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Research Article

# Neopterin as a Surrogate Marker for Coronary Artery Disease in Rheumatoid Arthritis

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**Abstract**

**Background:** Rheumatoid arthritis is an inflammatory illness. A complication of Rheumatoid arthritis is mainly linked to the risk of cardiovascular disease. High body weight has been implicated as a perilous risk factor for the development of Rheumatoid arthritis. Neopterin is secreted by monocytes and macrophages and is highly secreted from the coronary plaque. Moreover, high-sensitivity C-reactive protein is a biochemical analysis applied to quantify C-reactive protein with high sensitivity.
**Objectives:** To evaluate serum Neopterin and high-sensitivity C-reactive protein in patients with rheumatoid arthritis to predict the risk of coronary artery disease. In addition, a correlation between neopterin and high-sensitivity C-reactive protein and anthropometric measures.
**Methods:** The 120 women were included in the study; 60 of them had rheumatoid arthritis, and 60 of them were controls. Serum analysis included measurement of neopterin, sensitivity of C-reactive protein, and estimation of anthropometric measurements. Pearson's correlation between two quantitative variables was computed, and a t-test was applied. The correlation coefficient value demonstrates a positive or negative relation.
**Results:** The patients had significantly higher mean values of serum Neopterin, high-sensitivity C-reactive protein, body mass index, waist circumference, and waist-to-hip ratio compared to controls. were detected in the patient group, significant positive correlations were found between serum Neopterin and high-sensitivity C-reactive protein as well as body mass index, waist circumference, and waist-to-hip ratio.
**Conclusion:** The inflammatory markers neopterin and high-sensitivity C-reactive protein levels are higher in rheumatoid arthritis patients. The higher serum levels of these markers reflected the elevated level of systemic inflammation, and the patient was at risk of coronary artery disease. Also, there is a higher prevalence of obesity and overweight in rheumatoid arthritis patients and a positive correlation between neopterin levels and high anthropometric measures, which reflects an association between Neoperin and risk factors of coronary artery disease.

**Keywords:** High-sensitivity C-reactive protein; Neopterin; obesity; Rheumatoid arthritis; Coronary artery disease..

## Introduction

Rheumatoid arthritis (RA) is an inflammatory illness (1-3). The risk factors of RA are environmental, genetic, immune and hormonal factors that affect the hands, wrists, and knees most commonly. It causes pain, swelling, stiffness, and loss of function in these joints (4, 5).
Complication of RA is mainly linked to the risk of cardiovascular disease (CVD). Patients with RA have increased CVD morbidity and mortality due to several variables, such as higher systemic inflammation and hypercholesterolemia (6).
Higher body weight has been involved as a perilous risk factor for the development of RA and is increasingly commonly seen at the initial presentation of RA. There's growing evidence that a major mechanistic pathway linked to inflammatory states caused by RA and obesity. While Obesity may be a low-grade chronic inflammatory disorder due to the immune-modulatory features of adipose tissue (7).
Neopterin is a pteridine derivative that is secreted by monocytes and macrophages in response to interferon-γ stimulation (8) . It has been shown that neopterin is highly secreted by coronary injury in cases of unstable angina and also elevated in coronary artery disease. Previous studies demonstrated that elevated neopterin levels can predict cardiac incidents in individuals with chronic stable angina and acute coronary syndrome, as well as fatal cardiac events in diabetic patients (9).
High-sensitivity C-reactive protein (hs-CRP) is a biochemical test applied to quantify CRP with a high sensitivity. Hs-CRP is a new assay that measures a very small amount of CRP in blood. It can estimate the general inflammatory condition in the body.

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Hs-CRP is used as a predictive marker for assessing the risk of heart disease and stroke(10).
Due to the inflammatory condition in RA, this study aimed to evaluate serum neopterin and hs-CRP in RA patients to predict the risk of coronary artery disease and to find the the correlation between neopterin and hs-CRP as well as anthropometric measures.

