken after the patients' first PCI was performed.

5. Sequencing and Sequence Alignment of Product 6. 6.Amplification for LGALS3 Gene

In order to confirm the results of the electrophoresis of the PCR products of a *LGALS3* gene, twenty four samples of the amplified products (forward and rearward strand) of the *LGALS3* gene, ten for patients who underwent PCI (5 for once and 5 for more than once) and four for healthy people by forward direct sequencing to detect polymorphisms within these sequences and the other backward direct. These sequences were compared with the reference sequence of *LGALS3* in GenBank of NCBI.

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Table 8: Sequencing and Sequence Alignment for Homo Sapiens mRNA of *LGALS3* Protein Variant Protein with Sequence ID:AB 209391.1

No.	Type of Substitution	Location	Nucleotide	Identities
1	Transition	2412	T/C	99%
2	-------	-------	---------	100%
3	Gap	2367	C	97%
	Transversion	2389	G/T	
	Transition	2448	G/C	
4	Transversion	2405	G/A	99%
5	------	--------	---------	100%
6	Transversion	2389	G/T	98%
	Transition	2448	G/C	
7	Transversion	2389	G/T	98%
	Transition	2448	G/C	
8	Transition	2448	G/C	98%
	Transition	2451	T/C	
9	Gap	2430	C	99%
10	---------	---------	--------	100%
11	Transversion	2413	A/C	97%
	Transversion	2416	A/T	
	Transition	2448	G/C	
12	Gap	2430	C	99%
13	---------	--------	-------	100%
14	--------	--------	-------	100%
15	Transition	2448	G/C	99%

After alignment of product amplification of the *LGALS3* gene, the results are five samples with 100% concordance of two healthy subjects, two patients with one PCI and one patient with multiple PCI, so five samples have a 99% concordance of two healthy subjects, two patients with one PCI; and one patient with multiple PCI, three samples containing 98% for patients with Multi PCI and two samples having 97% for homo sapiens mRNA for *LGALS3* variant protein mRNA from GenBank using BioEdit software. It does not affect the production of any non-coding reverse protein. **Figure 1.1** for the first sample demonstrates the presence of transition in sit 2412 T/C where change the code TAC to TAT and both to amino acid Tyrosine.

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021 Number of Matches: 1, Range 1: 2374 to 2474.
Score =178 bits(197), Expect =1e-48, **Identities= 100/101(99%), Gaps= 0/101(0%)**, Strand= Plus/ Minus

```
Query  1     TGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTA T CTTTGAAAAAAAAAAAGCACA  60
             ||||||||||||||||||||||||||||||||||||||| | ||||||||||||||||||||
Subject  2374  TGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTA C CTTTGAAAAAAAAAAAGCACA  2433

Query  61    TACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  101
             |||||||||||||||||||||||||||||||||||||||||
Subject  2434  TACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Figure 1: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2374 to 2474 with Standard *LGALS3* from GenBank

Figures 2, 5, 10, 13 and 15 illustrated that their Identities are 100% without any change of nitrogen bases.

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Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1, Range 1: 2406 to2474.
Score =125 bits(138), Expect =1e-32, Identities= 69/69(100%), Gaps= 0/69(0%), Strand= Plus/ Minus

```
Query  1       CAAGTACCTTTGAAAAAAAAAAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCT  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2406  CAAGTACCTTTGAAAAAAAAAAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCT  2465

Query  61      CCAACAGCA  69
               |||||||||
Subject  2466  CCAACAGCA  2474
```

Figure 2: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2406 to2474 with Standard *LGALS3* from GenBank

Figure 3 shows the presence of a gap in the position 2367 of C, Transversion in site 2389 G/T where the code changed from TAT for Tyrosine to GAT for Aspartic acid, and Transition in the location 2448 G/C, where the code changed from CTC to CTG and both to Lucien. Transversion and Transition were frequent in samples 6 and 7 for each of the **Figures 3** and **4**.

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1
Length: 5021 Number of Matches: 1, Range 1: 2359 to 2474.
Score =125 bits(138), Expect =7e-53, Identities=113/116(97%), Gaps= 0/69 (0%), Strand= Plus/ Minus.

