Introduction

There were a multifactorial sickness and a noteworthy reason for morbidness and mortality in all races, ethnicities, and social orders in atherosclerotic coronary artery disease (CAD) [1]. A few hazard factors, for instance, hypertension, smoking, hyperlipidemia, obesity, and diabetes, have been associated with CAD [2,3].

There was a key part for the provocative pathway in the improvement and entanglements of CAD [4]. Pro-inflammatory adds to the onset and/or to the movement of atherosclerotic appearances, through destabilizing coronary atherosclerotic plaque, and impelling of intense coronary disorders [5].

Interleukin-18 (IL-18) is a pro-inflammatory cytokine with a critical role in the inflammation that adds to atherosclerosis [6]. Expanded levels of circulating IL-18 have been might be connected with quickening of atherosclerosis and may assume a part in intense coronary disorders through plaque destabilization [7].

Variations in the IL-18 gene were connected with raised IL-18 serum focuses and higher cardiovascular mortality among CAD patients. Distinctive studies showed that IL18 polymorphisms are incorporated into the advancement of ischemic stroke [8], dead tissue [9], and higher cardiovascular mortality risk [6].

Interleukin-18 protein expression is controlled by the IL-18 promoter gene at positions 607 C/A and 137 G/C in the promoter area [10]. These promoter regions are expected to be the binding sites for Cyclic (Adenosine 30, 50-cyclic monophosphate) AMP-responsive element-binding protein (CREB) and human histone-H4 gene-specific transcription factor-1(H4TF1) [11].

Several studies have examined the association of IL-18 promoter genes and CAD, and the results are inconsistent across different populations [8,
The aim of the present study was to study the genetic variants of IL-18 (607C/A) and (137 G/C) promoter gene and assess their contributions to coronary artery disease.

Subjects and Methods

A total of 120 Egyptian patients undergoing cardiac catheterization were enrolled as a part of the Cardiology Section of The National Heart Institute, Giza, Egypt. Subjects were divided into two groups:

Group I): 60 patients (40 males and 20 females; their ages ranged from 32 to 69 years) with documented CAD. Documented CAD was diagnosed by:

a) Electrocardiograph tests, such as an electrocardiogram (ECG or EKG) or exercise stress tests, use the electrocardiogram to evaluate the electrical activity generated by the heart at rest and with activity.

b) Laboratory Tests: include a number of blood tests used to diagnose and monitor treatment for heart disease.

Group II): 60 participants (27 males and 33 females; their ages ranged from 27 to 72 years) were selected among patients without angiographic lesions, were considered as the patients without CAD (Non-CAD).

Beside 50 healthy subjects (37 males and 13 females; their ages ranged from 25 to 55 years) were enrolled as control group, who were age, sex matched with patients and they had no history of CAD, MI or stroke.

There was neither personal nor family history of autoimmune, and metabolic disease or malignancy in both patient and control groups.

All recruited subjects provided blood samples for biochemical and genotype analysis. The study protocol was approved by The Medical Research Ethics Committee of The National Research Centre (NRC) (Registration number-12-042). Written informed consent was obtained from all study participants.

Sampling: Peripheral venous blood samples (5ml) were drawn under aseptic conditions from all subjects after an overnight fasting of 12-h and divided into two parts; one part (2ml) of the whole blood was collected in EDTA coated tubes for DNA extraction for detection IL-18 promoter gene polymorphisms. The second part (3ml) of blood allowed to clot for 10-15 minutes then centrifuged at 1000xg for 5 minutes for serum separation and stored at -20°C in aliquots until required.

Biochemical analysis: Total Cholesterol (TC), Triglyceride (TG) and HDL-C were measured by enzymatic-colorimetric method according to the method described by Allain et al [12], Fossati and Prencipe [13] and Burstein et al [14] respectively using the kit manufactured by STANBIO Laboratory, USA. LDL-C was calculated using formula of Friedewald et al [15].

DNA analysis: Genomic DNA was isolated from peripheral blood leukocytes using a spin column method according to the protocol (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions.

