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# Association of Serum Ferritin and Iron with C-reactive protein in Menopausal Women with Cardiovascular Disease in Erbil-City

**Nihayat Omar Ahmad**[1] , **Halala Hatem Mohammed**[2]

[1]*Department of Chemistry, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq*

[2]*Clinical Biochemistry, Hawler Medical University College of Medicine. Erbil, Iraq*

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## ABSTRACT

Cardiovascular diseases (CVD) are recognized as the number one cause of morbidity and mortality globally. The purpose of this research is to evaluate the impact of iron and ferritin levels in serum menopausal women and to determine the correlation between CRP with iron and ferritin in menopausal women that have CVD. This case-control study was carried out on (180) participants (40-69 years) of age with and without CVD. The following parameters were measured (iron, ferritin, and C-reactive protein). Menopausal women with CVD had significantly higher mean serum levels of iron (67.13±2.178 mg/dL *vs* 59.36±2.131 mg/dL) (p=0.0117) along with serum ferritin level (114.0±5.760 ng/mL *vs* 73.29±5.001 ng/mL) (p<0.0001) and CRP level (1.305±0.1584 mg/dL *vs* 0.4412±0.05976 mg/dL) (p<0.0001) in comparison with healthy menopausal women, and the results of this study shows a significant and positive correlation between Ferritin with CRP in menopausal women with CVD (r=0.37, p=0.0003). In conclusion, the serum level of ferritin, iron, and CRP higher significantly in menopausal patients compared with healthy menopausal women.

**Keywords:** Cardiovascular disease (CVD), C-reactive protein (CRP), Ferritin, Iron, and Menopausal women.

**Name:** Nihayat Omar Ahmad **E-mail:** nihayat.ahmad@su.edu.krd

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# العلاقة بين الفيريتين والحديد في المصل والبروتين التفاعلي سي لدى النساء في سن اليأس المصابات بأمراض القلب والأوعية الدموية في مدينة أربيل

**نهاية عمر احمد[1]، هةلالة حاتم محمد[2]**
*[1]قسم الكيمياء، كلية العلوم، جامعة صلاح الدين-أربيل، إقليم كردستان، العراق*
*[2]قسم الكيمياء الحيوية السريرية، كلية الطب بجامعة هولير الطبية، أربيل، العراق*

**الملخص**

تُعرف أمراض القلب والأوعية الدموية بأنها السبب الأول للوفيات والمرض على مستوى العالم. والغرض من هذا البحث هو تقييم تأثير مستويات الحديد والفيريتين في مصل النساء في سن اليأس وتحديد العلاقة بين البروتين التفاعلي سي والحديد والفيريتين في النساء في سن اليأس اللاتي يعانين من أمراض القلب والأوعية الدموية.

أُجريت دراسة الحالات والشواهد هذه على (180) مشاركًا (40–69 عامًا) من ذوي الأعمار يعانون من أمراض القلب والأوعية الدموية ومن غير المصابين بها. تم قياس المعلمات التالية (الحديد والفيريتين والبروتين التفاعلي سي).

كان لدى النساء في سن اليأس المصابات بأمراض القلب والأوعية الدموية مستويات مصل متوسطة أعلى بشكل ملحوظ من الحديد (67.13 ± 2.178 مجم / ديسيلتر مقابل 59.36 ± 2.131 مجم / ديسيلتر) ($p = 0.0117$)

إلى جانب مستوى الفيريتين في المصل (114.0 ± 5.760 نانوغرام / مل مقابل 73.29 ± 5.001 نانوغرام / مل)($p < 0.0001$)

ومستوى البروتين المتفاعل C–مجم / ديسيلتر (1.305 ± 0.1584 مقابل 0.4421 ± 0.05976 مجم / ديسيلتر) ($p < 0.0001$)

بالمقارنة مع النساء الأصحاء في سن اليأس.

وتُظهر نتائج هذه الدراسة وجود علاقة إيجابية مهمة بين الفيريتين مع البروتين المتفاعل C–في النساء في سن اليأس المصابات بأمراض القلب والأوعية الدموية ($r = 0.37$ ، $p = 0.0003$).

وفي الختام، فإن مستوى الفيريتين والحديد والبروتين التفاعلي سي في المصل أعلى بشكل ملحوظ في مريضات انقطاع الطمث مقارنة بالنساء الأصحاء في انقطاع الطمث.

