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Estimation the levels of B-type natriuretic peptide and interleukin-35 in some Iraqi patients suffering from rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a systemic autoimmune disorder associated with a chronic inflammatory process that can impact the joints as well as non-articular organs such as the heart, kidney, lung, digestive tract, eye, skin, and neurological system. This study was conducted at Private Nursing Hospital and Al Kadhimiya Educational Hospital, where blood samples were collected from 122 Iraqi women; seventy-fiveof them were apparently healthy controls whileforty-sevenwere patients who had long-standing RA. B-type natriuretic peptide(BNP) and interleukin-35(IL-35)wereevaluated utilizing an enzyme-linked immunoassay. Also, total cholesterol, triglyceride, andHigh-density lipoprotein(HDL)were determined using an enzymatic colorimetric method.When RApatients were compared to healthy controls, their serum levels of BNP significantly increased($p \leq 0.001$), but IL-35significantly decreased ($p<0.001$). In another hand, no significant differences were noted in total cholesterol, triglyceride, HDL,low-density lipoprotein(LDL)and verylow-density lipoprotein (VLDL)when the comparison between the patients with control group. It can be concluded thatelevated levels of BNP in patients with RA may increase the risk of cardiovascular disease. Moreover, IL-35 is a crucial player in the body's immune response, and its dysregulation has been implicated in the pathogenesis of rheumatoid arthritis, suggesting a complex interplay between immune function and disease development.

Keywords: Rheumatoid arthritis; Interleukin-35; B-type natriuretic peptide; cardiovascular disease.

تقدير مستويات الببتيد المدر للصوديوم من النوع–B والانترلوكين–35 لدى بعض المرضى العراقيين المصابين بمرض التهاب المفاصل الرثوي

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الخلاصة

التهاب المفاصل الرثوي (RA) هو اضطراب في المناعة الذاتية يرتبط بعملية التهابية مزمنة يمكن أن تؤثر على المفاصل وكذلك الأعضاء غير المفصلية مثل القلب والكلى والرئة والجهاز الهضمي والعين والجلد والجهاز العصبي. أجريت هذه الدراسة في مستشفى التمريض الخاص ومستشفى الكاظمية التعليمي، حيث تم جمع عينات الدم من 122 امرأة عراقية. خمسة وسبعون منهم كانوا من الاصحاء بينما سبعة وأربعون كانوا

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مرضى يعانون من التهاب المفاصل الرثوي منذ فترة طويلة. تم تقييم الببتيد المدر للصوديوم من النوع B- (BNP) والإنترلوكين 35 (IL-35) باستخدام المقايسة المناعية المرتبطة بالإنزيم. كما تم تقييم الكوليسترول الكلي والدهون الثلاثية والبروتين الدهني عالي الكثافة (HDL) باستخدام الطريقة اللونية الأنزيمية. عندما تمت مقارنة مرضى التهاب المفاصل الروثوي مع الأشخاص الأصحاء، ارتفعت مستويات الببتيد المدر للصوديوم من النوع B بشكل ملحوظ ($P \geq 0.001$) في الدم، ولكن انخفضت مستويات IL-35 بشكل ملحوظ ($P < 0.001$). ومن ناحية أخرى لم تلاحظ فروق ذات دلالة إحصائية في الكولسترول الكلي، الدهون الثلاثية، البروتين الدهني عالي الكثافة (HDL)، البروتين الدهني منخفض الكثافة (LDL) والبروتين الدهني منخفض الكثافة جدا (VLDL) عند المقارنة بين مجموعة الاشخاص الاصحاء مع أولئك الذين يعانون من التهاب المفاصل الرثوي. يمكن أن نستنتج أن المستويات المرتفعة من BNP في المرضى الذين يعانون من التهاب المفاصل الرثوي قد تزيد من خطر الإصابة بأمراض القلب والأوعية الدموية. علاوة على ذلك، يلعب IL-35 دورا حاسمًا في الاستجابة المناعية للجسم، كما أن خلل تنظيمه يتسبب في التهاب المفاصل الرثوي، مما يشير إلى وجود التفاعل المعقد بين وظيفة المناعة وتطور المرض.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease that primarily involves persistent inflammation of the joints. It can harm extra-articular organs such as the heart, skin, and nervous system [1]. Rheumatoid arthritis affects 0.5%–1.0% of the global population [2]. Rheumatoid arthritis is divided into two types: non-inflammatory arthritis (osteoarthritis) and inflammatory arthritis brought on by crystal deposition (pseudo gout, gout), either by bacterial and viral infections or by autoimmune processes. Systemic lupus erythematosus (SLE), spondyl arthritis (SpA), psoriatic arthritis (PsA), polymyositis (PM), and other conditions are also included in the group of RA diseases. Since signs and symptoms can align, differential diagnosis is paramount [3]. Many studies have reported that the prevalence of RA increases by three to five times in females compared to males [4].

