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RESEARCH ARTICLE

Study of serum levels of interleukine-18 (IL-18) and leptin in Iraqi patients with acute coronary syndromes

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Abstract

Background: Acute Coronary Syndromes (ACS) represents a pathological, diagnostic, and risk continuum from unstable angina through myocardial infarction (MI) with or without ST-segment elevation. These three conditions share a very similar pathology, although treatment differs. Interleukin -18 (IL-18) is a recently evaluated cytokine synthesized by Kupffer cells. It has been shown to activate macrophages and has direct pro-inflammatory activities of many cells including inflammatory and vascular cells**.** Adipose tissue also serves as an important endocrine organ by producing hormones such as leptin, resistin, and cytokine. Several traditional cardiovascular risk factors track with inflammatory biomarkers, in particular central obesity and body mass index.

Objective: The aim of this study was to investigate the predictive value of IL-18 and leptin in acute coronary syndromes. And to assess the relation of serum IL-18 & leptin levels with various cardiovascular risk factors.

Patients and Methods: 85 cardiac patients were admitted to the coronary care unit, Ibn-Alnafees Hospital and Al Kindy Hospital over the period July 2012 and March 2013 with the clinical diagnosis of acute coronary syndrome their ages range was (32-78) years, the number of male was (50), (58. 82 %) and female was (35), (41.18 %). 30 healthy control (age, sex-matched) were enrolled in this study. All cardiac patients have routine ECG, cardiac biomarkers measurements especially (Troponin), lipid profile test, cytokine profile (IL-18), and Leptin. 8 ml of blood needed for assessment of the above makers.

Results: The current study reported that the patients with ACS had significantly higher level of IL-18 & leptin levels than the healthy controls ,a significant positive correlation was found in mean serum IL-18 level with BMI in ACS (AMI & UA) while a significant positive correlation was observed between leptin with BMI in AMI but non-significant in UA patients. on the other hand a positive correlation was observed between IL-18 and Leptin in AMI, while a negative correlation was observed in unstable angina patients.

Conclusion: this study shows a significantly increase in IL-18 and leptin in the circulation of patients with ACS as compared with healthy control. These finding suggest these biomarkers may be useful in diagnosis of patients with ACS. Also Serum IL-18 may play an important role in the instability of atherosclerotic plaque

1. Introduction

Coronary atherosclerosis is the cause of CHD (1, 2). Typical symptoms are chest pain and dyspnoea on exertion and are the result of reduced blood flow to myocardium. The reduction in blood flow is, in turn, caused by atherosclerotic plaques narrowing the coronary vascular lumen and thus decreasing the nutritional blood flow. In the event of sudden rupture or erosion of the plaque, a thrombogenic mass bulges into the arterial lumen, activates thrombocytes and the coagulation system.

Acute myocardial infarction (AMI) is one of the major causes of mortality and morbidity in the world (3). The most common cause of an AMI is atherosclerotic coronary artery disease (CAD) with erosion or rupture of a plaque causing transient, partial or complete arterial occlusion.

Heart cannot continue to function without adequate blood flow, and if it is severely compromised, death is inevitable.).several risk factors for coronary heart disease have been well documented, including hypertension, hyperlipidemia, diabetes, appositive family history, smoking, obesity and inactivity. However, these factors explain only part of attributable cardiovascular disease.(4,5). Interleukin -18 (IL-18) is a recently evaluated cytokine synthesized by Kupffer cells which has been shown to activate macrophages(6), and has direct pro-inflammatory activities of many cells including inflammatory and vascular cells(7). IL-18 and its receptor expression were found to be increased in human atherosclerotic plaques and promote atherosclerotic plaques instability. Moreover, it has been observed that inhibition of IL-18 decreases plaque development and induces a switch to a stable plaque phenotype (8). This inflammatory mechanisms have an important role in the pathogenesis of atherosclerosis and the occurrence of acute coronary syndromes (ACS)(9). More recently, leptin was found to have multiple roles

in the cardiovascular system as a vasoactive substance, in coronary artery vasoreactivity, regulation of myocardial blood flow,(10) may also have a prothrombotic effect,(11) and may increase with acute myocardial infarction (AMI).(12) Serum leptin was independently associated with serum CRP, suggesting that body fat may be the most significant predictor of CRP.(13)

, the present study was undertaken to assess the serum levels of IL-18 and leptin in patients with ACS.