## INTRODUCTION

Among chronic conditions, heart failure (HF) and cardiovascular diseases are two of the most common causes of death that contribute to the mortality epidemic worldwide. Cardiovascular disease (CVD) was responsible for one-third of them specifically ischemic heart disease and stroke [1-3]. CVD known as the primary disease-causing death in the world, due to an array of risk factors that are both environmental and of a genetic nature. This chronic condition has a significant impact on the quality of life. Several factors such as being overweight, diabetes, smoking, high blood pressure, high cholesterol levels, and a family history of heart disease, can increase the likelihood of developing cardiovascular conditions [4, 5].

Menopause is a biological phenomenon between the 45-55 age groups in which the menstrual cycle ends as a result of low hormonal secretion, which is made up of the majority of estrogen hormone. Cardiovascular diseases that can contribute to dying from heart attack or stroke are becoming more common in the postmenopausal years [6, 7]. Previous research has indicated that premenopausal women are less likely than age-matched women to develop

atherosclerosis, a condition characterized by the formation of plaques composed of fatty deposits and other substances within arteries. Then there is the fact that Estrogen shields women against cardiovascular diseases but the protection goes away after menopause and usually, women in late postmenopausal stages over 65 years are more vulnerable to atherosclerosis [8, 9] .

Iron is needed for numerous physiological activities, it has a role in the supply of blood cells, secure catalytic process, DNA synthesis as well as mitochondrial energy cycling. Inessential iron excess or inadequacy is associated with a range of cardiovascular diseases, and decreases plasma antioxidant levels, which raises the risk of ischemic cardiovascular events.

It can therefore be connected to
the progression of atherosclerosis [10, 11].

As iron deficiency impedes the function of heart muscle cells and energy metabolism at the level of mitochondria, heart failure might be the consequence. Atherosclerotic lesions can form more quickly when there is an iron overload because it releases free radicals, additionally, excess iron might lead to the appearance of hydroxyl radicals employing the Haber–Weiss–Fenton processes. These damaging radicals can oxidase biologically valuable molecules such as lipids, proteins, and DNA. What is more, it has been clinically proven that apoptosis through the process of iron-induced cell death, which is also termed "Ferroptosis", causes cardiomyocyte damage and is, therefore, a very important factor for the development of cardiovascular diseases[12, 13].

The new suggestion of Sullivan is that the prevalence of CVD in women after menopause is linked to the fact that they usually accumulate more iron reserves in the body; while this very feature is lacking in women before menopause, which might explain why it is the males of the same age who have lower CVD incidence, one of the variables that determines body iron storage is dietary iron consumption [14, 15].

Elevated sensitivity A well-known indicator of inflammation, CRP is high-sensitivity C-reactive protein (hs-CRP) is utilized to determine the risk of cardiovascular events. Research has demonstrated that ferritin can alter the connection between arterial stiffness, a crucial marker of atherosclerosis, and inflammation (as determined by hs-CRP). People with elevated ferritin and CRP levels may be more susceptible to cardiovascular problems because ferritin can increase the effect of CRP on arterial stiffness [16]. Elevated levels of CRP can cause to reduced blood flow to the heart in individuals with unstable or stable angina, as well as in those undergoing percutaneous angioplasty and emergency department visits due to acute coronary syndrome (ACS) [17].

Avoiding certain risk factors through diet or lifestyle changes can help decrease the levels of serum CRP, serum CRP levels ≥3μg/mL are used as an unspecific marker for tissue damage, infection, and inflammation linked to an acute phase response in clinical settings [18].

This study aims to evaluate mean serum levels of ferritin, iron, and CRP, and to find the relationships between ferritin and iron with CRP levels in menopausal women that have CVD in Erbil-city.

## MATERIALS AND METHODS

In this research, 180 participants had blood samples collected at the Hawler Teaching Hospital and the Surgical Specialty Hospital-Erbil Cardiac Center. The study included 90 patients with stenosis (a narrowing of the artery by more than 50 percent) due to coronary atherosclerosis disease. Ninety individuals who were healthy and did not have stenosis had samples of their blood collected. The patient and healthy groups were aged between 40 and 69 years old. Blood samples were collected from September 2023 to March 2024. A face-to-face questionnaire was used to collect the required information, including demographic characteristics, risk factors, family history, BMI, hypertension, and diabetes mellitus. Blood samples were collected, let

it to coagulated, then centrifugated, after that placed in a freezer at -70°C before they were analyzed. Serum of samples was used to determine Irone, Ferritin and C-reactive protein by auto analyzer (Roche/Hitachi COBAS C-311, Germany), improved particle enhanced immunoturbidimetric assay.

