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Role of Interleukin-35 in Patients with Heart and Vascular Diseases

Conflict of interest: nothing to declare.
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Abstract

Introduction. Interleukin-35 (IL-35) is a currently labeled cytokine in the IL-12 family, that plays a key duty in the suppressive function of supervisory T containers.
Purpose. To explore the effect of Interleukin-35 on cardiovascular diseases.
Materials and methods. This study was conducted in the heart center in Thi-Qar Governorate, during the period between 15/3/2024 to 15/12/2024. The immune status was investigated for patients with heart and vascular diseases by measuring the levels of Interleukin-35 (IL-35) in the serum of blood using a technique Enzyme-Linked Immunosorbent Assay (ELISA). The investigation included 75 participants: 50 patients with heart and vascular diseases and 25 healthy subjects serving as controls.
Results. The statistical analysis showed a significant increase ($P \leq 0.05$) in serum the rate of concentration of (IL-35) in patients (21.11±65.13 pg/ml) compared to the healthy control group (5.53±12.43 pg/ml) with a statistically significant difference ($P=0.0001$). The findings of the study demonstrated that the IL-35 level was higher in smokers than in non-smokers, where the IL-17 level for smokers was 15.33±77.46, while it was 13.01±43.21 for non-smoking with no statistically significant difference.
Conclusion. The high level of cytokinesis 35 concentration has a fundamental and influential role in the emergence and development of cardiovascular diseases.
Keywords: heart and vascular diseases, IL-35, coronary heart disease, atherosclerosis, congestive heart failure

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Роль интерлейкина-35 в возникновении и развитии сердечно-сосудистых заболеваний

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Резюме

Введение. Интерлейкин-35 (IL-35) представляет собой цитокин семейства IL-12, существенно влияющий на супрессивную активность регуляторных Т-клеток.
Цель. Изучить влияние IL-35 на возникновение и развитие сердечно-сосудистых заболеваний.
Материалы и методы. Исследование проводили в кардиологическом центре в мухафазе Ти-Кар в период с 15.03.2024 по 15.12.2024. Исследовали иммунный статус пациентов с сердечно-сосудистыми заболеваниями путем измерения уровня IL-35 в сыворотке крови методом иммуносорбентного ферментного анализа (ИФА/ELISA). В исследовании приняли участие 75 человек: 50 пациентов с сердечно-сосудистыми заболеваниями и 25 здоровых добровольцев (контрольная группа).
Результаты. Статистический анализ выявил достоверное увеличение (P≤0,05) концентрации IL-35 в сыворотке крови пациентов с сердечно-сосудистыми заболеваниями (21,11±65,13 пг/мл) по сравнению со здоровыми добровольцами из группы контроля (5,53±12,43 пг/мл) со статистически значимой разницей (P=0,0001). Результаты исследования показали, что уровень IL-35 был выше у курящих испытуемых, в то время как уровень IL-17 у курящих составил 15,33±77,46 пг/мл, а у некурящих – 13,01±43,21 пг/мл при отсутствии статистически значимой разницы.
Заключение. Повышенный уровень концентрации IL-35 играет существенную роль в возникновении и развитии ССЗ.
Ключевые слова: сердечно-сосудистые заболевания, IL-35, ишемическая болезнь сердца, атеросклероз, застойная сердечная недостаточность

■ INTRODUCTION

Cardiovascular diseases (CVDs) represent the leading cause of death on a global scale. The 2013 Global Burden of Disease Study indicated that these diseases accounted for approximately 30% of all fatalities worldwide. Data from the UK Healthcare Information Centre revealed that in 2013, there were over 480,000 instances of heart failure, 1.2 million strokes, and nearly 2.3 million individuals in the UK diagnosed with coronary

heart disease. Furthermore, the global mortality rate experienced a 12.5% increase in 2016, attributed to various factors, including the persistent rise in cardiovascular diseases. This trend has resulted in significant economic challenges for nations, particularly those that are impoverished or still developing, due to the associated healthcare costs and workforce depletion. Atherosclerotic cardiovascular afflictions give reason for tens of millions of oblivion occurring, accompanying the most due to congestive heart failure (CHD). Heart failure atherosclerosis is ultimate common fundamental cause of CHD. Even though atherosclerosis is exceptionally fatal, late it ruptures or corrodes, followed by loss of consciousness from blockage in vein or artery, the risk of progress to acute heart failure condition increases piercingly [1]. Cardiovascular diseases (CVD) encompass a variety of disorders and abnormalities that impact the heart and blood vessels. This category includes numerous conditions, such as atherosclerosis and coronary heart disease (CHD). CHD specifically refers to a collection of ailments resulting from defects or injuries to the coronary arteries, which are responsible for delivering blood to the heart muscle. Such defects can result in the narrowing or obstruction of these arteries, potentially leading to severe complications for the heart muscle, with the risk of fatal outcomes [2].