**Patients, Materials and Methods**

This case-control study included 120 adult females aged between 23 to 64 years, who were Iraqi individuals who attended the Rheumatology and Rehabilitation Outpatients Clinic /Baghdad Teaching Hospital from November 2023 to February 2024. Informed consent was obtained from each participant. The research was approved by the Ethics Committee of the Department of Biochemistry, College of Medicine, University of Baghdad. The female participants were classified into the following groups: the first group consisted of 60 females with RA and the second group consisted of 60 healthy females as controls.
Biochemical tests, including serum Neopterin and hs-CRP, were determined using an enzyme-linked immunosorbent assay (ELISA) using kits supplied by CLOUD CLONE United States of America. Also, anthropometric measurements were done for each participant, including (11) :
1. Body Mass Index (BMI) is calculated by the following formula:[BMI = weight (kg) / (height (m)) $^2$ ].
2. Waist Circumference (WC): This is a simple anthropometric indicator of abdominal fat that provides a more accurate assessment of visceral obesity compared to BMI.
3. Waist to Hip Ratio (WHR): A WHR of ≥0.1 increases the risk of heart disease and other obesity-related health issues in both men and women.
Diagnosis of RA women was done according to criteria of the 2010 American College of Rheumatology/European League Against Rheumatism RA classification(12). However, patients with a history of diabetes, hypertension, hypothyroidism, malignant diseases, CVD, active infection, and other autoimmune diseases were excluded from this research.

**Statistical analysis**

Statistical analysis was done using IBM SPSS ver. 25.00 (IBM Corp., Armonk, NY, USA). Student's t-test was applied for comparison between studied groups, and quantitative data were stated as the mean and standard deviation (Mean±SD). Pearson correlation between two quantitative variables was computed and its t-test was applied. A positive or negative relation is demonstrated by the correlation coefficient value (r). significant result when the p-value is less than 0.05. Statistical analysis was done using IBM SPSS ver. 25.00.

**Results**

The result of this research showed a statistically significant difference in the mean levels of serum neopterin between the two study groups. Significantly higher levels of Neopterin were observed in RA women compared to controls (2.01 ng/ml vs 0.86 ng/ml; $p$= 0.001), as shown in Figure 1.

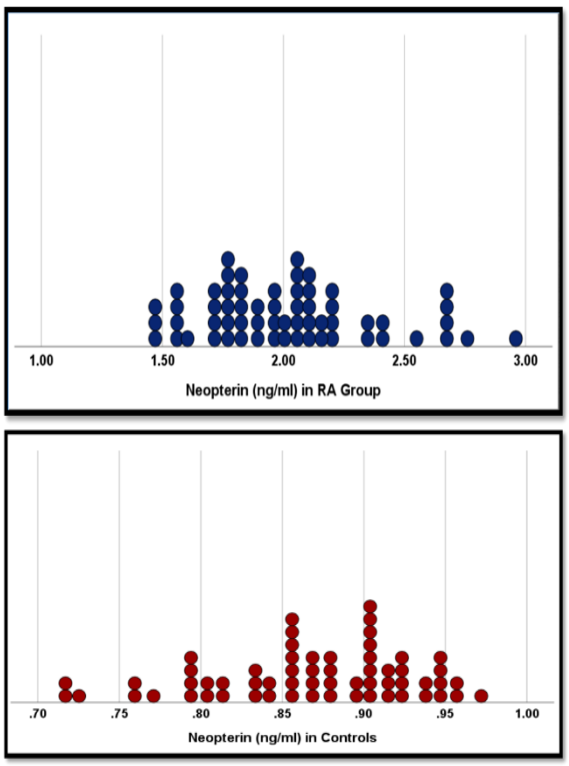


**Figure (1): Distribution of study groups according to serum Neopterin levels.**

The odds of Neopterin levels were significantly higher in RA patients than in healthy controls (OR= 1.71, C.I= 1.09 – 2.12, $p$= 0.001), as shown in Table 1.