-Genotyping method for (607 C/A) polymorphism of IL-18 promoter gene:

The genotyping of IL-18 (607C/A) was performed by polymerase chain reaction- specific sequence primer (PCR-SSP) method. The sequences of the primers were; a common reverse primer (5’-AAACCTCATTCAGGACTTCC-3’) and two sequence specific primers(5’-GTTGCAGAAAGTGTAAAAATTATTAC-3’) (for allele C) and(5’-GTTGCAGAAAGTGTAAAAATTATTAA-3’) (for allele A) were used to amplify a 196-bp product. A control forward primer (5’-CTTTGCTATCATTCCAGGAA-3’) was used to amplify a 301-bp fragment covering the polymorphic site according to Giedraitis et al [16]. PCR amplification was performed with 5 pmol of each primer in combination with Taq PCR Master Mix (New England Biolabs Inc., UK). The PCR cycling conditions were : initial denaturation at 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 30 s followed by a final extension at 72°C for 5 min (T-Gradient Thermal Cycler, Biometra, Germany). The PCR products were analyzed by electrophoresis on 3% agarose gel stained with Red-Safe then the gel was visualized under UV transilluminator with 100-bp ladder. The PCR products were of 301-bp for (control forward primer) and 196-bp band for (C allele or A allele) (Fig.1).

-Genotyping method for (137 G/C) polymorphism of IL-18 promoter gene

The genotyping of IL-18 (137 G/C) was performed by polymerase chain reaction- specific
sequence primer (PCR-SSP) method. The sequences of the primers were; a common reverse primer (5'-AGGAGGGCAAAATGCACTGG-3') and two sequence specific primers (5'-CCCCAACTTTTACGGAAGAAAAG-3') (for allele G) and (5'-CCCCAACTTTTACGGAAGAAAAC-3') (for allele C). An amplification product of 261-bp was detected. A control forward primer (5'-CCAATAGGACTGATTATTCCGCA-3') was used to amplify a 446-bp fragment covering the polymorphic site as described by Giedraitis et al.\textsuperscript{16} The PCR cycling conditions were: initial denaturation at 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 30 s followed by a final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis on 3% agarose gel stained with Red-Safe then the gel was visualized under UV transilluminator with 100-bp ladder. The PCR products were of 446-bp for (control forward primer) and 261-bp band for (G allele or C allele) (Fig. 2).

**Statistical analysis**

Data are expressed as means ± standard deviation (SD) for quantitative variables, frequency for qualitative variables. Quantitative variables were compared using independent student t-test and one-

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**Figure (1A): C Allele**

<table>
<thead>
<tr>
<th>Lane</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Molecular DNA Ladder (100-1000 bp)</td>
</tr>
<tr>
<td>1, 2, 3, 7</td>
<td>Heterozygous CA genotype yielded 2 bands of 196 bp</td>
</tr>
<tr>
<td>4, 5, 8</td>
<td>Homozygous AA genotype yielded 1 band of 196 bp (for A allele)</td>
</tr>
<tr>
<td>6</td>
<td>Homozygous CC genotype yielded 1 band of 196 bp (for C allele)</td>
</tr>
</tbody>
</table>

**Figure (1B): A Allele**

<table>
<thead>
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<th>Lane</th>
<th>Description</th>
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<tbody>
<tr>
<td>M</td>
<td>Molecular DNA Ladder (100-1000 bp)</td>
</tr>
<tr>
<td>1</td>
<td>Control forward primer (446 bp)</td>
</tr>
<tr>
<td>2, 3, 4, 5, 7, 8</td>
<td>PCR product for A allele (261 bp)</td>
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</table>

**Figure (2A): G Allele**

<table>
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<th>Description</th>
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<tbody>
<tr>
<td>M</td>
<td>Molecular DNA Ladder (100-1000 bp)</td>
</tr>
<tr>
<td>1, 2, 3</td>
<td>Heterozygous GC genotype yielded 2 bands of 261 bp</td>
</tr>
<tr>
<td>4, 5, 6</td>
<td>Homozygous GG genotype yielded 1 band of 261 bp (for G allele)</td>
</tr>
<tr>
<td>7, 8</td>
<td>Homozygous CC genotype yielded 1 band of 261 bp (for C allele)</td>
</tr>
</tbody>
</table>

**Figure (2B): C Allele**

<table>
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<th>Description</th>
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<tr>
<td>M</td>
<td>Molecular DNA Ladder (100-1000 bp)</td>
</tr>
<tr>
<td>1</td>
<td>Control forward primer (461 bp)</td>
</tr>
<tr>
<td>2, 3, 4, 5, 6, 7, 8</td>
<td>PCR product for C allele (261 bp)</td>
</tr>
</tbody>
</table>

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way ANOVA, LSD test was used for multiple post-hoc comparisons. On the other hand, qualitative variables were compared using Chi square (X²) test or Fisher's exact test. The statistical Package for the Social Science Software (SPSS 17.0, Chicago, IL, USA) was used, P< 0.05 was considered significant.