Interleukin-35 (IL-35) is a potent anti-inflammatory and immunosuppressive member of IL-12 family of cytokines. The two subunits of IL-35are IL-12A and Epstein-Barr virus-induced gene 3 (EBI3), which is chiefly released by regulatory T cells and regulatory B cells. This newlydiscovered heterodimeric IL-12 family member has the capability to suppress inflammation and immune responses [5, 6]. An essential anti-inflammatory function of IL-35 is to prevent a number of inflammatory and autoimmune disorders [7, 8]. IL-35 may have a role in regulating the RA pathogenesis, particularly when it comes to disease activity [9].

Cardiovascular events are approximately 50% more likely to occur in RA patients including myocardial infarction, cerebral stroke cardiac death and cardiovascular death [10]. Cardiovascular disease (CVD) is the primary comorbidity associated with mortality in RA, one thing that has been consistently demonstrated for decades and has been reaffirmed by many studies since the 1950s is the elevated risk of CVD in patients with RA [11, 12]. Females have higher incidence rates of myocardial ischemia and mortality compared to males of the same age [13].

Measurement of B-type natriuretic peptide (BNP) levels could serve as a useful complementary assessment to the clinical evaluation of patients suffering from rheumatic heart disease [14, 15]. Increases in volume or pressure cause the ventricles and the atrium to release BNP primarily [16]. Cardiomyocytes synthesize BNP as a precursor hormone, known as preproBNP, which contains an NH2-terminal signal peptide. Following the cleavage of the signal peptide, the pro-hormone peptide is divided into two segments: the longer segment, known as amino-terminal pro-BNP (NT-proBNP), contains the NH_2-terminus, while the

shorter fragment, usually knows as the COOH-terminus fragment, is considered as the active hormone, known as BNP. A previous study has shown the value of the BNP assay and the level of NT-proBNP plasma for prognosis in patients with heart failure and for ruling out suspicious symptoms [17].

This study aims to find out the risk of cardiovascular disease in patients with RA through measurement of BNP and IL-35 levels

2. Methods

The study involved the collection of 122 serum samples from Iraqi females for a period of three months, from October 2023 to January 2024, in the governorate of Baghdad. The specimens were chosen from Private Nursing Hospital and Al Kadhimiya Educational Hospital. The patients' diagnoses were determined by the hospital's consultant medical staff, who evaluated clinical symptoms and the results of laboratory investigations. Seventy-five healthy controls with mean age (40±13 year) were compared with 47 samples patients who had long-standing RA with mean age (47±12 year).

2.1. Excluded from this Study:

Patients suffering from chronic diseases, smokers, and pregnant women were excluded from this study.

2.2. Collection of Blood Samples:

Five millilitres of blood were collected and placed in gel tubes, and all of them were placed inside the centrifuge at 3000 rpm for 10 minutes to separate the blood and obtain the serum, then they were stored in the Eppendorf tube, at -20℃.

2.3. Laboratory Tests:

The BNP and IL-35 were evaluated utilizing an enzyme-linked immunoassay kit (My Bio Source, U.S.A). Also, total cholesterol, triglyceride and HDL were determined using an enzymatic colorimetric method, the kit provided (Biosystem, Spain) while LDL and VLDL were also calculated using the equation [18]:

VLDL = Triglyceride \5

LDL= (Total Cholesterol)-(VLDL+HDL)

2.4 . Analytical Statistics

The statistical analysis was conducted using the SPSS Statistical package (Version 22: SPSS). The sample t-test was employed to compare the variances between the research groups and Pearson analysis to find out the correlations between parameters of this study. Statistics deemed significant were those with a p-value of less than 0.05.

3. Results

The findings of the present study revealed a significant elevation in BNP levels ($p<0.001$) and a significant reduction in IL-35 levels ($p<0.001$) when comparing patients to the control group. However, no significant differences were observed in total cholesterol, triglyceride, HDL, LDL and VLDL between the two groups, as depicted in Table-1.

Table 1: The comparison of the studied parameters between the patients with control group.

