

## **Evaluation of IL-35 and hs-CRP in Iraqi Patients with Cardiovascular diseases**

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### **Abstract**

Interleukin-35 (IL-35) is a novel anti-inflammatory of IL-12 family member cytokine. High sensitive C- reactive protein (hs-CRP) is a marker of inflammation disease. This study was obtained to estimate the concentrations of IL-35 and hs-CRP in cardiovascular diseases Iraqi patients. The study contained 79 subjects divided into two groups, 47 (21 male and 26 female) suffering from cardiovascular diseases and 32 as control group. The concentration of IL-35 and hs-CRP was measured by enzyme- linked immune sorbent assay (ELISA). The results revealed that the concentrations of IL-35 and hs-CRP are increased in patients group. IL-35 is strongly expressed in human advanced plaque and hs-CRP. Therefore these two biomarkers could be useful as addition in diagnosis of cardiovascular diseases.

A significant increase of IL-35 and hs-CRP levels in patients group compared with controls ( $P < 0.005$ ) and there was a positive correlation between IL-35 with hs-CRP in patients group. The results of this study revealed that IL-35 is a potentially anti-inflammatory marker in cardiovascular diseases.

**Key Words:** Inflammatory markers, IL-35, hs-CRP, Cardiovascular diseases, Iraqi patients.

## Introduction

Inflammatory plays an important role in the pathogenesis of cardiovascular disease and various studies in

indicated that inflammatory markers appear to be protein of cardiovascular events such as interleukin-6, IL-10 and C-Reactive protein (CRP)[1-5].

Interleukin 35 (IL-35) is the newly identified member of IL-12 family[6,7]. IL-35 is a heterodimeric cytokine composed of p35 subunit of IL-12 $\alpha$  and the Epstein-Bar Virus induced gene3 (EBI3)[7,8]. EBI3 is expressed at high levels in human B lymphoblast cells, tonsils and spleen [9]. IL-35 is secreted by CD4<sup>+</sup> regulatory T (Treg) cells rather than CD4<sup>+</sup> effector T (Teff) cells in mice; therefore, IL-35 has been considered to be the characteristic factor of Treg cells[10–13]. Human Treg cells, placental trophoblast cells, endothelial cells, activated dendritic cells, and macrophages also express IL-35[14,15]. Additional evidence demonstrated that IL-35 is an important anti-inflammatory cytokine and can efficiently suppress the Teff cell (including Th1, Th2 and Th17 ) activity and reduce the progression of inflammatory diseases and autoimmune diseases [6,7,16,17].

C-reactive protein (CRP) is the prototype acute phase protein primarily synthesized in liver and its release is stimulated by interleukin6 (IL-6) and other pro inflammatory cytokines[18]. It is composed of five identical non-glycosylated polypeptide subunits each containing 206 amino acid residues. CRP is a hepatically derived pentraxin that plays a key role in the innate immune response[19,20].

Epidemiological studies have demonstrated that C-reactive protein (CRP) is a strong predictor myocardial infarction [21-23], stroke[21,22,24] sudden cardiac death[25], and peripheral arterial disease[24] in apparently healthy adults. High sensitive CRP (hs-CRP) is a marker of low grade chronic system inflammation and it can amplify the anti-inflammatory response through complement activation, tissue damage, and activation of endothelial cells [26-28].

There is no information mentioned to the relationship between the levels of plasma IL-35 and its potential role in cardiovascular disease for Iraqi patients.

This present study was carried out to assess the levels of serum IL-35 in heart diseases patients and the relationship to other parameters, including hs-CRP and total cholesterol (TC).

## Methods

The study consisted of 79 subjects divided into two groups, 47 subjects (21 male and 26 female, range age 48-68 years) suffering from Cardiovascular diseases. The other 32 health were controlled as control group (male 13 and 19 female, range age 44- 65 years). The patients were taken from CCU unit in AL-Kadymia teaching Hospital for period from January to April, while the healthy controls were volunteers.

Blood samples (5 ml) were drawn from patients and controls. The levels of serum IL-35 and hs-CRP were measured by using an enzyme- linked immune sorbent assay (ELISA), according to the manufacturer's instructions (CUSABBIO) and (DRG) respectively.

Serum total cholesterol (TC) was estimated by commercially available kit from BioSystems. It was estimated by enzymatic method.