```
Query  17      TTTTTCAC - TTAGTTTGTTGTACTGCCAAA  G ATTTTATTTGGAATAACAAGTACCTTTGA  75
               ||||||||   ||||||||||||||||||||    ||||||||||||||||||||||||||||
Subject  2359  TTTTTCAC C TTAGTTTGTTGTACTGCCAAA  T ATTTTATTTGGAATAACAAGTACCTTTGA 2418

Query  76      AAAAAAAAAAGCACATACAAACTTCAGCT G CCCTTTGAGGCTTAGCTCCAACAGCA  131
               |||||||||||||||||||||||||||||  ||||||||||||||||||||||||||
Subject  2419  AAAAAAAAAAGCACATACAAACTTCAGCT C CCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Figure 3: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2359 to2474 with Standard *LGALS3* from GenBank

For the sample 4, **Figure 4** show Transversion in position 2405 G/A where the code changed from AAC of Asparagine to AGC of Serine, while **Figure 5** has identities 100%.

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1, Range 1: 2366to 2474.
Score =193 bits(213), Expect =6e-53, Identities= 108/109(99%), Gaps= 0/109(0%), Strand= Plus/ Minus.

```
Query  1       CCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATA G CAAGTACCTTTGAAAAAAAA  60
               |||||||||||||||||||||||||||||||||||||||  ||||||||||||||||||||
Subject  2366  CCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATA A CAAGTACCTTTGAAAAAAAA  2425

Query  61      AAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  109
               |||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2426  AAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  247
```

Figure 4: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2366 to 2474 with Standard *LGALS3* from GenBank

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Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1, Range 1: 2375 to 2474.
Score =181 bits(200), Expect =9e-50, Identities= 100/100(100%), Gaps= 0/100 (0%), Strand= Plus/ Minus.

```
Query  1     GTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAAAAAGCACAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2375  GTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAAAAAGCACAT  2434

Query  61    ACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  100
             ||||||||||||||||||||||||||||||||||||||||
Subject  2435  ACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Figure 5: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2375 to2474 with Standard *LGALS3* from GenBank

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1,Range1:2364to2474.
Score =192 bits(212), Expect =2e-52, Identities= 109/111(98%), Gaps= 0/111 (0%), Strand= Plus/ Minus.

```
Query  20    CACCTTAGTTTGTTGTACTGCCAAA G ATTTTATTTGGAATAACAAGTACCTTTGAAAAAA  79
             ||||||||||||||||||||||||| | ||||||||||||||||||||||||||||||||
Subject  2364  CACCTTAGTTTGTTGTACTGCCAAA T ATTTTATTTGGAATAACAAGTACCTTTGAAAAAA  2423

Query  80    AAAAAGCACATACAAACTTCAGCT G CCCTTTGAGGCTTAGCTCCAACAGCA  130
             ||||||||||||||||||||||||   ||||||||||||||||||||||||||
Subject  2424  AAAAAGCACATACAAACTTCAGCT C CCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Figure 6: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2364 to2474 with Standard *LGALS3* from GenBank

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1,Range1:2366 to **2474. Score =188 bits(208), Expect =2e-51, Identities= 107/109(98%), Gaps= 0/109(0%), Strand= Plus/ Minus.**

```
Query  19    CCTTAGTTTGTTGTACTGCCAAA  G  ATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAA  78
             |||||||||||||||||||||||  |  ||||||||||||||||||||||||||||||||||||
Subject 2366  CCTTAGTTTGTTGTACTGCCAAA  T  ATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAA  2425

Query  79    AAAGCACATACAAACTTCAGCT  G  CCCTTTGAGGCTTAGCTCCAACAGCA  127
             ||||||||||||||||||||||  |  ||||||||||||||||||||||||||
Subject  2426  AAAGCACATACAAACTTCAGCT  C  CCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Figure 7: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2366 to2474 with Standard *LGALS3* from GenBank

Figure 8 shows the Identities was **98%** because Transition in the location 2448 G/C where the code changed from CTC to CTG and both to Lucien, in site 2451 T/C the code is also different from CCC to CCT of same amino acid is Proline.

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1,Range1: 2390 to2474. Score=145 bits(106), Expect=6e-39, Identities= 83/85(98%), Gaps= 0/85(0%), Strand= Plus/ Plus.