2. Subjects and Methods

The population consisted of 115 subjects divided into three groups, 25 with UA their age range (21-70), 60 with AMI their age range (39-70). The other 30 subjects age and sex matched healthy subjects were studied as controls (This group includes 30 subjects who had no history or clinical evidence of cardiac diseases or any chronic disease).All patients had been admitted to the Coronary Care Units (CCU) of Ibn-Alnafees Hospital and Al kindy Hospital, between July 2012 and March 2013.The clinical examination and diagnosis were performed by physician specialized in in Ibn Al-Nafees Cardiac Specialty Teaching hospital and AL-Kindy Teaching hospital. Blood collection and laboratory analysis. From each patient and control, five(5) ml venous blood were aspirated from a suitable vein. Samples were collected between (8-9 A.M.) after 12 hours fast. Blood samples were divided into two parts, three ml transferred to a plain tube for (lipid Profile and troponin .The remaining of blood was transferred to another sterile plain tubes for storage to measure leptin and IL-18. The non-heparinized blood in the plain tubes were left to clot and then centrifuged at 4000 rpm for 5 minutes to separate the serum and dispensed into tightly closed Eppendorf tubes in 1.0 ml aliquot, and stored at -20 C° until assayed. Each serum sample was analyzed for Lipid profile were measured by using standard methods with reagents from BioMaghreb Company – Tunisia, leptin and IL-18 were measured by using ELISA kits from United States Biological-Company.

Statistical Analysis

All data were expressed as mean ± SEM. The statistical significance was evaluated by Student's t- test using Statistical Package for the Social Sciences (SPSS Cary, NC, USA) version 12.0.

3. Result

Serum IL-18 and leptin were estimated in 85 patients with ACS, (60 AMI & 25 UA) compared with 30 healthy control group, age and sex matched.as expected, the patients with ACS had significantly higher level of IL-18 and leptin levels than the healthy controls. see figure (1) and figure (2), a significant positive correlation was found in mean serum IL-18 level with BMI in ACS while a significant positive correlation was observed between leptin with BMI in AMI but non-significant in UA patients.as shown

in figures 3,4,5 and 6. On the other hand a positive correlation was observed between IL-18 and leptin in AMI see figure 8, while a negative correlation observed in unstable angina patients. See figure 7 and table. 2. The level of IL-18 and leptin in normal healthy subjects and ACS subjects was depicted in (Table.1).

Table- 1: The Anthropometric and biochemical variables among the three studied groups.
AMI (acute myocardial infarction), UA(unstable angina), ACS (acute coronary syndromes), X versus C (control)

Parameters	Control	Unstable angina	Acute myocardial infarction	P(ANOVA)-(T-Test)
NO.	**30**	**25**	**60**	……
Mean±SEM	**33.95± 6.57**	**47.07 ±12.49**	**56.70±1.58**	AMI x UA: p< 0.01 ACS x C: P<0.0001
Male Gender(%)	**73%**	**56%**	**83.3%**	AMI x UA: p< 0.460 ACS x C: P<0.001
Leptin(ng/ml)	**27.33±7.22**	**55.26±19.36**	**66.11±13.88**	AMI x UA: p<0.211 ACS x C: P<0.01
BMI (kg/m²)	**22.9 ± 2.6**	**26.912**	**30.449**	AMI x UA: p<0.001 ACS x C: P<0.00001
IL-18 (pg/ml)	**108.37±25.4**	**368.47±47.7**	**751.76±92.9**	AMI x UA: p<0.05 ACS x C: P<0.001

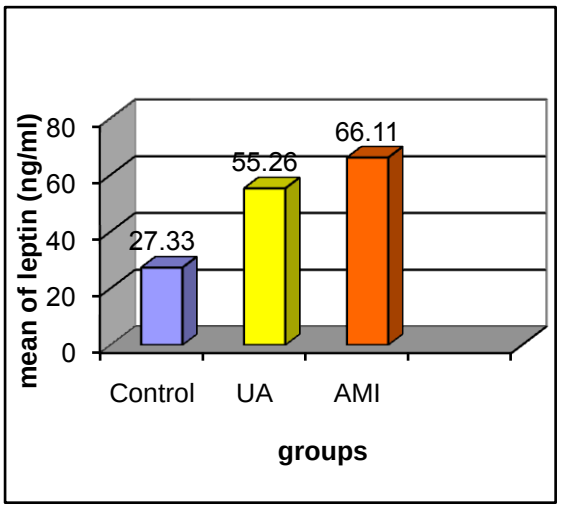

Figure.1 (Serum leptin concentration among among the study groups).