## Statistical Analysis

All data are given as the mean  $\pm$  SD and where analyzed by an ANOVA. Person's correlation was used to examine the relation between IL-35 with hs-CRP and TC. A value of P was considered to be statically significant ( $P < 0.005$ ).

## Results

The distribution of sex, mean age and total cholesterol for patients and control groups are shown in table 1. The mean age was ( $50 \pm 4.3$ ) years in Iraqi patients and ( $56 \pm 6.9$ ) in control group. There was no significant difference in age and gender between the Iraqi patients with cardiovascular diseases and control group as in Table 1.

Serum total cholesterol showed significant differences between patients and control groups ( $P < 0.005$ ).

Mean value of serum total cholesterol (TC) was  $228.6 \pm 21.05$  mg/ dl in patients group while in control group it was  $175.34 \pm 12.05$  mg/ dl.

Table 2 showed mean value of serum IL-35 for patients and healthy control groups which it was  $36.21 \pm 3.52$  pg/ dl and  $22.21 \pm 2.01$  pg/ dl respectively. Mean values of hs-CRP were  $4.36 \pm 0.76$  mg/ L and  $0.77 \pm 0.25$  mg/L for patients and control groups respectively.

## Discussion

The levels of anti-inflammatory cytokine IL-35 were measured in cardiovascular diseases for Iraqi patients. The results showed that the elevated concentration of IL-35 was associated with cardiovascular diseases and positively correlation.

The levels of anti-inflammatory cytokine IL-35, hs-CRP and total cholesterol were investigated in cardiovascular diseases for Iraqi patients. The results showed that the levels of IL-35 and hs-CRP were significantly higher in patients than in control group.

The results showed that higher IL-35 levels were positively correlated with CRP ( $R = 0.11$ ), (figure 3) whereas higher TC levels were negatively correlated with IL-35 ( $R = -0.1$ ) in patients with cardiovascular diseases (figure 4).

The serum anti-inflammatory cytokine concentrations levels are still controversial. Some studies observed that these levels were increased other studies observed significantly decreased [10-18,29,30]. These studies indicate that the concentrations of anti-inflammatory cytokines can predicate the incidence of cardiovascular events.

IL-35 is an IL-12 family member cytokine composed of an  $\alpha$  chain p35 and a  $\beta$  chain EB13 [6,7]. EB13 and p35 are expressed in almost all advanced plaque lesions and are co-expressed in atheroma vascular smooth muscle cell, indicating that IL-35 may be secreted by vascular smooth cells and is involved in the atherosclerosis [31-34].

In addition, higher IL-35 concentrations showed a positive correlation while higher TC concentration showed a negative correlation with IL-35 in patients with cardiovascular diseases, suggestion a potential role of IL-35 in prognosis of cardiovascular diseases.

It was found that elevated hs-CRP was significantly correlated with coronary artery disease [33,34]. A significant difference has been observed regarding the value of hs-CRP in cardiovascular disease subjects compared to normal subjects in this study. This reveals that there is an association between hs-CRP and cardiovascular diseases [27].

In conclusion: The results that were drawn from this research may confirm the relationship between high levels of IL-35 and hs-CRP with cardiovascular diseases.

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**Table No.(1): Clinical characteristics of Iraqi patients and control groups**

Characteristics	Patients n=47	Control n= 32	P value
Age (years)	50± 4.3	56± 6.9	N.S
Sex (male/ female)	21/26	13/19	N.S
TC (mg/ dL)	228.61± 21.05	175.34± 12.05	P < 0.005

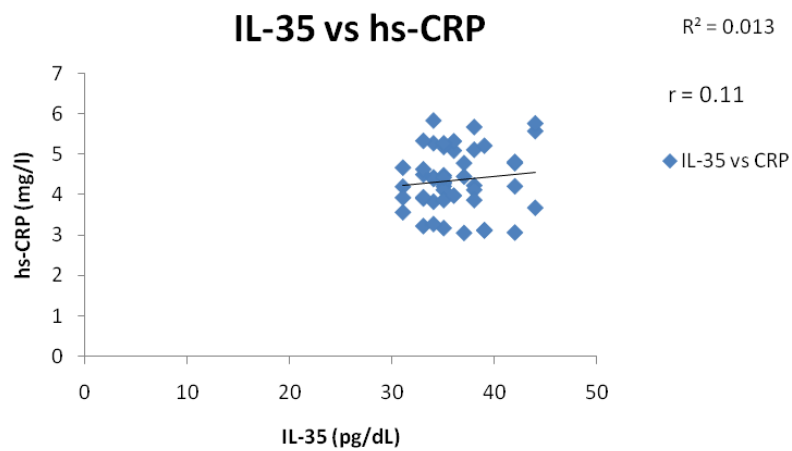
The data are given as the mean ±SD.