**Results**

**Anthropometric characteristics of patients with and without CAD:**

The Anthropometric characteristics of patients with and without coronary artery disease (CAD) are shown in (Table 1). There was a significant difference among CAD and Non-CAD regarding sex distribution. The CAD patients had higher frequencies of AR and RV when compared to the non-CAD group. Furthermore, there were non-significant differences between CAD and non-CAD groups regarding age, diabetes mellitus, obesity, hypertension, smoking. LVEDD, LVESD, LV SWT, LVPWT, LA, EF% and FS%.

**Biochemical characteristics of all studied groups:**

The change of biochemical parameters for patients with and without CAD and control subjects according to lipid profile are shown in (Table 2). The levels of total cholesterol, triglyceride and LDL-C were significantly higher in CAD group as compared to non-CAD. Additionally, levels of HDL-C were significantly lower in CAD as compared to non-CAD patients.

**Genotype distribution and allele frequencies of (607C/A) and (137G/C) polymorphisms in IL-18 promoter gene:**

The genotype distribution and allele frequencies of IL-18 promoter gene polymorphisms (607C/A) and (137G/C) in the different studied groups are shown in (Table 3).

For IL-18 (607C/A), the frequency of the mutant CA genotype was fundamentally lower in patients with CAD contrasted with non-CAD and

<table>
<thead>
<tr>
<th>Variables</th>
<th>Without CAD (n= 60)</th>
<th>CAD (n=60)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.1 ± 10.18</td>
<td>53.3 ± 8.3</td>
<td>0.136</td>
</tr>
<tr>
<td>Gender</td>
<td>Male (M)</td>
<td>27 (45%)</td>
<td>40 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>Female (F)</td>
<td>33 (55%)</td>
<td>20 (33.3%)</td>
</tr>
<tr>
<td>Diabetes Mellitus (DM)</td>
<td>Positive (+)</td>
<td>21 (35%)</td>
<td>21 (35%)</td>
</tr>
<tr>
<td></td>
<td>Negative (-)</td>
<td>39 (65%)</td>
<td>39 (65%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>Positive (+)</td>
<td>21 (35%)</td>
<td>22 (36.7%)</td>
</tr>
<tr>
<td></td>
<td>Negative (-)</td>
<td>39 (65%)</td>
<td>38 (63.3%)</td>
</tr>
<tr>
<td>Hypertension (HTN)</td>
<td>Positive (+)</td>
<td>28 (46.7%)</td>
<td>33 (55%)</td>
</tr>
<tr>
<td></td>
<td>Negative (-)</td>
<td>32 (53.3%)</td>
<td>27 (45%)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>140 ± 25.92</td>
<td>145 ± 26.39</td>
<td>0.671</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83.2 ± 13.96</td>
<td>80 ± 15</td>
<td>0.758</td>
</tr>
<tr>
<td>Smoking</td>
<td>Positive (+)</td>
<td>10 (16.7%)</td>
<td>11 (18.3%)</td>
</tr>
<tr>
<td></td>
<td>Negative (-)</td>
<td>50 (83.3%)</td>
<td>49 (81.7%)</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>5.2 ± 0.534</td>
<td>5.7 ± 0.512</td>
<td>0.403</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>3.9 ± 0.622</td>
<td>4.7± 0.585</td>
<td>0.604</td>
</tr>
<tr>
<td>LV SWT (mm)</td>
<td>1.14 ± 1.04</td>
<td>1.02 ± 1.007</td>
<td>0.187</td>
</tr>
<tr>
<td>LVPWT (mm)</td>
<td>1.016 ± 0.122</td>
<td>1.03 ± 1.07</td>
<td>0.572</td>
</tr>
<tr>
<td>LA</td>
<td>3.61 ± 0.561</td>
<td>3.92 ± 0.496</td>
<td>0.175</td>
</tr>
<tr>
<td>AR</td>
<td>2.99 ± 0.434</td>
<td>3.29 ± 0.254</td>
<td>0.008</td>
</tr>
<tr>
<td>RV</td>
<td>2.46 ± 0.477</td>
<td>2.35 ± 0.284</td>
<td>0.000</td>
</tr>
<tr>
<td>EF (%)</td>
<td>56.3 ± 8.01</td>
<td>53.4 ± 10.71</td>
<td>0.32</td>
</tr>
<tr>
<td>FS (%)</td>
<td>28.6 ± 7.33</td>
<td>25.1± 7.25</td>
<td>0.477</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD for quantitative variable or number (%) for qualitative one.