**Table 1: Comparison of Neopterin levels between study groups**

| Variable | Study group | | Odds ratio | C.I | *P* - Value |
|---|---|---|---|---|---|
| | RA Group Mean ± SD | Control Group Mean ± SD | | | |
| Neopterin (ng/ml) | 2.01 ± 0.34 | 0.86 ± 0.06 | 1.71 | 1.05 – 2.12 | 0.001 |

Also, in this study, the mean levels of hs-CRP were significantly different between the two study groups. RA group had significantly higher levels of hs-CRP compared to controls (261.33 pg/ml vs 121.2 pg/ml; $p$= 0.001), as shown in Figure 2.

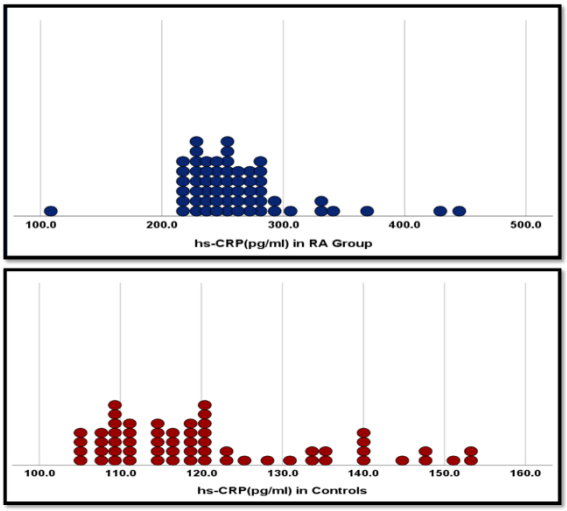


**Figure 2: Distribution of study groups according to serum hs-CRP levels.**

In addition, there was a significant rise in the mean value of BMI ($P$=0.014), WC ($P$=0.001), and WHR ($p$=0.001) in RA patients compared to controls, as shown in **Table 2.**

In the Pearson correlation analysis, there was a significant positive relation ($P$ <0.05) between Neopterin levels with hsCRP and all anthropometric measurements, as shown in Figures (3 and 4), respectively.

**Table 2: Comparison between study groups according to the anthropometric measurements**

| Variable | Study group | | $p$ = Value* |
|---|---|---|---|
| | RA Group (n=60) Mean ± SD | Control Group (n=60) Mean ± SD | |
| BMI (kg/m$^2$) | 30.60 ± 4.98 | 26.63 ± 3.86 | 0.014 |
| Waist Circumference (cm) | 97.82 ± 10.15 | 88.27 ± 9.32 | 0.001 |
| Waist / Hip Ratio | 0.98 ± 0.21 | 0.70 ± 0.07 | 0.001 |

* Significant difference between two independent means using Students-$t$-test at 0.05 level.