**Statistical Analysis:**

Data were analyzed by GraphPad Prism (version 10), and MedCalc is a statistical software package for the biomedical sciences. The mean and standard error of the mean (Mean ± SE) were the data's representations. Pearson's correlation on the other for determining relationships between variables. There has been established statistical significance with the p-value level ($p<0.05$) of significance. ROC curve (Receiver Operating Characteristic) analysis used for diagnostic efficiency.

## RESULTS AND DISCUSSION

Two groups were participated in this study healthy menopausal women and menopausal women that have CVD the mean ages are (56.80± 0.6171 and 59.29± 0.6550) respectively, each of them was about (50%) of the study population. According to the Chi-square test, there were a significant difference in duration of menopause, BMI, MAP, family history, obesity, smoking, and hypertension between healthy and menopausal women with CVD ($p<0.05$) (Table 1).

**Table 1: General characteristics of the study population**

| Variables | Case<br>Mean± SE (N%) | Control<br>Mean± SE (N%) | p-value |
|---|---|---|---|
| Age (years) | 59.29± 0.6550 | 56.80± 0.6171 | 0.0063 |
| Duration of menopause (years) | 10.71± 0.5900 | 6.289± 0.4835 | <0.0001 |
| BMI ($Kg/m^2$) | 30.74± 0.4325 | 26.89± 0.4660 | <0.0001 |
| MAP (mmHg) | 37.60± 0.4692 | 34.52± 0.2823 | <0.0001 |
| Family history<br>• Positive<br>• Negative | <br>50(55.56%)<br>40(44.44%) | <br>21(23.33%)<br>69(76.67%) | <0.0001 |
| Obesity<br>• Healthy Weight<br>• Overweight<br>• Obese | <br>8(8.888%)<br>28(31.11%)<br>54(60.0%) | <br>35(35.71%)<br>38(38.77%)<br>25(25.51%) | <0.0001 |
| Smoking<br>• Ex-smoker<br>• Non-smoker | <br>34(37.78%)<br>56(62.22%) | <br>3(3.33%)<br>87(96.67%) | <0.0001 |
| Hypertension<br>• Yes<br>• No | <br>81(90.00%)<br>9(10.00%) | <br>0(0.0%)<br>90(100.0%) | <0.0001 |
| Data are mean ± SE. BMI: body mass index; SBP: systolic blood pressure;<br>DBP: diastolic blood pressure; MAP: Mean Arterial Pressure; MAP=DBP+1/3(SBP-DBP). | | | |

Serum ferritin is viewed as the most reliable measure of stored iron levels, which are markers of iron levels in the body. The iron-heart disease theory suggests that an increased amount of iron in the body can lead to oxidative stress, which can increase the risk of developing chronic illnesses such as heart disease [19, 20]. The level of ferritin in the blood, often measured in studies looking at biomarkers, is a well-recognized indicator of iron deficiency and has been linked to risk factors for heart conditions, including diabetes, high insulin levels, and high blood pressure [21].

Studies examining the relationship between the amount of iron consumed through diet and the

presence of CVD produced varied findings. There was a clear link between high iron intake in the diet and an increased risk of cardiovascular disease in some cases, while in others, no such connection was seen. When the body's iron content increases, vital organs like the heart start to absorb iron, and excess iron can lead to myocardial diseases, pericarditis, and arrhythmias [22, 23]. The present study illustrated that menopausal women with CVD had significantly higher mean serum iron levels than healthy women (case: 67.13±2.178 mg/dL; control: 59.36±2.131 mg/dL) ($p<0.01$), this is consistent with [24, 25].