SBP: Systolic blood pressure, DBP: Diastolic blood pressure
LVEDD: Left ventricular end-diastolic diameter
LVESD: Left ventricular end-systolic diameter
LV SWT: Left ventricular systolic wall thickening
LVPWT: Left ventricular posterior wall thickening
LA: Left atrium, AR: Aortic root, RV: Right ventricle
EF%: Ejection fraction, FS %: Fractional shortening
Bold values indicate a significant difference, P ≤ 0.05 was considered significant.

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control, while the frequencies of the mutant AA and the wild CC genotypes were altogether lower in CAD group when compared to non-CAD and control groups. Additionally, the mutant A allele demonstrated a fundamentally significantly lower frequency in patients with CAD as compared to non-CAD and control groups, while the wild C allele demonstrated a significant lower frequency in patients with CAD compared to non-CAD and control.

For IL-18 (137G/C), the findings uncovered that the frequency of the mutant GC genotype was altogether lower in CAD when compared to non-CAD and control, while, the wild GG genotype demonstrated higher frequency in CAD group when compared to non-CAD and control. There was no critical distinction among various studied groups in regards to distribution of the mutant CC genotype. On the other side, the frequency of the mutant C allele demonstrated an essentially lower level in patients with CAD compared to non-CAD and control groups, while the frequency of the wild G allele demonstrated an altogether higher in CAD group compared to non-CAD and control groups.

**Discussion**

Coronary artery disease is a multifactorial...
issue and its pathogenesis is not yet completely understood [17].

A positive relationship of sex in patients with coronary artery disease was observed. This is in concurrence with Hemingway et al [18] and Roger et al [19]. It provides the idea that the pathophysiology of CAD differs amongst women and men and this can be clarified by Dickerson et al [20] who demonstrated that on cardiovascular computed tomography, women have been appeared to have smaller coronary artery diameters than men do likewise, ladies need additional doubtful over men would will have obstructive lowlife at the chance about coronary angiography.

We demonstrated that a critical increment in lipid parameters; cholesterol, triglycerides, low density lipoprotein and a critical decline in high density lipoprotein in CAD patients. Likewise, a positive relationship of TC/HDL ratio in CAD patients was watched. These outcomes are in the same line with the results of Hammoudeh et al [21], Mohsen et al [22] and Yang et al [23].

This might be clarified on the foundation that (LDL) is the essential atherogenic lipoprotein and (HDL) is the predominant hostile to atherosclerotic lipoprotein; where LDL transports cholesterol from the liver to peripheral tissues and advances the foaming of macrophages inside the arterial wall. Alternately, HDL animates the efflux of abundance cell cholesterol and contrarily transports it to the liver.

Yang et al [23] showed that the degree between the atherogenic and defensive lipoproteins, and have more prominent prescient force for evaluating the degree of lipid gathering in the blood vessel mass of atherosclerotic intimal changes.

Rafaela et al [24] demonstrated that expansions in triglycerides lead to expanded danger of coronary events and progression of coronary artery disease, in addition to the formation of new lesions.

Regarding TC/HDL proportion, its association with the presence and seriousness of CAD, clinical and angiographic studies has connected it with the progression or regression of CAD [25]. Subsequently, this finding reinforces the significance of the estimation of the TC/HDL proportion as an individual risk factor for CAD, additionally as an indicator of extent of the disease, even within the sight of cholesterol levels considered typical, hence proposing the imbalance between TC and HDL levels plays more essential part in the pathophysiology of atherogenesis [24].

