

# Gene Polymorphism and Serum Levels of Interlukine-18 in Patients with Coronary Artery Disease and Type-2 Diabetes Mellitus

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## ABSTRACT

Atherosclerosis is a multifactorial disease that develops from childhood and ultimately can lead to death. The clinical manifestations of atherosclerosis include Myocardial Infarction (MI), stroke and Sudden Cardiac Death (SCD). The prevalence of the disease is high, and approximately 50% of all deaths globally can be attributed to atherosclerosis.

**Keywords:** Whey protein; Milk; Casein; Cheapest protein

## INTRODUCTION

Atherosclerosis was known as an inflammatory disease, several researches have been done on blood markers of inflammation, including some cytokines. Interleukins are the most important group of cytokines that are divided into several families. Interleukin 18 (IL-18), a new member of IL-1 family, is a cytokine that plays a central role in the inflammatory process and acts in both innate and acquired immunity [1].

IL-18 is a pro-inflammatory cytokine that plays an important role in the inflammatory cascade. Some evidence suggests that its expression may be related to atherosclerotic plaque progression and vulnerability [2].

The aim of this work was to assess the serum levels of IL-18 and its gene polymorphism in Coronary Artery Disease (CAD) and in diabetic patients and correlate these findings with clinical, echocardiographic, laboratory, and coronary angiographic findings.

## METHODS AND MATERIALS

A cross-sectional study was conducted on 180 patients; they were enrolled in the study after obtaining their written informed consent and approval of the local ethical committee of the hospital.

The study population was divided according to presence of CAD and/or type II DM into 4 groups;

Group I (n=41 patients): Non diabetic subjects without CAD,  
Group II (n=51 patients): Non diabetic patients with the CAD,  
Group III (n=40 patients): Diabetic patients without the CAD,  
Group IV (n=48 patients): Diabetic patients with CAD.

Diabetes mellitus was diagnosed according to World Health Organization and the American Diabetes association as fasting blood glucose  $\geq 126$  mg/dl ( $\geq 7$  mmol/L) or 2 hour post-load blood glucose  $\geq 200$  mg/dl ( $\geq 11.1$  mmol/L), or random blood glucose  $\geq 200$  mg/dl and presence of diabetes symptoms [3].

Diagnostic coronary angiography was performed for patients who had any of the following; typical ischemic chest pain, Electro Cardiographic (ECG) changes suggesting CAD, previous cardiac interventions e.g. Percutaneous Coronary Intervention (PCI), Coronary Artery Bypass Graft (CABG) or other investigations suggesting CAD such as positive exercise stress test, positive myocardial perfusion imaging or echocardiographic regional wall motion abnormalities.

Patients with type I DM, more than mild valvular heart disease, congestive heart failure NYHA class III or IV, atrial fibrillation, history of malignancy or autoimmune diseases were excluded from the study.

Diagnostic coronary angiography was carried out in all patients using Judkin's technique. Quantitative analysis of the coronary arteries was done using the computer-assisted coronary angiography analysis system; end diastolic frames were selected for analysis. The percentage diameter stenosis was assessed in

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different projections and the highest value for each lesion was chosen.

The coronary tree was divided into 16 segments as the following: the left main coronary artery (LM) as one segment; the Left Circumflex artery (LCX) was divided into: Proximal, mid, and its distal segments beside two marginal branches, the Left Anterior Descending artery (LAD) was divided into: Proximal, mid and its distal segments beside two diagonals; the Right Coronary Artery (RCA) was divided into proximal, mid, and distal segments beside Posterior Descending Artery (PDA) and postero-lateral branch.

Vessel score was defined as one vessel disease, two vessel disease or three vessel disease; according to the number of vessels with >70% luminal diameter stenosis [4].