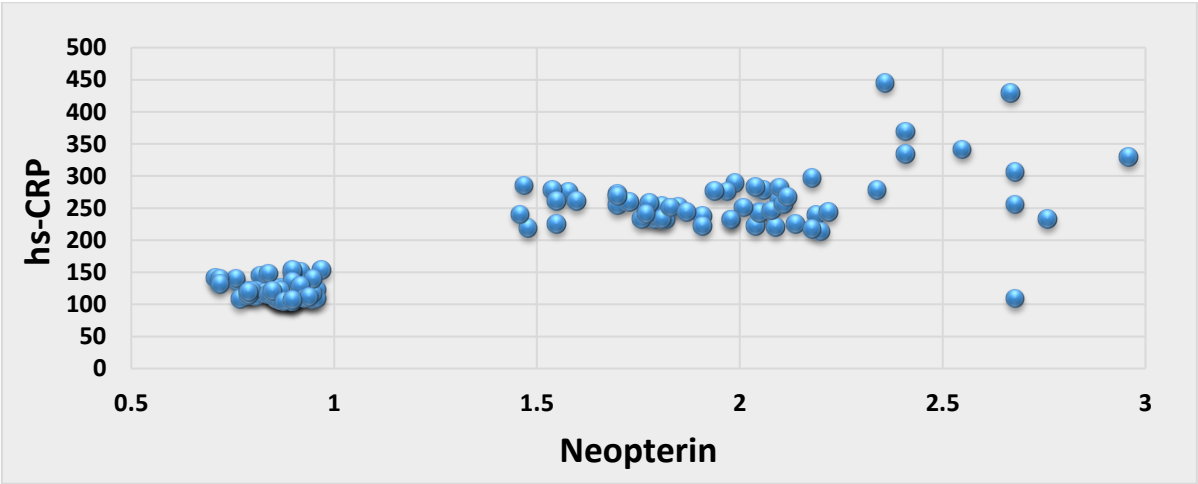


**Figure (3): Positive correlation between serum levels of Neopterin and hs-CRP (r=0.86, $p$= 0.001).**

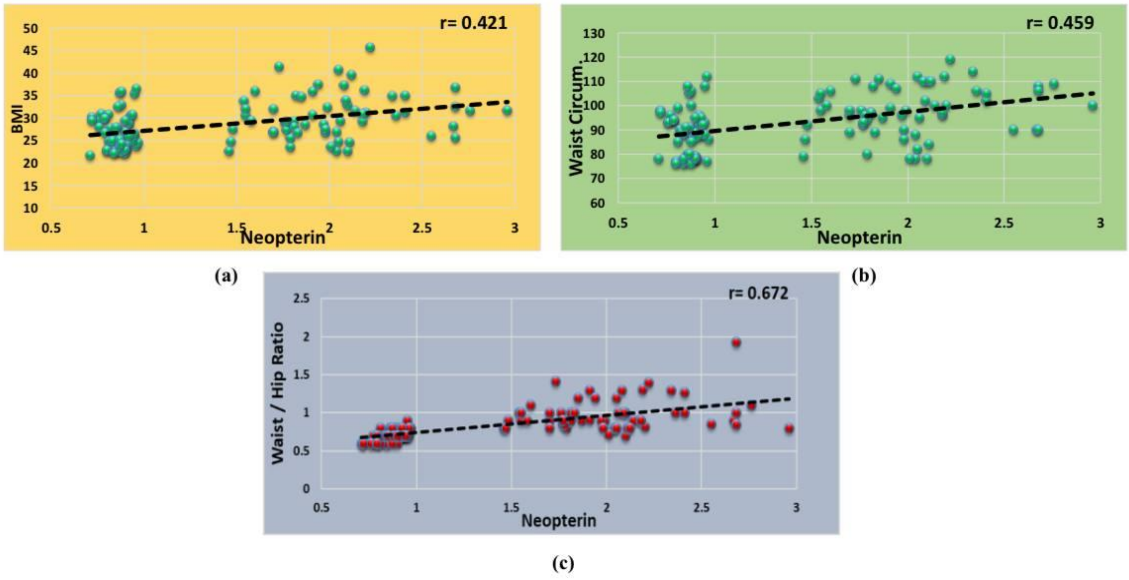


**Figure (4): Positive correlation between serum levels of Neopterin and BMI, WC, and WHR.**

**Discussion:**

Neopterin is also referred to as a monocyte/macrophage activation marker. Neopterin has been shown to have antioxidant properties and its plasma concentrations are increased in patients with coronary artery disease (13). Complications of chronic autoimmune disease are associated with the risk of CVD (14). This study showed higher serum levels of Neopterin in RA patients compared to controls similar to a previous study which found reported elevated Neopterin levels in RA patients (15). Also, Gamal et al(8) . showed that serum Neopterin can be utilized as a sensitive indicator for assessing inflammation in patients with RA(8). In addition, Husein and Mansoor(16) showed that Neopterin is considered a marker for innate immune activity in RA cases(16). A study by (17) demonstrated that Neopterin expression is higher in coronary artery lesions and plasma levels are higher in coronary artery disease (CAD) patients than in non-CAD patients(17). This study also showed that the odds ratio of Neopterin levels was significantly higher in RA patients compared to healthy controls, meaning that patients had a 71% higher chance of having elevated Neopterin levels compared to healthy controls. This suggests that Neopterin could be a useful biomarker for assessing immune activation and predicting heart disease in RA.

