Evaluation of Serum Interleukin-6 levels and Lipid Profile as Biomarkers in Egyptian Patients with Atherosclerotic Coronary Artery Disease

Ibrahim H. Borai, Olfat G. Shaker, Nahla S. Hassan, Esmat Ashour, Mohammed El Badrawy, Olfat M. Fawzy, Lamiaa Mageed

Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt
Medical Biochemistry Department, Faculty of Medicine, Cairo University, Egypt
Biochemistry Department, National Research Centre, ElBuhous St, P.O. J2622, Dokki, Cairo, Egypt.
The National Heart Institute, Giza, Egypt.

ABSTRACT

Background: Inflammatory processes are now recognized to play a central role in the pathogenesis of atherosclerosis and its complications. Plasma levels of several markers of inflammation have been found to be associated with future cardiovascular risk in a variety of clinical settings. Vascular lipid accumulation and inflammation are hallmarks of atherosclerosis and perpetuate atherosclerotic plaque development. Mediators of inflammation such as interleukin-6 (IL-6), are related to the arterial wall inflammatory process and may contribute to the pathogenesis of atherosclerosis.

Objective: To assess the association of serum interleukin-6 [IL-6] with atherosclerosis and risk factors such as diabetes, hypertension, dyslipidemia, and obesity in Egyptian patients.

Subjects: A total of 120 Egyptian Subjects [60 patients with CAD and 60 patients without CAD] were included in the study besides age - sex matched 50 healthy controls were taken for comparison.

Methods: Lipid profiles [total cholesterol, triglyceride, HDL-C] were measured by enzymatic colorimetric method and serum IL-6 levels were determined using ELISA.

Results: Our data revealed a significant association between serum IL-6 level and lipid profiles [total cholesterol, triglyceride, HDL-C and LDL-C] and elevation of serum IL-6 level was observed in atherosclerotic patients compared with healthy subjects.

Conclusion: The present study showing a strong association between IL-6 and dyslipidemia as an important risk factor of atherosclerosis and a markedly high level of IL-6 in Patients with atherosclerosis, based on which suggests that IL-6 may be served as an early and susceptibility biomarker in the pathogenesis of atherosclerosis.

INTRODUCTION

Atherosclerotic coronary artery disease [CAD] is characterized by the accumulation of atheromatous plaques within the walls of the coronary arteries. In the development of coronary atherosclerosis, as an indicator of atherosclerotic disease, calcium precipitates in the coronary artery wall and preventing the progression of atherosclerosis is therefore important [1]. However, in atherosclerotic high-risk patients, the conventional management of traditional risk factors is insufficient to prevent the development of atherosclerosis.

Cholesterol fractions, such as low density lipoprotein cholesterol [LDL-C] and high density lipoprotein cholesterol [HDL-C], are commonly measured markers in clinical medicine. The lipoprotein system has been linked to atherosclerotic disease, as LDL-C is related to lesion formation and growth, and HDL-C is protective against atherosclerosis [2 & 3].

IL-6, a circulating multifunctional cytokine, that is produced by several cell types, including activated macrophages, endothelial cells and smooth muscle cells, has been recognized as a potential marker linked to cardiovascular events [4]. It is considered to be a key mediator in both low-grade inflammatory process and the onset of inflammatory crisis. Large epidemiologic studies have demonstrated that increased levels of IL-6 are associated with several diseases such as hypertension, malnutrition, cardiovascular events and atherosclerosis [5 & 6].

Corresponding Author: Esmat Ashour Biochemistry Department, Faculty of Science, Ain Shams University, P. O. J2622, Cairo, Egypt.
E-mail: esmatashour3@gmail.com
IL-6, which is produced and secreted by macrophages, endothelial cells and T lymphocytes, is measurably elevated in acute and chronic CAD [7]. IL-6 is also increased during muscle contraction and may play a role in insulin resistance [8]. It has been associated with autoimmune disorders, such as rheumatoid arthritis [9], which is itself an independent risk factor for CAD [10]. IL-6 plays a number of important roles in the immune system, including up regulation of CRP [7], but its role in predicting CAD onset and progression is still being evaluated. Intra-individual variation and short half-life of IL-6 may limit the clinical utility of IL-6 measurement, and additional studies are needed to fully characterize its role as an inflammatory biomarker for CAD [8].