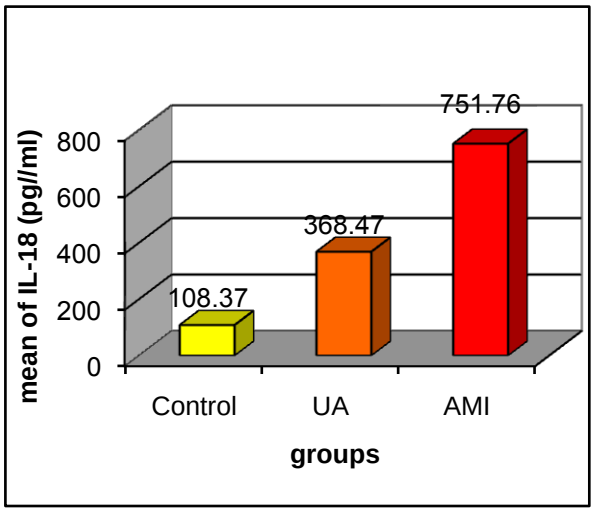

Figure.2 (Serum IL-18 concentration the study groups).

UA

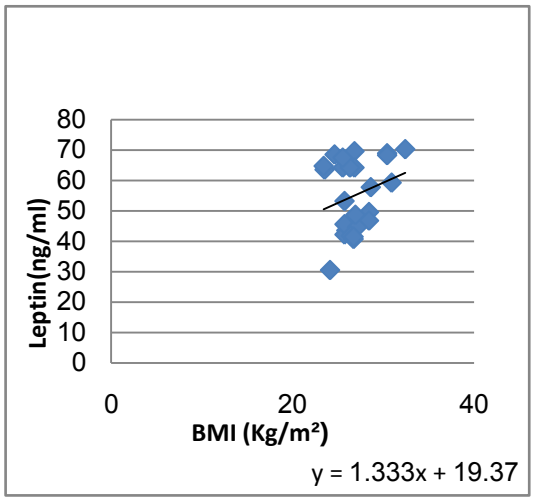

Figure.3 Correlation between Serum leptin Level and BMI in unstable angina patients (n=25)

AMI

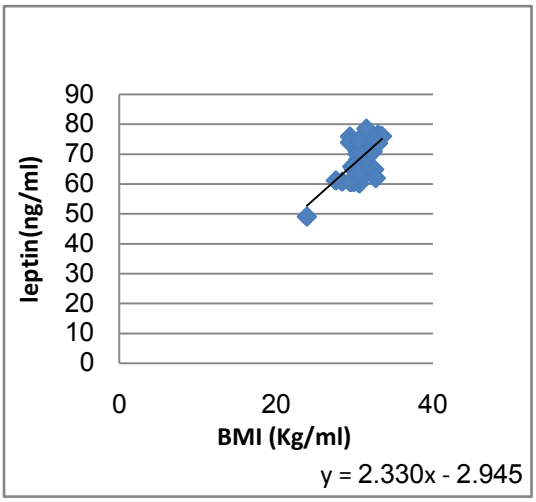

Figure.4 Correlation between Serum leptin Level and BMI in AMI patients (n=60)

UA

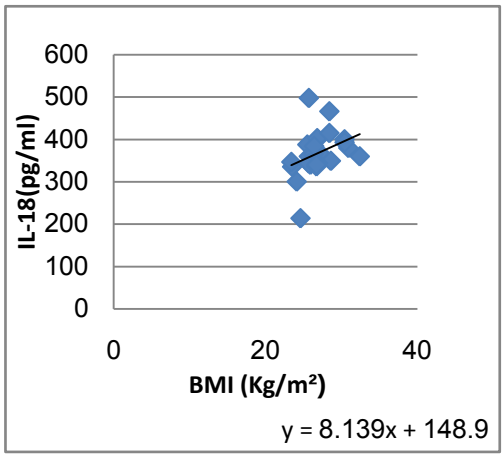

Figure.5 Correlation between Serum IL-18 Level and BMI in unstable angina patients (n=25)