```
Query  1     ATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAAAAAGCACATACAAACTTCAGCT G C  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| | |
Subject  2390  ATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAAAAAGCACATACAAACTTCAGCT C C  2449

Query  61    C T TTTGAGGCTTAGCTCCAACAGCA  85
             | | ||||||||||||||||||||||||
Subject  2450  C C TTTGAGGCTTAGTCCAACAGCA  2474
```

Figure 8: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2366 to2474 with Standard *LGALS3* from GenBank

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Figure 9 reveals that the Identities ratio is **99%**, and this is a result for the presence of a gap in the position 2430 caused by the process of deleting of the Cytosine nitrogen base, this result is similar to what recorded in the sample 12 (**Figure 12**).

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021 Number of Matches: 1,Range 1: 2400 to2474. Score=128 bits(141), Expect=5e-33, Identities= 74/75(99%), Gaps= 0/75 (0%), Strand= Plus/ Plus.

```
Query  1     GAATAACAAGTACCTTTGAAAAAAAAAAAG - ACATACAAACTTCAGCTCCCCTTTGAGGC  59
             |||||||||||||||||||||||||||||  | |||||||||||||||||||||||||||
Subject  2400  GAATAACAAGTACCTTTGAAAAAAAAAAAG C ACATACAAACTTCAGCTCCCCTTTGAGGC  2459

Query  60    TTAGCTCCAACAGCA  74
             |||||||||||||||
Subject  2460  TTAGCTCCAACAGCA  2474
```

Figure 9: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2400 to2474 with Standard *LGALS3* from GenBank

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021 Number of Matches: 2,Range 1: 2366 to2474. Score=197 bits(218), Expect=4e-54, Identities= 109/109(100%), Gaps= 0/109(0%), Strand= Plus/ Plus.

```
Query  20    CCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAA  79
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2366  CCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAA  2425

Query  80    AAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  128
             ||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2426  AAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Range 2: 2459 to 2474. Score=30.1 bits(32), Expect=0/001, Identities= 16/16(100%), Gaps= 0/16(0%), Strand= Plus/ Plus.

```
Query  261   CTTAGCTCCAACAGCA  276
             ||||||||||||||||
Subject  2459  CTTAGCTCCAACAGCA  2474
```

Figure 10: Alignment Sequence rs1009977 SNP of *LGALS3* at The Positions2366 to2474 and2459 to 2474with Standard *LGALS3* from GenBank

It was also noted that sample 11 in **Figure 11** has a Identities that equal to 97%, and this is a result of the Transversion presence in position 2413 A/C where the code alteration from CTT of Lucien to ATT of Isoleucin, also in site 2416 A/T, the change was from TGA of Stop code to AGA of Arginine, while in 2448 position, it was Transition where the different G/C to produce CTG instead of CTC and both to Lucien.

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021 Number of Matches: 1,Range 1: 2374 to 2474. Score= 169 bits (187), Expect=6e-46, Identities= 98/101 (97%), Gaps = 0/101 (0%), Strand = Plus/ Plus.

```
Query  1     TGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTAC A TT A GAAAAAAAAAAAGCACA  60
             |||||||||||||||||||||||||||||||||||||||| || | |||||||||||||||||
Subject  2374  TGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTAC C TT T GAAAAAAAAAAAGCACA  2433

Query  61    TACAAACTTCAGCT G CCCTTTGAGGCTTAGCTCCAACAGCA  101
             |||||||||||||| | ||||||||||||||||||||||||||
Subject  2434  TACAAACTTCAGCT C CCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Figure 11: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2374 to 2474 with Standard *LGALS3* from GenBank

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Figure 12 illustrates Identities to 99% as a result of deleting and creating a gap.

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1,Range 1: 2405 to2474. Score=119 bits (187), Expect=2e-30, Identities= 69/70 (99%), Gaps= 1/70 (1%), Strand= Plus/ Plus.

```
Query  1     ACAAGTACCTTTGAAAAAAAAAAAG - ACATACAAACTTCAGCTCCCCTTTGAGGCTTAGC  59
             ||||||||||||||||||||||||||   ||||||||||||||||||||||||||||||||
Subject  2405  ACAAGTACCTTTGAAAAAAAAAAAG C ACATACAAACTTCAGCTCCCCTTTGAGGCTTAGC  2464

Query  60    TCCAACAGCA  69
             ||||||||||
Subject  2465  TCCAACAGCA  2474
```

Figure 12: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2405 to 2474 with Standard *LGALS3* from GenBank

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 1,Range 1: 2366 to 2474. Score=197 bits(218), Expect=2e-54, Identities= 109/109(100%), Gaps= 0/109 (0%), Strand= Plus/ Plus.