Objective:
The current study aimed to investigate the association of serum IL-6 level with atherosclerosis and risk factors including diabetes, arterial hypertension, hyperlipidemia and obesity in Egyptian patients. As well as to determine the lipid profile and to identify the correlation between Lipid profile and coronary artery disease [CAD].

Subject And Method:

Study Subjects:

A total of 120 Egyptian patients undergoing cardiac catheterization were enrolled as 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.

c) Invasive Testing, such as cardiac catheterization, involve inserting catheters into the blood vessels of the heart in order to get a closer look at the coronary arteries.

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.

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 National research Centre [NRC] and National Heart Institute [NHI] Research Ethics Committees. All subjects gave written informed consent to participate in this study.

Methods:

A) Sample Collection:

Laboratory assays were measured using venous blood obtained after a 12-h fast. Three milliliters fasting venous blood were collected the morning after enrollment in the hospital, and within 30 min, all blood samples were centrifuged at 4°C and at 3000 rpm for 10 min. The serum was separated, and stored at -70°C for detection within 6 months.

Biochemical Analyses:

Lipid Profile measurement:

Total Cholesterol [TC], Triglyceride [TG] and HDL-C were measured by enzymatic-colorimetric method according to the method described by Allain et al., [11], Fossati and Prencipe, [12] and Burstein et al., [13] respectively using the kit manufactured by STANBIO Laboratory, USA. LDL-C was calculated using Friedewald formula: LDL-cholesterol=TC-[HDL+TG/5] [14].

IL-6 measurements:

Serum level of IL-6 was determined by the Enzyme-Linked immunosorbent Assay [ELISA] kit by using AviBion Human IL-6 ELISA kit [15] according to the manufacturer’s instruction.

Statistical Analysis:

Data are expressed as means ± standard deviation for quantitative variables, frequency for qualitative variables. Quantitative variables were compared using independent student t-test and one-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 Fischer'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:

Demographic and Clinical data for the patient groups:

The demographic and clinical characteristics of patients with and without coronary artery disease [CAD] are shown in Table 1.

The coronary artery disease group [CAD] was compared with patients without coronary artery disease [Non-CAD] regarding age, Systolic blood pressure [SBP], diastolic blood pressure [DBP], diabetes mellitus, obesity, hypertension and smoking. There were no statistical significant differences among studied groups [CAD & Non CAD] regarding ages [53.3 ± 8.3 and 52.1 ± 10.18, years respectively], SBP [145 ± 26.39, 140 ± 25.92 mmHg respectively] and DBP [80 ± 15, 83.2 ± 13.96 mmHg respectively]. As well as distribution of both diabetes mellitus [35%, 65% and 35%, 65%, respectively], obesity [36.7%, 63.3% and 35%, 65%, respectively], hypertension [55%, 45% and 46.7%, 53.3%, respectively], and smoking [18.3%, 81.7% and 16.7%, 83.3%, respectively] in CA and Non CAD.

Furthermore, there was a significant difference among CAD, Non-CAD regarding sex distribution [M/F: 40/20 and 27/33, respectively].

Table 1: Demographic data of patients with and without coronary artery disease.