AMI

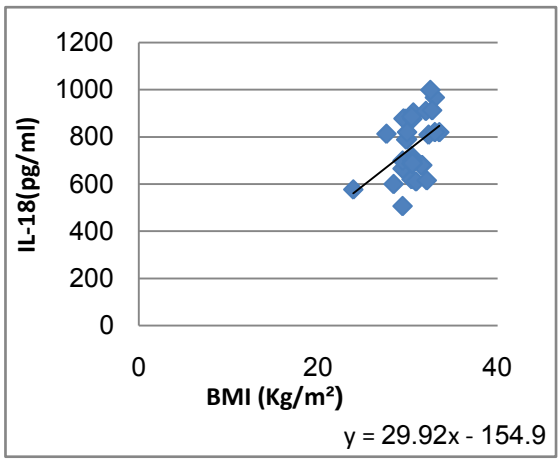

Figure.6 Correlation between Serum IL-18 level and BMI in AMI patients (n=60)

UA

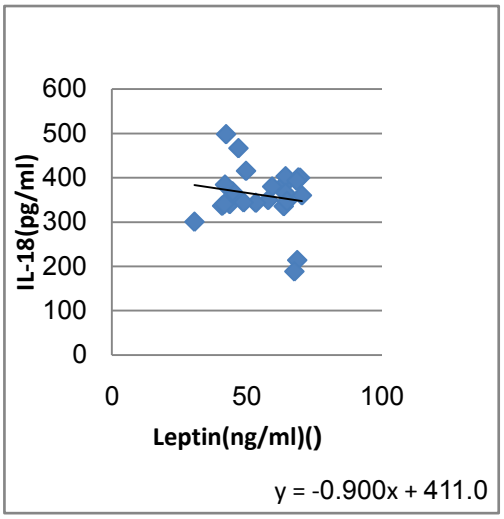

Figure.7 Correlation between Serum IL-18 Level and leptin in unstable angina patients (n=25)

AMI

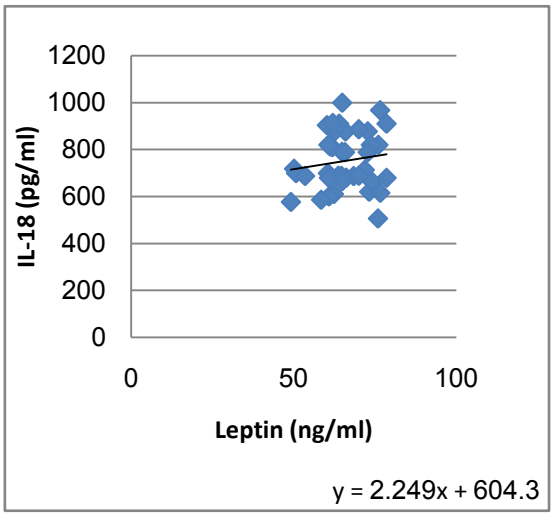

Figure.8 Correlation between Serum IL-18 level and leptin in AMI patients (n=60)

Table 2. Correlation, Coefficient (r), and significant value (p) of Serum IL-18 vs. BMI, leptin vs. BMI, IL-18 vs. Leptin in acute coronary syndromes (ACS), UA (unstable angina), AMI (acute myocardial infarction).

Correlation IL8 & BMI	Type	r	P	Significance
UA	positive	0.347	0.05	**significant**
AMI	Positive	0.439	0.001	**significant**
Correlation leptin & BMI				
UA	positive	0.256	0.166	**Non-significant**
AMI	Positive	0.624	0.002	**significant**
Correlation IL8 & leptin				
UA	Negative	-0.167	0.370	**Non-significant**
AMI	positive	0.145	0.430	**Non-significant**