To estimate the Severity score, the coronary circulation was divided into eight proximal segments. The eight proximal segments scored included LM, LCX up to the junction of the middle and distal thirds of the vessel, the proximal third of the major obtuse marginal branches, LAD up to the junction of the middle and distal thirds of the vessel, the proximal third of the major diagonal branches, RCA up to and including the origin of the PDA, the proximal third of the PDA. In cases in which the PDA was supplied by the LCX (LCX dominance), lesions in the LCX up to the origin of the PDA were included. Diseases in the distal segments were not considered because of difficulty to quantify the severity of lesions in these areas. The percentage of stenosis for each lesion in the proximal coronary circulation was assessed according to the maximal narrowing of the diameter in all projections, the severity of the proximal coronary disease was estimated by assigning points to each lesion; less than 50% stenosis of the luminal diameter, 1 point; 50% to 74% stenosis, 2 points; 75% to 99% stenosis, 3 points; total obstruction, 4 points. The points for each lesion were summed and the score for severity was obtained [5].

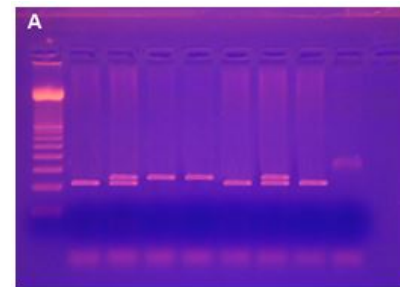
The SYNTAX score was calculated by a computer program consisting of sequential and interactive self-guided questions. The algorithm consists of 12 main questions. The first three questions determine the dominance, the total number of lesions and the vessel segments involved per lesion, the maximum number of lesions allowed is twelve and each lesion is characterized by a number, 1 to 12. The last nine questions refer to adverse lesion characteristics and are repeated for each lesion. The first question is referring to a total occlusion. If a total occlusion is scored, answers should be given to detailed sub-questions. The last of these sub-questions refers to the absence or presence of side branches and their size. If multiple lesions were less than 3 vessel reference diameters apart, these lesions were scored as being one lesion. However, lesions at a greater distance from each other (more than 3 vessel reference diameters), were scored as separate lesions [6].

The total SYNTAX score was derived from the summation of these individual scorings. After the completion of the algorithm a report was automatically generated summarizing all the adverse characteristics and the individual scoring of each lesion as well as the total SYNTAX score.

All study population underwent 12 lead Electrocardiogram (ECG) and full conventional echocardiographic examination. Laboratory investigations were done which included serum urea and creatinine, fasting and postprandial blood sugars, glycated Hemoglobin (HbA1C), lipid profile, serum IL-18 level by ELISA and genotyping of IL-18 at 137 G/C and 607 C/A Single Nucleotide Polymorphisms (SNP).

PCR amplification was done using "Applied Biosystem 2720 thermal cycler, UK". PCR condition was done as first Initial denaturation at 94°C for 10 minutes then 35 cycles were performed (denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 45 seconds). Then Final extension step at 72°C for 7 minutes.

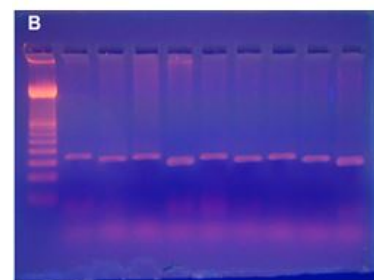
The PCR products were digested with the appropriate restriction enzymes overnight and then separated on 4% agarose gels. For the 137 G/C site, the product sizes digested with BglIII were 105 and 36 bp for the G 141 bp for the C allele (Figure 1A) [7].



**Figure 1A:** IL-18 genotyping at 137 G/C SNP by PCR-RFLP analysis.

PCR amplification was done using "Applied Biosystem 2720 thermal cycler, UK". PCR condition was done as first Initial denaturation at 94°C for 10 minutes then 35 cycles were performed (denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 45 seconds). Then Final extension step at 72°C for 7 minutes.