Since ferritin is a protein that reacts to inflammation, it might help explain why there's so much debate over the link between high levels of cardiac arrest ferritin and this condition. When it comes to serum ferritin, which is the gold standard for noninvasive probing of body iron stores, acute-phase proteins including ferritin can be raised during inflammation [26, 27], results of this study demonstrated a significant difference ($p<0.0001$) in serum ferritin levels between menopausal women with cardiovascular disease and healthy women (case: 114.0±5.760 ng/mL; control: 73.29±5.001 ng/mL), similar results have been obtained by the researchers [28-30]. In our study, the data analysis showed that when the mean serum ferritin level was considered a standalone factor, individuals with higher ferritin levels had increased iron levels in cardiovascular diseases.

Arterial hardening and heart diseases are predominantly linked to inflammation, as supported by many pieces of evidence. Some studies suggest that the accumulation of iron plays a crucial role in raising markers of inflammation, such as C-reactive protein (CRP). In our research, we found that CRP measurements alone could accurately forecast the occurrence of major heart conditions and major cardiovascular diseases later. This observation is agreement with most of the research on heart diseases, covering both overt and hidden cases, where higher CRP levels are strongly associated with heart diseases [31, 32]. The mean value of serum CRP levels in this study are higher in menopausal women with CVD compared to the healthy group significantly (case: 1.305±0.1584 mg/dL; control: 0.4412±0.05976 mg/dL) ($p<0.0001$), this results agreement with the results of previous study [28, 33], that showed in (Table 2) and (Fig. 1).

**Table 2: Biochemical parameter means of subjects in the study population.**

| Biochemical parameters | Mean± SE | 95% CI Lower- Upper | p-value |
|---|---|---|---|
| Iron (mg/dL) | | | |
| • Case (n=90) | 67.13±2.178 | 62.80-71.45 | 0.0117 |
| • Control (n=90) | 59.36±2.131 | 55.13-63.60 | |
| Ferritin (ng/mL) | | | |
| • Case (n=90) | 114.0±5.760 | 100.5-127.4 | <0.0001 |
| • Control (n=90) | 73.29±5.001 | 63.35-83.23 | |
| CRP (mg/dL) | | | |
| • Case (n=90) | 1.305±0.1584 | 0.9900-1.620 | <0.0001 |
| • Control (n=90) | 0.4412±0.05976 | 0.3225-0.5600 | |

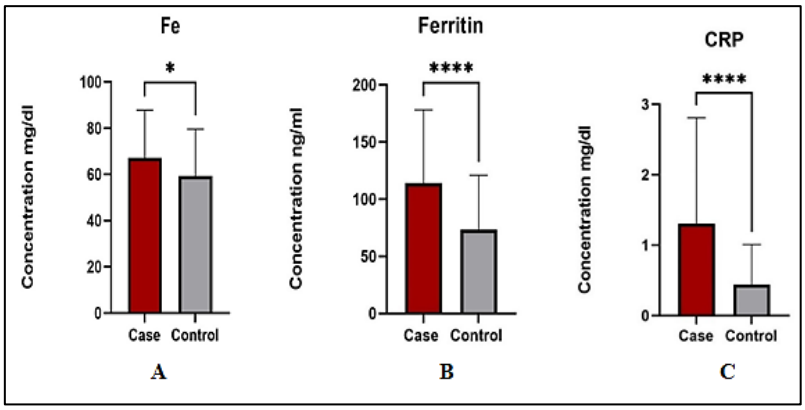


**Fig. 1: Compare serum levels of (A) Iron, (B) Ferritin, and (C) CRP between healthy menopausal and menopausal women with CVD.**

Among menopausal women with CVD, there was a positive and significant correlation between the serum ferritin with CRP (r=0.3754; p=0.0003). Whereas iron was correlated with CRP positively and non-significantly (r= 0.06458, p>0.05), as designated in (Fig. 2).