Hs-CRP is utilized as a predictive sign for heart problems and stroke in apparently healthy individuals with or without risk factors for CAD. The hs-CRP test also detects lower levels of inflammation and suggests the risk for stroke and heart disease (10, 18). The present study showed higher serum levels of hs-CRP in RA patients as compared to controls similar to a study by (19) who demonstrated that hs-CRP can be used alone or in association with other markers for early detection of RA, prediction of disease duration, and evaluation of response to therapy (19). Moreover, Albabawaty et al. it was noted that hs-CRP is an important parameter for early diagnosis of RA (18).

The evaluation of obesity was based on anthropometric measurements. Traditionally, BMI which provides information on total body fat, has been the most commonly used index for determining the prevalence of high body weight in humans. However, the abdominal obesity indexes demonstrated by WC and WHR precisely reflect the distribution of central body lipids and are more closely correlated to the risk of cardiovascular disease than BMI (20). Moreover, abdominal obesity is a medical state linked to metabolic defects such as high blood pressure, high blood sugar, and lipid abnormalities, collectively called metabolic syndrome (21).

The present study showed that patients in the RA group had significantly higher means of BMI, WC, and WHR when compared with the control group, similar to a study by Ohno et al (2020). who showed higher BMI and WC were linked with a higher risk of RA. The positive association between BMI in midlife and RA was only found in women, with no discernible correlation in men. The increased frequency of RA in women may be caused by interactions with hormone-related variables (22). Additionally, increased BMI is associated with an increased risk of RA, as well as worse treatment efficacy and quality of life. Obesity may raise the risk of CVD and rheumatic disease due to an insufficient release of inflammatory adipokines (23). Research by Wan et al. in 2021 showed Neopterin levels were positively linked with BMI, higher in obese individuals than in non-obese individuals, and decreased following weight loss. Similarly, serum Neopterin levels increased with WHR greater than 0.9. In addition, neopterin and BMI may be associated with chronic low-grade inflammation in obese people (24). Moreover, recent research by Andonian in 2023 showed that higher body weight has a variety of negative effects which are associated with the development of RA, high disease activity, poor therapeutic response, and high degree of insulin resistance (25). The increased prevalence of obesity in early RA is linked to worse disease activity as well as health-associated quality of life, with significant adverse effects on obtaining low DAS 28 (11). In addition, obesity is linked with lipid abnormalities and may increase the risk of developing CAD. Furthermore, a study by Mandviwala et al. (year) showed that high body weight is both a peril factor and a risk sign for cardiac failure, arterial fibrillation, and subclinical and clinical CAD (26).

**Limitation:**

Small sample size and limited time of this study as we were not able to do electrocardiogram (ECG) and echocardiography (ECHO) for our patients to confirm heart problems.

**Conclusion:**

The inflammatory markers, Neopterin and high sensitivity-C reactive protein are found in higher levels in rheumatoid arthritis patients. The higher serum levels of these markers reflect the elevated level of systemic inflammation and prediction of coronary artery disease in Rheumatoid arthritis patients. Also, the higher prevalence of obesity and overweight in rheumatoid arthritis patients and the positive correlation between Neopterin levels and high anthropometric measures reflect an association between Neoperin and risk factors of coronary artery disease.

**Authors' declaration:**

We confirm that all figures and Tables in the manuscript are original to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical considerations' Approval-Ethical Clearance: The project was approved by the local ethical committee in the Department of Chemistry, College of Medicine, Universitr of Baghdad. according to the code number (80) on (04/ 03/ 2024).