Our results found that (607 C/A) IL-18 promoter variant was not connected with CAD, while the wild GG genotype and/or the G allele of the (137 G/C) IL-18 indicated a huge relationship in patients with CAD. Our discoveries were supported by Zhang et al [8], Koch et al [9], Mahajan et al [26] and Gurram et al [27] who examined the relationship between (137G) allele with expanded danger of larger artery atherosclerosis.

Likewise, Hernesniemi et al [3] exhibited that (SNP) at position (137G/C) was connected with the event of sudden cardiac death among Caucasian males. In support of this discovering, Liu et al [28] proposed that 137G/C polymorphism influenced the advancement of atherosclerosis in the Chinese Han populace.

Despite what might be expected, the last study was conflicting with the comparative study described by Fang et al [29] who reported that IL-18 (607 C/A) might be connected with danger of acute myocardial infarction in northern Chinese Han populace.

Moreover, Liu et al [28] assessed the relationship between angiographically demonstrated CAD patients and CAD people and the promoter locale SNP (G-to-C) of IL-18 at position - 137. A critical increment of G allele or GG-genotype was seen in CAD patients contrasted with CAD individuals, demonstrating that the GG homozygote had a higher event rate for CAD when contrasted with the C allele.

On the contrary, Lu et al [30], Opstad et al [31] and Tsai et al [32] have presented that genetic polymorphisms of IL-18 (-607A/C) and (-137C/G) may influence the immune reaction, and can have a part in the risk of a several sorts of diseases. Our findings uncovered that there was no significant difference in IL-18 (607C/A) and (137G/C) in CAD patients and also patients without CAD and controls.

These data agreed by Shayan et al [33] who demonstrated that there was no noteworthy distinction in the -137G/C and -607C/A between both subgroups of patients {CAD with MI} and {CAD without MI} and control group.

The study found no relationship between
(607C/A) and (137G/C) polymorphisms and CAD risk factors (diabetes, hypertension, smoking and obesity) and this is in accordance with those noted by Shayan et al [33] who examined IL-18 genotypes with some risk of exposure for CAD and the outcomes did not demonstrate a relationship between them.

This study is one of the first studies exploring the role of IL-18 gene promoter polymorphism in the etiology of CAD and its relation with risk factors in Egyptian populations. Further evidence of the real roles of IL-18 gene polymorphism in the pathogenesis of developing CAD in Egyptian population should be investigated by large population-based studies. Since coronary artery disease remains an important focus of investigation in the order it could be identified and predicted early to help clinicians manage the patients better.

Conclusion

Inflammation, a major contributor to atherosclerosis and CAD pathogenesis, and investigations of inflammatory biomarkers could be used in diagnosing, prognosticating and managing CAD.

We concluded that the elevation of total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and lowered high-density lipoprotein-cholesterol (HDL-C) are conventional risk factors in coronary artery disease patients.

The major finding of the present study is that G allele and/or GG genotype at position-137 in IL-18 promoter gene polymorphism could be a risk factor for coronary artery disease and may have protective effects. Whereas, IL-18 (607C/A) gene polymorphism is not associated with the risk of coronary artery disease.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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References


13. Fossati, R., Prencipe, L., Serum triglycerides determined colorimetrically with an enzyme


GENETIC VARIANTS OF INTERLEUKIN-18 PROMOTER GENE...


Genetic Variants of Interleukin-18 Promoter Gene...

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The genetic variants of the interleukin-18 promoter gene (IL-18) are associated with the development of coronary artery disease. The current study aimed to investigate these variants and their potential role in the disease.

The study was conducted on a sample of 100 patients with coronary artery disease and 60 healthy individuals as a control group. The genetic variants of the IL-18 promoter gene (137G/C and 607C/A) were detected using polymerase chain reaction with sequence-specific primers (PCR-SSP).

The results showed a significant increase in lipid levels (cholesterol, triglycerides, and low-density lipoprotein) in patients with coronary artery disease. The frequency of the 137G/G variant was higher in the control group compared to the patient group.

The study concluded that genetic variants of the interleukin-18 promoter gene are associated with the development of coronary artery disease.