TC: total cholesterol, N.S: nonsignificant.

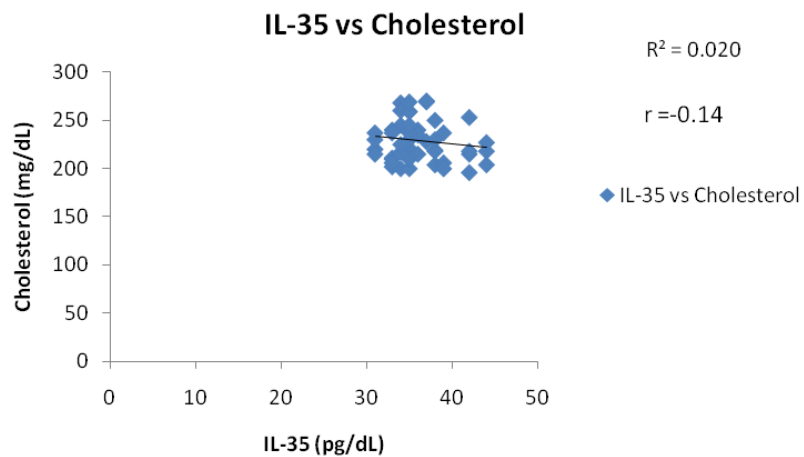
**Table No.(2): plasma IL-35 levels and hs-CRP in patients and control group**

Characteristics	Patients	Control	P value
IL-35 (pg/dl)	36.21± 3.52	22.21± 2.01	P < 0.005
hs-CRP (mg/l)	4.36± 0.76	0.77± 0.25	P < 0.005

Note: The data are given as the mean ±SD



**Figure No. (1): The correlation between IL-35 and hs-CRP in patients groups**



**Figure No. (2): The correlation between IL-35 and TC in patients groups.**

## تقدير انترلوكين-35 وبروتين سي عالي الحساسية للمرضى العراقيين المصابين بأمراض الأوعية القلبية

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### الخلاصة

انترلوكين-35 عضو جديد من عائلة انترلوكين-12 ضد الألتهابات. بروتين سي عالي الحساسية هو مؤشر للألتهابات. أجريت هذه الدراسة تقدير تركيز انترلوكين-35 وبروتين سي عالي الحساسية عند المرضى العراقيين المصابين بأمراض الأوعية القلبية. تضمنت الدراسة 79 حالة، قسمت على مجموعتين: 47 حالة ( 21 ذكراً و 26 انثى) يعانون من امراض الأوعية القلبية و 32 حالة غير مرضية مجموعة السيطرة. قيس تركيز انترلوكين-35 وبروتين سي عالي الحساسية بطريقة الإيلايزا. اظهرت النتائج زيادة في تركيز انترلوكين-35 وبروتين سي عالي الحساسية في مجموعة المرضى. انترلوكين-35 وبروتين سي عالي الحساسية يُعبر عند الأشخاص بشدة في الحالات المتقدمة، لذا يمكن أن يكون هذان المؤشران الحياتيان مفيدتين في تشخيص امراض الأوعية القلبية. حصلت زيادة معنوية في مستوى انترلوكين-35 وبروتين سي عالي الحساسية في مجموعة المرضى مقارنة بمجموعة السيطرة ( $P < 0.005$ ) وهناك علاقة موجبة بين انترلوكين-35 وبروتين سي عالي الحساسية لمجموعة المرضى. نتائج الدراسة أظهرت انترلوكين-35 مؤشر محتمل ضد الألتهابات في امراض الأوعية القلبية.

**الكلمات المفتاحية:** دالات الألتهابات، انترلوكين-35، بروتين سي عالي الحساسية، امراض الأوعية القلبية.