```
Query  21    CCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAA  80
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2366  CCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAA  2425

Query  81    AAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  129
             |||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2426  AAAGCACATACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Figure 13: Alignment Sequence rs1009977 SNP of *LGALS3* at The Position 2366 to 2474 with Standard *LGALS3* from GenBank

Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1 Length: 5021
Number of Matches: 2,Range 1: 2375 to 2474. Score=181 bits(200), Expect=3e-49, Identities= 100/100 (100%), Gaps= 0/100 (0%), Strand= Plus/ Plus.

```
Query  1     GTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAAAAAGCACAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2375  GTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAAAAAAAAGCACAT  2434

Query  61    ACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  100
             ||||||||||||||||||||||||||||||||||||||||
Subject  2435  ACAAACTTCAGCTCCCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Range 2: 2459 to 2474, Score=30.1 bits(32), Expect= 0.001, Identities= 16/16(100%), Gaps= 0/16 (0%), Strand= Plus/ Plus.

```
Query  233   CTTAGCTCCAACAGCA  248
             ||||||||||||||||
Subject  2459  CTTAGCTCCAACAGCA  2474
```

Figure 14: Alignment Sequence rs1009977 SNP of *LGALS3* at The Positions 2375 to 2474 and 2459 to 2474with Standard *LGALS3* from GenBank

It is noted in **Figure 15** of sample 15 that the apparent identity is equal 99% and this is a result of the presence Transition of 2448 site G/C to produce CTG instead of CTC code and both to Lucien [Baynes, 2019].

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Homo sapiens mRNA for *LGALS3* protein variant protein, chromosome 14, Sequence ID: AB209391.1
Length: 5021 Number of Matches: 2,Range 1: 2363 to 2474.
Score=198 bits(219), Expect=3e-54, Identities= 111/112(99%), Gaps= 0/112(0%), Strand= Plus/ Plus.

```
Query  1     TCACCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject  2363  TCACCTTAGTTTGTTGTACTGCCAAATATTTTATTTGGAATAACAAGTACCTTTGAAAAA  2422

Query  61    AAAAAAGCACATACAAACTTCAGCT G CCCTTTGAGGCTTAGCTCCAACAGCA  112
             ||||||||||||||||||||||||| ||||||||||||||||||||||||||
Subject  2423  AAAAAAGCACATACAAACTTCAGCT C CCCTTTGAGGCTTAGCTCCAACAGCA  2474
```

Range 2: 2459 to 2474
Score=30.1 bits (32), Expect=0.001, Identities= 16/16(100%), Gaps= 0/16 (0%), Strand= Plus/ Plus.