<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>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male [M]</td>
<td>27 [45%]</td>
<td>40 [66.7%]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Female [F]</td>
<td>33 [55%]</td>
<td>20 [33.3%]</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus [DM]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive [+ ]</td>
<td>21 [35%]</td>
<td>21 [35%]</td>
<td>0.576</td>
</tr>
<tr>
<td>Negative [- ]</td>
<td>39 [65%]</td>
<td>39 [65%]</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive [+ ]</td>
<td>21 [35%]</td>
<td>22 [36.7%]</td>
<td>0.500</td>
</tr>
<tr>
<td>Negative [- ]</td>
<td>39 [65%]</td>
<td>38 [63.3%]</td>
<td></td>
</tr>
<tr>
<td>Hypertension [HTN]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive [+ ]</td>
<td>28 [46.7%]</td>
<td>33 [55%]</td>
<td>0.233</td>
</tr>
<tr>
<td>Negative [- ]</td>
<td>32 [53.3%]</td>
<td>27 [45%]</td>
<td></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></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive [+ ]</td>
<td>10 [16.7%]</td>
<td>11 [18.3%]</td>
<td>0.500</td>
</tr>
<tr>
<td>Negative [- ]</td>
<td>50 [83.3%]</td>
<td>49 [81.7%]</td>
<td></td>
</tr>
</tbody>
</table>

SBP= Systolic blood pressure  DBP= Diastolic blood pressure

Data are expressed as mean ± SD for quantitative variable or number [%] for qualitative one

*Significantly different from patients without CAD.

*Bold values indicated significant difference.

P value < 0.05 was considered significant.

1.1. Biochemical characteristics of the all studied groups:

The change of biochemical parameters for patients with and without coronary artery disease [CAD] and healthy control subjects according to lipid profiles [total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total C/HDL-C] and interleukin-6 [IL-6] are shown in Table 2 and [Fig. 1&2].

Table 2: The change of lipid profiles and IL-6 levels in patients with and without coronary artery disease and control groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control [n=60]</th>
<th>Without CAD [n= 60]</th>
<th>CAD [n=60]</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol [mg/dl]</td>
<td>172 ± 24.25</td>
<td>212 ± 24.16</td>
<td>279 ± 45.25 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL Cholesterol [mg/dl]</td>
<td>80.98 ± 10.39</td>
<td>63.65 ± 10.55</td>
<td>48.61 ± 9.92</td>
<td>0.765</td>
</tr>
<tr>
<td>LDL Cholesterol [mg/dl]</td>
<td>61.9 ± 16.3</td>
<td>120.7 ± 13.49</td>
<td>192.9 ± 41.31*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride [mg/dl]</td>
<td>115.7 ± 13.8</td>
<td>155.6 ± 24.95</td>
<td>186.6 ± 35.39*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total C/HDL-C</td>
<td>2.1 ± 0.188</td>
<td>3.37 ± 0.52</td>
<td>5.9 ± 1.32*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IL-6 [pg/ml]</td>
<td>12.6 ± 6.25</td>
<td>11.6 ± 3.86</td>
<td>23.5 ± 6.68*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Fig. 1: Lipid Profiles in all Different Studied Groups.
Regarding lipid profiles, the levels of total cholesterol, triglyceride and LDL cholesterol were significantly higher in CAD group \(279 \pm 45.25, 186.6 \pm 35.39\) and \(192.9 \pm 41.31\), respectively when compared to non-CAD \(212 \pm 24.16, 155.6 \pm 24.95\) and \(120.7 \pm 13.49\), respectively] [P<0.05]. Furthermore, levels of HDL-cholesterol were significantly decreased in CAD patients \(48.61 \pm 9.92\) as compared to non-CAD \(63.65 \pm 10.55\) and control groups \(80.98 \pm 10.39\) [P<0.05]. Also, serum IL-6 level was significantly higher in CAD patients \(23.5 \pm 6.68\) compared to non-CAD group [11.6 ± 3.86] and controls [12.6 ± 6.25] [P<0.05].