The PCR products were digested with the appropriate restriction enzymes at 37°C for one hour. The draI digestive products were run by 4% agarose gel electrophoresis for 30 minutes and stained with ethidium bromide, and the bands were visualized under ultraviolet light. Digested PCR products were 154 bp for the C allele and 125 and 28 bp for the A allele (Figure 1B).



**Figure 1B:** IL-18 genotyping at 607 C/A SNP by PCR-RFLP analysis.

## STATISTICAL ANALYSIS

Data was statistically described in terms of mean, Standard Deviation (SD), frequencies (number of cases), median and percentages when appropriate. Data was analyzed using International Business Machines Corporation (IBM) SPSS software package, version 20 (IBM Corp., Armonk, New York, USA). Comparison of quantitative variables between the studied groups was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square test was used. Exact test was used instead when the expected frequency is less than 5. A Probability value (P value) less than 0.05 was cystic considered statistically significant.

## RESULTS

The clinical and baseline characteristics of the study population are depicted in Table 1. Patients with CAD had higher LVEDD (P=0.03), lower EF (P=0.01) than patients without CAD. Serum levels of Total Cholesterol (TC), Triglycerides (TG) and Low Density Lipoprotein (LDL) were significantly higher in diabetic groups (groups III and IV) when compared to other groups, while High Density Lipoprotein (HDL) level was significantly lower in the same groups than other groups (P<0.01) (Table 1).

Mean SD	Group I N=41	Group II N=51	Group III N=40	Group IV	Test	P value
LVEDD (mm)						
Sex	Female N (%)	18 (43.9%)	25(49.02%)	20(50%)	25(52.08%)	0.625 0.891
	Male N (%)	23(56.1%)	26(50.98%)	20(50%)	23(47.92%)	
Age (years)	55.7 ± 6.2	56.4 ± 6.7	53.9 ± 7.9	56.1 ± 6.7	1.097	0.352
	50.1 ± 5.7	53.5 ± 5.6	50.9 ± 6.3	51.9 ± 4.5	3.11	0.03*
EF (%)	62.08 ± 7.3	57.3 ± 8.2	59.6 ± 7.3	57.5 ± 7.3	3.7	0.01*
HbA1C %	5.9 ± 0.32	5.8 ± 0.38	8.09 ± 0.59	8.76 ± 0.72	360.46	0.001*
TC (mg/dl)	164 ± 17.8	185.8 ± 42.4	224.7 ± 50.8	235.3 ± 40.5	30.01	0.001*
TG ( mg/dl)	119.4 ± 41.4	128.3 ± 51.6	178.5 ± 81.04	189.9 ± 79.5	12.23	0.001*
LDL (mg/dl)	93.5 ± 17.8	115 ± 40.1	145.7 ± 48.1	152.6 ± 37.4	22.91	0.001*
HDL (mg/dl)	46.6 ± 7.4	45.1 ± 9.6	43.9 ± 8.1	41.04 ± 7.6	3.75	0.012*

**Abbreviations:** EF: Ejection Fraction; HbA1C: Glycated Hemoglobin; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; LVEDD: Left Ventricular End Diastolic Diameter; SD: Standard Deviation; TC: Total Cholesterol; TG: Triglycerides.

\* Significant P value

**Table 1:** Basic clinical, echocardiographic and laboratory data of the study population.

As regard serum IL-18 level, there was highly significant differences between all studied groups. CAD groups (groups II and IV) showed higher levels of IL-18 than non-CAD groups (groups I and III) (P<0.001) (Table 2).