**Conflict of Interest:** None
**Funding:** None

**Authors' contributions:**
Study conception & design: (Maryam Khaery kamil and Rana Ali Hamdi ). Literature search: (Maryam Khaery kamil). Data acquisition: (Maryam Khaery kamil and Rana Ali Hamdi). Data analysis & interpretatio: (Maryam Khaery kamil and Rana Ali Hamdi). Manuscript preparation: (Maryam Khaery kamil and Rana Ali Hamdi ). Manuscript editing & review:(Maryam Khaery kamil and Rana Ali Hamdi).

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# دور العلامات الالتهابية (النيوبتيرين والبروتين التفاعلي C عالي الحساسية) في التسبب في مرض الشريان التاجي لدى النساء المصابات بالتهاب المفاصل الرثوي

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**خلاصة:**

**الخلفية:** الخلفية: التهاب المفاصل الروماتويدي هو مرض التهابي. ترتبط مضاعفات التهاب المفاصل الرثوي بشكل أساسي بخطر الإصابة بأمراض القلب والأوعية الدموية. ان زياده الوزن له دور اساسي في تطور التهاب المفاصل الرثوي. النيوبترين يفرز من الخلايا الوحيدة والبلاعم.وقد تبين أن النيوبترين يفرز بشكل كبير عن طريق الشريان التاجي . كذلك ، فإن البروتين المتفاعل عالي الحساسية هو تحليل كيميائي حيوي يتم تطبيقه لتحديد كمية البروتين التفاعلي بحساسية عالية.

**الأهداف:** تقييم مستوى النيوبترين والبروتين المتفاعل عالي الحساسية في مصل الدم لدى المرضى الذين يعانون من التهاب المفاصل الرثوي للتنبؤ بخطر الإصابة بمرض الشريان التاجي. ايضا دراسة العلاقة بين النيوبترين والبروتين المتفاعل عالي الحساسية بالإضافة إلى القياسات الخاصة بالاوزان.

**المواد وطرق العمل:** شاركت 120 امرأة في هذة الدراسة ، 60 منهم مصابات بالتهاب المفاصل الرثوي، و60 منهم أصحاء. شملت التحليل الكيميائيه الحيوية في مصل الدم النيوبترين والبروتين المتفاعل عالي الحساسية بالأضافه للقياسات الخاصة بوزن الجسمز

**النتائج:** وجدت نتائج هذه الدراسة زيادة واضحة في متوسط قيم النيوبترين في مصل الدم

البروتين المتفاعل عالي الحساسة, مؤشر كتلة الجسم ,محيط الخصرونسبة الخصر إلى الورك في المرضى الذين يعانون من التهاب المفاصل الروماتيزمي مقارنة مع مجموعة الاصحاء . وايضا وجدت هذه الدراسة وجود علاقات إيجابية واضحة بين مستوى النيوبترين والبروتين المتفاعل عالي الحساسي، مؤشر كتلة الجسم ، محيط الخصر، ونسبة الخصر إلى الورك ,

**الاستنتاج:** إن العلامات الالتهابية ، النيوبترين والبروتين المتفاعل عالي الحساسية وجدت في مستويات أعلى في مرضى التهاب المفاصل الرثوي .و تعكس هذه المستويات المرتفعة مدى وجود الاتهابات الشاملة ومدى علاقتها بخطر الإصابة بمرض الشريان التاجي لدى مرضى التهاب المفاصل الرثوي. كما أن ارتفاع معدل انتشار الوزن الزائد والسمنة لدى مرضى التهاب المفاصل الرثوي و وجود علاقات ايجابية بين مستوى النيوبترين والقياسات الخاصة بزيادة الاوزان مما يعكس العلاقة بين النيوبرين وعوامل خطر الإصابة بمرض الشريان التاجي.

**الكلمات المفتاحية:** التهاب المفاصل الرثوي، نيوبترين، البروتين المتفاعل عالي الحساسية، السمنة.