The serum IL-6 and both of cholesterol, triglyceride, and LDL cholesterol levels were positively correlated with each other \(r = 0.550, 0.460\) and \(0.583\), respectively] in all cases of patients [Non-CAD+CAD] group [Fig 3, 4&5]. While there was negative correlation between IL6 and HDL –cholesterol \(r=-0.413\) in all cases of patients [Non-CAD+CAD] group [Fig 6].

### Table [3] represents the sensitivity [Sn], specificity [Sp], positive predictive value [PPV], negative predictive value [NPV], accuracy, positive likelihood ratio [PLR] and negative likelihood ratio [NLR] calculated for IL-6 level. At IL-6 value 8.80 [Pg/ml], the sensitivity and NPV values were 100% and 83.3% respectively, but the specificity [30%] and PPV [78.9%] decreased. In ROC analysis, the area under the receiver characteristic curve for IL-6 value [8.80] indicating the ability of IL-6 values for distinguishing coronary artery disease from without coronary artery disease group [Figure 1]. For CAD group, positive and negative likelihood
ratios [PLR, NLR] for serum IL-6, showed small changes but there was no significant changes observed in PLR and NLR of Patient without CAD.

![Figure 5](image_url)

**Fig. 5:** Correlation between IL-6 level and LDL-Cholesterol conc. in all studied patient with Atherosclerosis diseases.

![Figure 6](image_url)

**Fig. 6:** Correlation between IL-6 level and HDL-Cholesterol conc. in all studied patient with Atherosclerosis diseases.

**Table 3:** Cut off, percent sensitivity, specificity, positive and negative predictive values [PPV, NPV], accuracy, positive and negative likelihood ratios [PLR, NLR] for IL-6 level of patient groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut off</th>
<th>Sensitivity % [95% CI]</th>
<th>Specificity % [95% CI]</th>
<th>PPV % [95% CI]</th>
<th>NPV % [95% CI]</th>
<th>Accuracy %</th>
<th>PLR [95% CI]</th>
<th>NLR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 level</td>
<td>8.80</td>
<td>100</td>
<td>30</td>
<td>78.9</td>
<td>83.3</td>
<td>84</td>
<td>1.34</td>
<td>0.74</td>
</tr>
</tbody>
</table>

![Figure 7](image_url)

**Fig. 7:** Receiver Operating Characteristic [ROC] curve for IL-6 level.

**Discussion:**
Since inflammation is believed to have a role in the pathogenesis of cardiovascular events, measurement of markers of inflammation has been proposed as a method to improve the prediction of the risk of these events [16].
Dyslipidemia is one of the primary causes for coronary artery disease [CAD]. Elevated total cholesterol [TC], triglycerides [TG], low-density lipoprotein-cholesterol [LDL-C] and lowered high-density lipoprotein-cholesterol [HDL-C] are conventional risk factor in myocardial infarction patients [17].

Most international studies emphasized the importance of elevated LDL-C and TC levels in the development of CAD. They considered these factors as more important than the other risk factors studied [hypertension, smoking and diabetes]. Although observational studies show that different lipid fractions have opposing associations with risk of myocardial infarction, the magnitude of the atherogenic/protective power of these factors is not known. In risk estimation, changes in ratios have been shown to be better indicators of successful CHD risk reduction than changes in absolute levels of lipids or lipoproteins [18 & 19].

A main underlying pathology of coronary artery disease is the deposition of cholesterol in the arteries supplying blood to the heart that leads to stenosis and myocardial infarction. We tested if dyslipidemia is a risk factor for coronary artery disease in the Egyptian patients, and studied the role of the total cholesterol/HDL-cholesterol [TC/HDL-C] ratio as a biological marker of coronary artery disease.

Our result indicated that the all lipid profile except HDL-Cholesterol is higher in patients with coronary artery disease than patient without CAD. This results agreement with Conkbayir et al. [20].

Ishikawa et al. [21] examined the clinical relevance of high-density lipoprotein cholesterol [HDL-C] efflux capacity from macrophage [cholesterol efflux capacity] as a predictor of atherosclerotic coronary artery disease [CAD] in comparison with that of conventional coronary and lipid risk variables in Japanese daily practice.