	Group I (N=41)	Group II (N=51)	Group III (N=40)	Group IV (N=48)	Test	P value
IL-18 (pg/ml) Mean ± SD	292.7 ± 21.3	312.7 ± 17.2	306.6 ± 15.2	323.1 ± 24.8	F 17.5	<0.001*
137 G/C SNP	CC N 25 (60.9%)	7 (13.7%)	21 (52.5%)	6 (12.5%)	X2 54.4	<0.001*
	GC N 12 (29.2%)	12 (23.5%)	10 (25%)	13 (27%)		
	GG N 4 (9.7%)	32 (62.7%)	9 (22.5%)	29 (60.4%)		
607 C/A SNP	C allele N (%) 62 (75.6%)	26 (25.4%)	52 (65%)	25 (26%)	X2 73.2	<0.001*
	G allele N (%) 20 (24.3%)	76 (74.5%)	28 (35%)	71 (73.9%)		
	CA N 18 (43.9%)	26 (50.9%)	23 (57.5%)	25 (52%)		
	AA N 2 (4.8%)	7 (13.7%)	4 (10%)	5 (10.4%)		
	C allele N (%) 60 (73.1%)	62 (60.7%)	49 (61.2%)	61 (63.5%)	X2 3.7	0.29
	A allele N (%) 22 (26.8%)	40 (39.2%)	31(38.7%)	35 (36.4%)		

**Abbreviation:** IL-18: Interleukin 18; SD: Standard Deviation; SNP: Single Nucleotide Polymorphism.

\* Significant P value

**Table 2:** Comparison between all study groups regarding IL-18 level, 137 G/C SNP, 607 C/A SNP genotypes and individual allele distribution.

There was high significant difference between four groups regarding 137 G/C genotype distribution with predominance of CC genotype in non-coronary groups (groups I and III), accounting 25 patients (60.98%) in group I and 21 patients (52.5%) in group III and predominance of GG genotype in

coronary groups (groups II and IV), accounting 32 patients (62.75%) in group II and 29 patients (60.42%) in group IV (P<0.001) (Tables 2).

Regarding distribution of individual G and C alleles in the study population, C alleles were more prevalent in group I and group III; group I had 62 C alleles (75.6% of all C alleles) and group III had 52 C alleles (65% of all C alleles) while G alleles were more prevalent in group II and group IV; group II had 76 G alleles (74.5% of all G alleles) and group IV had 71 G alleles (73.9% of all G alleles) (Table 2). On the other hand, we didn't find any significant differences among the studied group regarding 607 C/A SNP genotype distributions and no significant difference in allele distribution (Table 2).

Serum level of IL-18 was higher significantly in patients with predominant GG genotype when compared to other genotypes (P<0.001) (Table 3).

Variables	CC	GC	GG	Annova	
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	F	P value
IL-18	293.71 ± 18.06	306.9 ± 17.15	324.03 ± 20.1	43.78	<0.001*
Severity score	0.86 ± 1.93	3.89 ± 4.57	7.04 ± 5.04	36.69	<0.001*
SYNTAX score	1.11 ± 3.24	7.79 ± 8.68	16.56 ± 12.27	46.83	<0.001*

Abbreviation: IL-18: Interleukin 18.

\* Significant P value

**Table 3:** Comparison between different genotypes of 137 G/C SNP regarding serum IL-18, severity and SYNTAX scores.

137 G/C SNP	Vessel score					P value
	Normal vessels	One VD	Two VD	Three VD	X2	
CCN (%)	46 (56.7%)	10 (34.4%)	2 (6.2%)	1 (2.6%)	71.61	<0.001*
GCN (%)	22 (27.1%)	4 (13.7%)	13 (40.6%)	8 (21%)		
GGN (%)	13 (16%)	15 (51.7%)	17 (53.1%)	29 (76.3%)		