Interleukin-35 (IL-35) is a currently labeled cytokine in the IL-12 family, that plays a key duty in the suppressive function of supervisory T containers (Tregs). Interleukin-35 (IL-35) is a heterodimeric cytokine collected of Epstein-Barr virus-inferred deoxyribonucleic acid 3 (EBI3) and IL-12p35 that has recently happened proved to play various and important duties in the carcinoma microenvironment (TME). Owing to allure immunosuppressive action and capability to promote lump tumor and progression, IL-35 is widely acknowledged as a key peacemaker of TME rank. Immune containers are key mediators of various tumor-connected phenotypes, and immunosuppressive cytokines in the way that IL-35 can advance tumor and often major in TME [3]. In 2009, Kempe et al. [4] examined the verbalization of the IL-35 subunit EBI3 cruel atherosclerosis and allure transcriptional regulation in vascular containers, professed allure verbalization in diseased endothelial containers, smooth power containers and macrophages.

This is the first study to reveal the connection 'tween IL-35 and atherosclerosis. They further demonstrated that the different IL-35 subunit, p35, is also present in atherosclerosis and is co-signified accompanying EBI3. The localization of EBI3 in atherosclerotic vascular smooth influence containers suggested that local discharge of EBI3 can have atherosclerotic and/or securing effects.

■ PURPOSE OF THE STUDY

To explore the effect of Interleukin-35 on cardiovascular diseases.

■ MATERIALS AND METHODS

Design of the Study

Fifty samples were collected from patients attending the heart center in Thi-Qar Governorate who were hospitalized in the center's halls, as well as those who were hospitalized in the Coronary Care Unit (CCU) with cardiovascular diseases (CVD), and 25 samples from healthy people as a control group in Thi-Qar Governorate. From 15/3/2024 to 15/12/2024 for both sexes, a questionnaire was filled out to collect information about patients and healthy people.

Collection of Blood Samples

Tow (ml) of venous blood was drawn from all participants by using a disposable syringe, and was transferred into a gel tube, after that, to obtain blood serum, we transferred the blood samples to the centrifuge and ran it at 4000 r/min for 10 minutes to separate the blood serum from the rest of its components. Then we transfer the separated blood serum to Eppendorf tubes and store it at (–20° C) until it is used to estimate the interleukin-35 level. The Enzyme-Linked Immunosorbent Assay (ELISA) technique was used to measure the levels of interleukin-35in the serum of all samples. The ELISA kit is manufactured by (Fine test – China).

Principle of Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-IL-35 antibody was pre-coated onto the 96-well plate. The biotin conjugated anti IL-35 antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with IL-35 conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of IL-35 in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

Statistical Analysis

The data underwent statistical analysis using SPSS, version 23.0. Data are represented as the mean ± standard deviation (SD). Comparison of a group of differences in numerical variables was estimated by t-test Where the level of significance was measured by the P value at a significant level (P.value ≤0.05).

■ RESULTS

Interleukin-35

The current study showed that the level of IL-35 in patients with cardiovascular diseases was 21.11±65.13 pg/ml and was very high compared to the control group, whose IL-35 level was 5.53±12.43 pg/ml and with a significant difference. Statistical significance (P=0.0001) (Table 1).

The effect of smoking on the IL-35 level

When the samples of the patients under study were distributed according to smoking status and IL-35 level, the findings of the study demonstrated that the IL-35 level was higher in smokers than in non-smokers, where the IL-17 level for smokers was 15.33±77.46, while it was 13.01±43.21 for non-smoking with no statistically significant difference (Table 2).