4. Discussion

Inflammatory mechanisms have an important role in the pathogenesis of atherosclerotic disease and the occurrence of acute ischemic syndromes.1 However, because of our limited knowledge about the critical inflammatory pathways involved in the pathogenesis and progression of ischemic heart disease in humans, no specific anti-inflammatory treatment can be advocated at this time, Interleukin (IL)-18, originally identified as an interferon (IFN) gamma inducing factor in Kupffer cells and macro-phages(5). It plays a central role in the inflammatory cascade and in the processes of innate and acquired immunities because of its ability to induce IFN-gamma production in T lymphocytes and natural killer cells which is believed to play a crucial role in atherosclerotic plaque rupture (14). Furthermore, IL-18 acts in synergy with IL-12 to promote the development of T helper 1 (Thl) responses (7). Recently, increased IL-18 expression has been reported in human atherosclerotic plaque (8), mediating INF-gamma release locally. Furthermore, the beneficial effect of inhibiting IL-18 on plaque progression and composition was reported by Whitman et al., in 2002(15).Our study showed that IL-18serum levels were statistically significantly higher in acute coronary syndromes (ACS) patients than that of healthy control as a whole and even when stratified as UA and AMI groups. These results are agreement with other result done by Blankenberg et al., in 2003 (16).

In the United States population, increased leptin concentrations was significantly associated with increased risk of myocardial infarction and stroke in men and women, independent of traditional cardiovascular risk factors and obesity status (17). Study by Stangl et al., in 2000 (18) concluded that patients with coronary artery disease exhibited higher serum leptin concentrations than controls matched for age, gender & BMI, suggesting that leptin could contribute to the development of cardiovascular disease, possibly via activation of the sympathetic nervous system. Leptin might be a marker of risk of coronary artery disease, at least in men, and contributes to the risk profile in subjects with insulin resistance. Body weight. Wallace et al. (19) documented that leptin is an independent risk factor for coronary artery disease using data from the west Scotland coronary prevention study. It has been suggests that leptin might participate in the catabolic state leading to development of cardiac cachexia in the course of congestive heart failure (20).

This study demonstrates elevation of serum leptin levels in the acute coronary syndromes with high levels in acute myocardial infarction and it peaks at 36 hours after admission .This study is concordant to the study in Poland, which included 35 patients with AMI and showed that plasma leptin levels in diabetic patients were significantly higher in AMI than in healthy control. These findings suggest that leptin may play an important role in the metabolic changes taking place during the first days of AMI (21).this results are agreement with other result done by Herminia González et al., in 2009 (22) who demonstrated that leptin stimulates vascular smooth cell proliferation ,accelerates vascular calcification, induces oxidative stress in endothelial cells that may contribute to atherogenesis, and promotes coagulation by increasing platelet adhesiveness. (22)

The predictive power of leptin on cardiovascular disease was addressed in a report from the Quebec cardiovascular study(23), eighty-six patients who developed ischemic heart disease were compared with referent matched for a number of traditional cardiovascular risk factor including body mass index. Leptin did not emerge as a predictor in coronary artery disease. However, fundamental differences between the two studies, first and most importantly, patients in our study were all proven first ever AMI cases according to newly defined criteria (24), whereas in Quebec, the study group constituted a mixture of stable and unstable angina (23). Furthermore Fujimaki et al.in 2001(25) correlated serum leptin with other myocardial infarction markers and interleukin level in 15 aged-matched controls and found a significant negative correlation between these two markers. These studies again suggest that leptin may play an important role in the metabolic changes taking place during the first days of myocardial infarction. This study demonstrate that serum leptin is positively correlated with IL-18,this result are agreement with other result done by GöranHallmans., et al., in 2008 (26) reported that the presence of the leptin receptor in the heart suggests that leptin could modulate cardiac function directly. Leptin may also exert other pro-inflammatory effects like maturation of lymphocytes into more pro-inflammatory phenotypes, characterized by production of pro-inflammatory cytokines. The most convincing evidence related to endothelial dysfunction or damage is associated with an activation of macrophages located in the vessel wall with a continued release of cytokines and proteolytic vessel wall degrading enzymes. (26).

In conclusion, our study shows a significant positive correlation was found in mean serum IL-18 level with BMI in ACS, a significant positive correlation of serum leptin with BMI in AMI but non-significant in UA patients. On the other hand a positive correlation was observed between IL-18 and Leptin in AMI, while a negative correlation observed in unstable angina patients. Therefore these biomarkers may be useful diagnosis of patients with ACS.

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