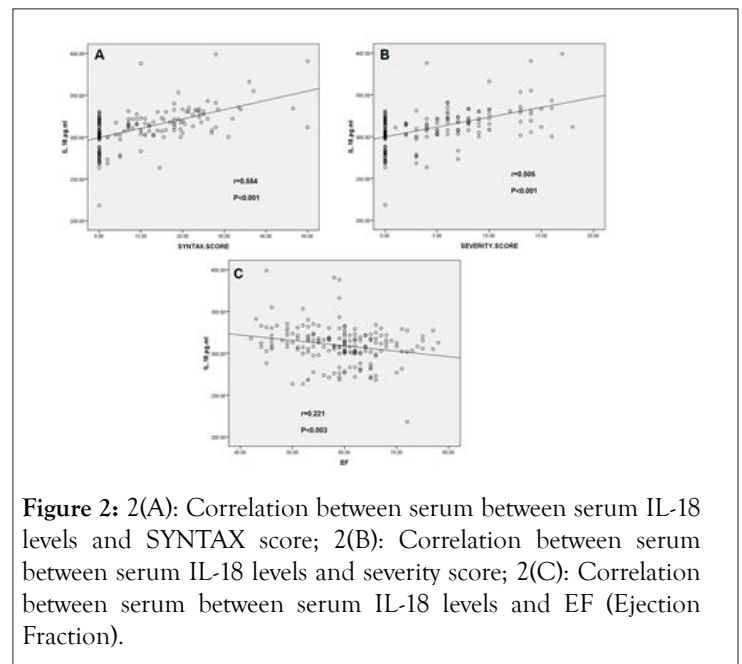
Abbreviation: SNP: Single Nucleotide Polymorphism; VD: Vessel Disease.

\* Significant P value

**Table 4:** Comparison between different genotypes of 137 G/C SNP regarding vessel score.

Furthermore, patients with GG genotype had higher vessel score and higher prevalence of three vessel disease compared to GC and CC genotypes (Table 4).

In all study population, a strong positive correlation was noted between serum IL-18 level and coronary angiographic SYNTAX score (r=0.554, P<0.001) and severity score (r=0.505, P<0.001) (Figure 2A and Figure 2B), while there was a significant inverse correlation between serum IL-18 level and EF (r=-0.221, P<0.05) (Figure 2C).



**Figure 2:** 2(A): Correlation between serum between serum IL-18 levels and SYNTAX score; 2(B): Correlation between serum between serum IL-18 levels and severity score; 2(C): Correlation between serum between serum IL-18 levels and EF (Ejection Fraction).

## DISCUSSION

Atherosclerosis is usually influenced by various inflammatory processes; IL-18 is one of the inflammatory biomarkers that lately have been in focus among researchers in cardiovascular disease. Being a member of the cytokine family, the molecule plays an important role in the inflammatory cascade [8].

Elevated circulating levels of IL-18 is associated with atherosclerotic lesions, type 2 DM, metabolic syndrome, hypertension and CAD [9,10].

The levels of IL-18 are affected by many factors of which genetic polymorphism may contribute. Studies have shown that some genetic variants of IL-18 may influence the risk and prognosis of CAD although the available genetic data suffers from disparity [11,12].

The present study was designed to evaluate serum levels of IL-18 and gene polymorphism at 137 G/C site of IL 18 gene in patients as regard absence or presence of coronary artery disease and type 2 DM.

In our study there was highly significant difference between all study groups regarding serum IL 18 levels especially between CAD groups (II and IV) and non-CAD groups (I and III). These findings run in parallel with the results of the study where IL-18 serum concentration was higher significantly in CAD patients



compared to healthy control individuals also IL-18 levels were found to be higher significantly when diabetic CAD patients were compared to non-diabetic CAD patients, and when a subgroup of the triple-vessel disease CAD diabetics was compared to the triple-vessel disease CAD non-diabetics. The lowest levels were seen in the control group, intermediate levels in the non-diabetic CAD patients, and higher levels in the diabetic CAD ones [13]. The highest levels were found in the diabetic CAD patients with triple-vessel disease. This implies that the type 2 DM patients with CAD, especially with multi-vessel disease, are characterized by the highest score of systemic inflammation and these patients are at great risk of further cardiovascular events and therefore secondary prevention should be taken particularly seriously.

Furthermore, our results are in agreement with the results of the study done who demonstrated that the IL-18 concentrations in non-CAD subjects were lower significantly than patients with stable angina. In patients with unstable angina, the IL-18 concentrations were higher significantly than those in the control group or the group with stable angina and significantly lower than patients with acute myocardial infarction [14].