Table 1
Comparison of the levels of IL-35 among two studied groups

Parameter	Patients	Control	t-test	p-value
	SD±Mean			
IL-35	21.11±65.13	5.53±12.43	4.04	<0.0001*

Table 2
Comparison of the effect of smoking on the L-35 level among studied groups

Parameter	Non-smoking patients (N=18)	Smoking patients (N=32)	t-test	p-value
	SD±Mean			
IL-35	13.01±43.21	15.33±77.46	–1.84	0.034*

■ DISCUSSION

IL-35 is an anti-inflammatory cytokine and a member of IL-12 family. It is a heterodimer composed of p35 (IL-12A) and EBI3 subunits [5]. IL-35 cytokine has been associated with the development of several inflammatory diseases. In fact, the study on this molecule points out its probable protective role against atherosclerosis [6].

Some studies in Thi-Qar Governorate, such as (Thamer, 2023), indicated an effective role for inflammatory cytokines in the development of cardiovascular diseases [7]. The current study also agrees with Attia and Salman [8], which indicated that the role for inflammation, immunity, and chemical compounds of the immune system, such as chemokines, in the emergence and development of cardiovascular diseases. The current result indicates a significant elevation of IL-35 levels in patients with cardiovascular disease compared to a control group. These findings align with several contemporary studies that endorse the role of IL-35 as a potential biomarker and therapeutic target in cardiovascular pathology. Su et al. [9] shown that IL-35 is synthesized by regulatory T cells and is essential in regulating inflammatory responses, which are critical in the progression of atherosclerosis and other cardiovascular diseases. A study conducted by Oflar et al. [10] revealed that increased IL-35 levels correlated with enhanced clinical outcomes in patients with coronary artery disease, indicating a preventive function against inflammation and plaque development. Moreover, IL-35 has been associated with the suppression of pro-inflammatory cytokines, potentially diminishing the likelihood of cardiovascular events [11]. A further study indicated that IL-35 may augment the stability of atherosclerotic plaques, which is essential for averting acute cardiovascular incidents, as described by Huang et al. [12]. A study by Lin et al. [13] found that elevated IL-35 levels correspond with reduced occurrences of major adverse cardiovascular events, validating the hypothesis that IL-35 may function as a protective factor in cardiovascular disease. The research by Ye et al. [14] indicated that although IL-35 may seem beneficial initially, its sustained rise could result in immunological dysregulation and worsen atherosclerosis. This viewpoint is corroborated by evidence indicating that persistent inflammation, marked by heightened IL-35 levels, may lead to plaque instability and augmented cardiovascular risk [15]. A study by Zhu et al. [16] found no significant difference in IL-35 levels between patients with cardiovascular illnesses and healthy controls, suggesting that IL-35 may not serve as a viable biomarker for assessing cardiovascular risk. Moreover, the function of IL-35 in facilitating Treg development may induce an imbalance in immunological responses, potentially causing detrimental effects in chronic inflammatory disorders; IL-35 levels may fluctuate considerably based on the disease context and stage [17].

The study indicates a significant increase in IL-35 levels in smokers with cardiovascular disease compared to non-smokers. Taskaldiran et al. [18] demonstrated that smoking induces a chronic inflammatory state, resulting in elevated levels of various cytokines, including IL-35. The research conducted by Pan et al. [19] indicated that smokers

displayed elevated levels of pro-inflammatory cytokines, implying that smoking intensifies inflammatory responses, which may result in heightened IL-35 production as a compensatory method to mitigate inflammation. Furthermore, Oflar et al. [10] discovered that IL-35 levels were markedly increased in patients with chronic inflammatory disorders, including cardiovascular disease, suggesting a possible involvement of IL-35 in the pathophysiology of smoking-associated cardiovascular problems. Conversely, the research of Sun [20] indicates that although IL-35 may initially seem to alleviate inflammation, its sustained rise in smokers could lead to immunological dysregulation and worse cardiovascular outcomes. A study by Yuvashri et al. [21] found no significant difference in IL-35 levels between smokers and non-smokers, suggesting that variables other than smoking status may affect IL-35 production. This inconsistency underscores the intricacy of IL-35's function in cardiovascular disease and indicates that additional research is necessary to elucidate its effects in smokers.

■ CONCLUSION

The high level of cytokinesis 35 concentration has a fundamental and influential role in the emergence and development of cardiovascular diseases.

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