Parameters	Control group (No.=75) (Mean ±SD)	Patients group (No.=47) (Mean ±SD)	P- value
Cholesterol (mg/dL)	137.89±33.59	145.72±24.72	0.142
Triglycerides (mg/dL)	148.21±45.96	142.26±41.87	0.463
HDL (mg/dL)	41.69±6.41	41.89±9.53	0.899
LDL (mg/dL)	67.4147±32.69	75.51±27.57	0.145
VLDL (mg/dL)	30.03±9.50	28.31 ± 8.37	0.299
IL-35 (ng\mL)	297.97±45.30	95.69±11.34	0.001
BNP (IU\mL)	120.9±68.32	318.76±105.08	0.001

The difference is significant at $p<0.05$, highly significant at $p<0.01$,and non-significant at $p>0.05$

The association between BNP and IL-35 levels in healthy controls reveals a non-statistically significant correlation between the two variables in healthy controls. Also, there was non-statistically significant correlation between the levels of BNP and IL-35 in RA patients as demonstrated inTable2

Table 2: Person Correlation between BNP with IL-35 levels in the healthy control people and patients.

Groups	BNP		IL-35	
	r	(P value)	r	(P value)
Patients	0.006	(0.969)	0.006	(0.969)
control	0.065	(0.577)	0.065	(0.577)

Correlation is significant at the 0.05

Discussion

According to the results of this study, those patients suffering from RAhave BNP levels that are noticeably greater than those in the control groupas shown in Table-1.These results may suggest a possible link between RA and a molecular profile modification that may be associated with CVD, which appears to be elevated in newly diagnosed patients and persisted over an extended period of disease [19]. This finding is consistent with study that found higher blood BNP levels in RA patients without a history of cardiac illness than in participants in a healthy control group. Increased blood BNP levels are correlated with ventricular strain and a higher risk of cardiac events and death in the general population[20]. Inflammation may be a crucial mechanism for the BNP secretion in patients with RA, myofibroblasts that the cells that synthesize natriuretic peptides in the human heart, play in inflammatory responses [21].

The present study found significant decrease in IL-35 levels in patients with RA compared to control group shown in the Table 1. These results are consistent with research showed a noteworthy decline in serum IL-35 levels and the proportion of regulatory T cells (Treg.) in

patients with RA. This study suggests that , IL-35 may inhibit T cell activation during RA-induced peripheral immunological responses [22]. Previous studies have demonstrated that the levels of IL-35 in patients were notably lower compared to healthy control individuals. Furthermore, IL-35 levels were lower in individuals with erosive arthritis or active illness than in those with inactive disease or no erosive arthritis [22, 23]. Elevated cytokine levels are a hallmark of RA and may serve as a valuable diagnostic marker. Pro-inflammatory cytokines play a pivotal role in the inflammatory processes that drive joint damage, destruction, and the development of comorbidities associated with RA [24]**.** Interleukin-35 induces the production of some pro-inflammatory cytokines in mononuclear cells, supporting the notion that IL-35 is pro-inflammatory in RA [25].

Patients with RA exhibited significantly higher BNP level compared to those in the control group. In contrast, they had markedly lower levels of IL-35,the result has not revealed a link between BNP and IL-35 depending on the correlation between BNP and IL-35 levels.

There were no statistically changes in lipid profile levels of patients with RA compared to healthy controls. The existing literature on lipid profiles in patients with RA reports inconsistent findings. Some studies have shown elevated levels of TC, LDL-C and HDL-C compared to healthy controls, while other research has found reduced or similar levels of these lipids between RA patients and controls [26]. Research has revealed that individuals with RA often exhibit an altered lipid profile, a phenomenon referred to as the "lipid paradox." This paradox occurs when lower total cholesterol and LDL levels, combined with higher HDL levels, do not necessarily translate to a reduced risk of CVD. However, the presence of confounding factors, such as medication, can make it challenging to determine the independent impact of inflammation on lipid metabolism in RA patients [27]. In contrast, another study found that RA patients had elevated levels of TC and LDL, as well as decreased HDL-C values [28].

Conclusions

On this basis, it can be concluded that individuals with rheumatoid arthritis who have elevated BNP levels may be at risk for cardiovascular disease. Significantly, IL-35 holds an influential position in the body's immunological response mechanism and this is linked to the development of rheumatoid arthritis.

Conflict of Interest

There is no conflict of interest.

Ethical Clearance

The Iraqi ministries of the environment, health, higher education, and scientific research have approved the Research Ethical Committee for scientific research.

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