```
Query  233   CTTAGCTCCAACAGCA  248
             ||||||||||||||||
Subject  2459  CTTAGCTCCAACAGCA  2474
```

Figure 15: Alignment Sequence rs1009977 SNP of *LGALS3* at The Positions 2363 to 2474 and 2459 to 2474with Standard *LGALS3* from GenBank

Comparison of Galectin-3 Levels with The Genotype rs1009977 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 9** 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 of rs1009977 SNP. In addition to that, its observed that the highest level of galectin-3 was recorded in TG genotype for patients.

Table 9: Comparison of Galectin-3 Levels of MI Patients and Controls with The Genotype of rs1009977 SNP in The *LGALS3* Gene Under The Combined Control Model

Parameters	Subjects		*p-value*
	Patients 70	Controls 30	
	TT(16) TG(40) GG(14) Mean ± S.D.	TT(8) TG(16) GG(6) Mean ± S.D.	
Galectin-3	9.21±2.20	6.63±1.48	*0.000* *for TT,TG & GG*
	10.15±1.71	7.93±2.35	
	10.14±2.65	3.82±1.38	

Table 10 shows the results of comparison galectin-3 of patients subgroups (MI patients with one PCI and those who underwent to multi PCI) and healthy controls for the three proven genotypes in the rs1009977 SNP of the *LGALS3* gene. Highly significant (p=0.000) elevations in the galectin-3 levels of two patients subgroups comparison to controls, while the current work failed to find significant differences in the galectin-3 levels between the patients subgroups. The maximum galectin-3 levels were recorded in GG genotype for patients with multiple elective PCI.

Table 10: Comparison of Galectin-3 Levels of Patients with One and Multi PCI as well as Controls to The Genotype of rs1009977 SNP in The *LGALS3* Gene Under The Combined Control Model

Parameters	Subjects			*p-value*
	One PCI 30	Multi PCI 40	Controls 30	
	TT(9) TG(16) GG(5)	TT(7) TG(24) GG(9)	TT(8) TG(16) GG(6)	

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	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	
Galectin-3	8.61±2.08	9.98±2.26	6.64±1.48	0.153 for1*vs*2
	10.17±2.03	10.13±1.51	7.93±2.35	0.000 for 1*vs*3
	9.24±3.25	10.64±2.31	3.82±1.38	0.000 for 2*vs*3

1: Patient with One PCI, 2:Patients with More PCI, 3: Healthy

Table 11 shows the results of comparison among galectin-3 levels of MI patients with one PCI and controls subgroups after classified according to gender, in the three diagnosed genotypes of the rs1009977 SNP of the *LGALS3*gene. Generally, the detailed results show a raise of galectin-3 level at the females than males subgroups parallel to the three detected genotypes in the present study, in addition; significant ($p<0.05$)variations were observed when the females in TG and GG genotypes as well as males patients in TG genotype compared to their matching gender in the healthy subgroups, while in the same patients group was increase significant ($p=0.004$) in TG genotype. Moreover; the highest galectin-3 levels (Mean ± S.D.=12.38±1.91) of MI female patients subgroup were correlated to GG genotype.

Table 11: Comparison of Galectin-3 Levels of Both Genders in Patients with One PCI and Controls to The Genotype of rs1009977 SNP in The *LGALS3* Gene Under The Combined Control Model

Parameters	Subjects				*p-value*
	One PCI 30		Controls 30		
	(6) TT (3) (5) TG (11) (2) GG (3) Mean ± S.D.		(4) TT (4) (8) TG (8) (1) GG (5) Mean ± S.D.		
	Female	Male	Female	Male	
Galectin-3	8.74±1.36	8.33±3.55	6.36±1.88	7.04±1.06	0.023 for 5*vs*7
	11.30±2.15	9.65±1.84	8.55±2.31	7.17±2.41	0.021 for 6*vs*8
	12.38±1.91	7.15±1.72	4.81±0	2.84±1.49	0.004 for 9*vs*10 0.002 for 9*vs*11

1: Female With One PCI For (TT); 2: Male With One PCI For (TT); 3: Healthy Female For (TT); 4: Healthy Male For (TT); 5: Female With One PCI For (TG); 6: Male With One PCI For (TG); 7: Healthy Female For (TG); 8: Healthy Male For (TG); 9: Female With One PCI For (GG); 10: Male With One PCI For (GG); 11: Healthy Female For (GG); 12: Healthy Male For (GG)

Results of the current study illustrated significant elevations ($p<0.05$) in the galectin-3 of females of MI patients who underwent at least two elective PCI group comparison to their matching sex in the controls group, for all of the three identified genotypes of rs1009977 SNP, also to males in (TG and GG) genotype, where was found that the highest galectin-3 levels of galectin-3 (which recorded in the MI females subgroup)were closely related to TT genotype **Table 12**, while there is no significant between males and females in the same disease group.

Table 12: Comparison of Galectin-3 Levels of Both Genders in Patients with Multi PCI and Controls to The Genotype of rs1009977 SNP in The *LGALS3* Gene Under The Combined Control Model

Parameters	Subjects				*p-value*
	Multi PCI 40		Controls 30		
	(4) TT (3) (6) TG (18) (3) GG (6) Mean ± S.D.		(4) TT (4) (8) TG (8) (1) GG (5) Mean ± S.D.		