Regarding the gene polymorphism of IL-18 at 137 G/C SNP, our study demonstrated that there was high significant difference between four groups regarding 137 G/C genotype distribution with predominance of CC genotype in non-CAD groups accounting 60.98% in non-diabetic non-CAD group and 52.5% in diabetic non-CAD group, and predominance of GG genotype in CAD groups accounting 62.75% in non-diabetic CAD group and 60.42% in diabetic CAD group. Furthermore, there was high significant difference between four groups regarding allele distribution with predominance of C allele in non-coronary groups and predominance of G allele in coronary groups. Serum IL-18 levels were higher in GG predominant patients than in GC and CC predominant patients. This data was in agreement with study done by who found that C allele is associated with diminished transcriptional activity of the gene and diminished production of mature IL-18. The carriers of the C allele are less prone to develop occlusions in the main branches of their coronary arteries. our results also go in agreement who found that IL-18 gene has a functional 137 G/C polymorphism (rs187238) in this promoter region and the GG genotype or G allele of 137 G/C polymorphism results in markedly higher transcription activity, leading to higher levels of the IL-18 protein than those made by the CC genotype or C allele [12].

The effects of 137 G/C polymorphisms of the IL-18 gene on patient susceptibility to In-Stent Restenosis (ISR), a significant increase of G allele or GG genotype was observed in ISR patients compared to non-ISR individuals, indicating that the GG homozygote had a higher occurrence rate for ISR when compared to the C allele carriers. The levels of serum IL-18 of GG homozygotes are obviously higher than C allele carriers. It indicates that IL-18 promoter 137 G/C polymorphism influences IL-18 levels and the occurrence of ISR.

On the other hand, in our study we didn't find a significant difference between all studied groups regarding 607 C/A genotype distribution, and there was no predominance of any

CC, CA, or AA genotype in any of the all studied groups, denoting that gene polymorphism at SNP 607 C/A is not functioning and was not associated with IL-18serum levels.

These data was in agreement with study who found no significant difference between patients with and without CAD at 607 C/A SNP site. But these data was against the results of the study who reported that the SNP polymorphism at position 607 C/A is functional and associated with IL-18 levels and associated with risk of myocardial infarction in Chinese patients, this conflict in results may be due to different ethnic groups and different environmental factors among various populations. Furthermore, genetic background of individuals from different ethnic groups as well as the environmental factors might be a reason for controversial results in different studies; moreover linkage between the selected SNPs and their continuous variants can influence the result of the study and might further explain different results among various studies.

Interestingly, in our study there was high significant correlation between serum levels of IL-18 and presence of CAD as regard severity and SYNTAX scores, these results are in agreement with described direct and continuous relationship between serum level of IL-18 and CAD severity [7].

In a IL-18 level was significantly correlated with components of the metabolic syndrome and Body Mass Index (BMI). IL-18 also showed strong correlations with measures of insulin resistance, glucose levels and lipid profile.

Furthermore, in our study we found that EF was higher in group I (non-diabetic non-CAD group) and group III (diabetic non-CAD group) than group II (non-diabetic CAD group) and group IV (diabetic CAD group), and there was negative significant correlation between serum IL-18 levels and EF. These results are in concordance with described a positive correlation between serum IL-18 levels and LVEDD and negative correlation with EF in patients with CAD.

## STUDY LIMITATIONS

First, the maintenance medications for the patients were not standardized before randomization; they were on different types and doses of statin therapy that may have a potential effect on the inflammatory process and levels of cytokines. Second, the study lacked a large validation population. Further prospective studies are thus needed to confirm our results.

## CONCLUSION

IL-18 is an independent risk factor for CAD and it is correlated with the extent and severity of CAD. Gene polymorphism of IL-18 at 137 G/C site could be a candidate risk factor for development of CAD, presumably by increasing serum IL-18 levels. Individuals carrying the G/G genotype and the G allele of IL-18 have an increased risk to develop CAD.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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