The association between the serum levels of total cholesterol and low-density lipoprotein cholesterol [LDL-C] in the development of CAD has been well established, whereas low serum levels of HDL-c have been considered a major risk factor for CHD [8].

Inflammatory biomarkers have been shown to be associated with and to predict the onset of cardiovascular events [22, 23 & 1]. The predictive value of these markers, including interleukin-6 [IL-6], has been demonstrated for subjects with existing coronary artery disease [CAD] and apparently healthy subjects [24 & 25].

Previous studies have shown that pro-inflammatory cytokines, such as interleukin-6 [IL-6], play a central role in the pathogenesis of atherosclerosis [26]. Interleukin-6 [IL-6] is a pleiotropic cytokine with important roles in both immunoregulation and non-immune events in a variety of cell types and tissues outside of the immune system [27]. IL-6 and its downstream signaling factors contribute to both atherosclerotic plaque development and plaque destabilization [28 & 29]. Also, IL-6 has been shown to inhibit lipoprotein lipase activity and stimulate lipolysis, which affects lipid profiles which could contribute to the pathogenesis of atherosclerotic disease [30].

Our data indicated that serum IL-6 was significantly increased in all patients with atherosclerosis, when compared with its level in healthy subjects. These results were in good agreement with recent results of Hudzik et al. [31] who have reported a strong correlation between IL-6 and the extent of asymptomatic left ventricular ejection fraction [LVEF] in patients with documented CAD. Also, Xiao et al. [32] reported that chronic elevation of IL-6 in the systemic circulation was consistently linked with an increased risk of cardiovascular morbidity and mortality in the independent of other factors of systemic inflammation. In another study, Sarwar et al. [33] found that elevated serum IL-6 could significantly predict the risk of acute myocardial infarction. Furthermore, Swerdlow et al. [34] showed that IL-6 level was the best predictor of critical coronary stenosis with the highest sensitivity and specificity.

In our study, we found that serum level of IL-6 was positively associated with TC, TG, and LDL in patients with atherosclerosis, indicating the intimate connection of IL-6 and blood lipid profile in atherosclerosis. Our results could be explained on the basis that dyslipidemia is one of components in cardiovascular disease [CVD], and is a well acknowledged risk factor for the progression of atherosclerosis [35].

Conclusion:

Inflammation, a major contributors to atherosclerosis and CAD pathogenesis, and markers of inflammation being potentially useful in predicting the course of CAD have led to the investigation of biomarkers as tools that could be used in diagnosing, prognosticating and managing CAD and many other conditions.

The present study supports that indicate the elevation of the serum IL-6 is associated with the pathogenesis of atherosclerosis and may be used as a quantitative biomarker in atherosclerosis. As this study is limited with less number of cases and controls, hence, further studies are warranted to explore the relationship between IL-6 and other risk factors. Finally, further trials are needed to confirm the potential benefits of statins amongst individuals with elevated IL-6 levels.

The study concludes that the importance of assessing the lipid profile and their ratio even in a normal individual as these are atherogenic factors for development of myocardial infarction and other coronary complications. The major finding of the present study is that elevated total cholesterol [TC], triglycerides [TG], low-density lipoprotein-cholesterol [LDL-C] and lowered high-density lipoprotein-cholesterol [HDL-C] are conventional risk factor in coronary artery disease patients.
In this analysis, HDL-C is an even stronger predictor for CAD than some other major classical risk and has a strong association with coronary artery disease [CAD].

ACKNOWLEDGMENTS

This work was supported by grants from the National Research Centre [NRC] and by Academy of Scientific Research and Technology [ASRT] Fund [For PhD, Code no. R6], Egypt. We thank the patients and volunteers who participate in the study.

Conflict of Interests:
The authors declare that they have no conflict of interests.

REFERENCES