	Female	Male	Female	Male	
Galectin-3	11.11±2.48	8.48±0.48	6.36±1.88	7.04±1.06	0.002 for 1*vs*3
	10.87±1.00	9.89±1.59	8.55±2.31	7.17±2.41	0.043 foe 5*vs*7 0.007 for 6*vs*8

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	10.69±2.30	**10.62±2.53**	**4.81±0**	**2.84±1.49**	**0.011 for9*vs*11 0.000 for10*vs*12**

1: Female With More PCI For (TT); 2: Male With Multi PCI For (TT); 3: Healthy Female For (TT); 4: Healthy Male For (TT); 5: Female With More PCI For (TG); 6: Male With Multi PCI For (TG); 7: Healthy Female For (TG); 8: Healthy Male For (TG); 9: Female With More PCI For (GG); 10: Male With Multi PCI For (GG); 11: Healthy Female For (GG); 12: Healthy Male For (GG)

Table 13 illustrates the outcome of comparison of galectin-3 levels for the patients subgroups (MI patients with one PCI and those who underwent to multi PCI) in the three diagnosed genotypes of rs1009977 SNP of the *LGALS3* gene, after classified patients according to their genders. For TT genotype, on significant differences (p>0.05) were observed when the levels of galectin-3 of males subgroups compared together, as well as, when the females patients subgroups compared together. Level elevation of thegalectin-3 in the females patients underwent to at least two elective PCI than males of the same group, while no significant variation was recorded, also, males and females patients underwent to one PCI. Statistically, results of TG genotype, significant elevation in the galectin-3 levels of females comparison to males in the same group. Insignificant results were recorded when males as well as females subgroups were compared together. Genotyping of GG showed significant variations in the galectin-3 levels of two genders subgroups of patients underwent to the first PCI, while the results in the two sexes of patients with multi PCI were reverse to these in the first patients group. So high significant (p=0.014) when comparison between the male in GG genotype for to patient subgroups. Furthermore; the highest levels of galectins-3 of MI females patients who experience to the first PCI were recorded in GG genotype.

Table 13: Comparison of Galectin-3 Levels of Both Genders in Patients with One and Multi PCI to The Genotype of rs1009977 SNP in The *LGALS3* Gene Under The Combined Control Model

Parameters	**Subjects**				***p-value***
	One PCI 30		**Multi PCI 40**		
	(6) TT (3) (5) TG (11) (2) GG (3) Mean ± S.D.		**(4) TT (3) (6) TG (18) (3) GG (6) Mean ± S.D.**		
	Female	**Male**	**Female**	**Male**	
Galectin-3	**8.74±1.36**	**8.33±3.55**	**11.11±2.48**	**8.48±0.48**	**0.004 for 9*vs*10 0.014 for 10*vs*12**
	11.30±2.15	**9.65±1.84**	**10.87±1.00**	**9.89±1.59**	
	12.38±1.91	**7.15±1.72**	**10.69±2.30**	**10.62±2.53**	

1: Female With One PCI For (TT); 2: Male With One PCI For (TT); 3: Female With Multi PCI For (TT); 4: Male With Multi PCI For (TT); 5: Female With One PCI For (TG); 6: Male With One PCI For (TG); 7: Female With Multi PCI For (TG); 8: Male With Multi PCI For (TG); 9: Female With One PCI For (GG); 10: Male With One PCI For (GG); 11: Female With Multi PCI For (GG); 12: Male With Multi PCI For (GG)

High levels of galectin-3 in the blood of patients with one PCI can be attributed to an increase in the level of LDL, which is oxidized by oxidative stress and affects the endothelial cells that are at the beginning of their inflammation, which motivates the increased expression of galectin-3 formation. During chronic inflammation conditions, continued activation of endothelial cells (ECs) by inflammatory stimuli reasons modifications in normal function of endothelial, resulting in endothelial dysfunction which has been considered to be the origin and primary step of atherosclerosis, the most common cause of cardiovascular diseases. Several studies have shown that oxidized low-density lipoprotein (ox-LDL) induced endothelial cell injury by changing proinflammatory gene expression. Accumulating evidence has demonstrated that Gal-3 aggravated ox-LDL-mediated endothelial injury by inducing inflammation [Li, 2017]. Ox-LDL induces endothelial dysfunction with focal inflammation which in turn causes increased expression of atherogenic signaling molecules that promote the adhesion of monocytes to the arterial endothelium and their penetration into the intimae. Some studies have indicated that the synthesis and expression of galectin-3 are associated with differentiation and activation of macrophage. indicated that galectin-3 can be expressed on the surface of normal human peripheral blood monocytes. This finding indicated that galectin-3 levels increased significantly as monocytes differentiated into macrophages *in vitro* [Gao, 2020].

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