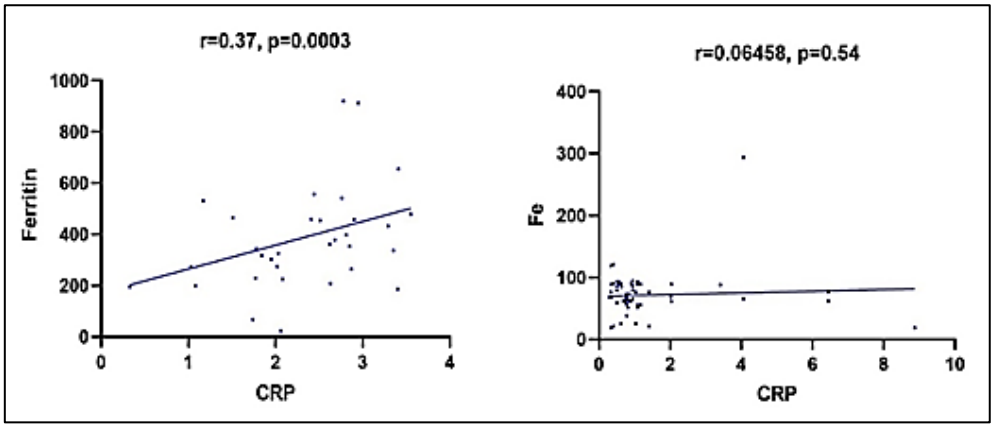


**Fig. 2: Correlation between CRP with Ferritin and Iron in studied group.**

From the Pearson correlation study, ferritin and CRP were significantly correlated to the duration of menopause in CVD (p< 0.05) as shown in (Table 3).

**Table 3: Correlation between duration of menopausal women with Fe, Ferritin and CRP in studied groups.**

| Variables | Pearson correlation (r) | Fe | Ferritin | CRP |
|---|---|---|---|---|
| Duration of menopause | r | 0.07168 | 0.4657 | 0.3444 |
| | p-value | 0.6967 | 0.0072 | 0.05 |
| MAP | r | 0.4109 | 0.3762 | 0.3631 |
| | p-value | 0.0195 | 0.0338 | 0.0411 |
| BMI | r | 0.1526 | 0.4080 | 0.1061 |
| | p-value | 0.4045 | 0.0204 | 0.5633 |

While Fe, Ferritin, and CRP have a positive and significant correlation with MAP (p<0.05). Results show a positive and significant correlation between BMI with Ferritin (p=0.02). Concerns that increase the risk of high blood pressure encompass markers of inflammation such as C-reactive protein (CRP), and several observational research efforts have confirmed their significance in the development of

hypertension [34]. Multiple research studies on both humans and animals have shown that inflammation is key to the onset of high blood pressure and is forecasted by levels of C-reactive protein (CRP) in the blood [35, 36]. The present study shows that MAP has positive and significant correlation with iron, ferritin, and CRP (Table 3).

The Body Mass Index (BMI) reflects our overall body structure, linked to the amount of energy expended. The connection between dietary iron intake and energy consumption was also explored during this analysis[7], people with a large BMI tend to consume more iron. It's important to investigate how this affects their levels of ferritin in the blood and iron reserves. In the regions of Kurdistan and Iraq, certain lifestyle factors like physical work, BMI, smoking, cholesterol levels (HDL and LDL), triglyceride levels, and blood pressure (including systolic and diastolic measurements) are significant risk factors for heart disease among women in menopause. Obesity-related visceral fat is a major source of chemicals that contribute to metabolic diseases. Inflammatory cytokines released in visceral fat elevate serum CRP levels, that have been associated to several metabolic alterations, including cardiovascular disease. BMI has positive and significant correlation with ferritin (r= 0.4080, p=0.02) (Table 3).

According to ROC curve analysis for evaluating the diagnostic biomarker, the Area Under the Curve (AUC) values for serum CRP is 84.4%, with significant p<0.0001, it indicates that it is very good biomarkers for diagnosing cardiovascular diseases in menopausal women. The AUCs for ferritin and Iron are 71% and 70% respectively and significantly p<0.0001, making them a good biomarker for menopausal women with cardiovascular diseases, in this study, we also found that CRP was the most sensitive and specific biomarker to detect CVD when compared to ferritin and iron (Table 4) and (Fig. 3).

**Table 4: Showing sensitivity, specificity, PPV and NPV of CVD biomarkers.**

| Biochemical Parameters | Cut off** | Sensitivity (%)** | Specificity (%)** | PPV (%)** | NPV (%)** | Accuracy** | %AUC* | p-value* |
|---|---|---|---|---|---|---|---|---|
| CRP | >0.47 | 88.89 | 76.67 | 79.2 | 87.3 | 0.6556 | 84.4 | <0.0001 |
| Ferritin | >53.4 | 92.22 | 51.11 | 65.4 | 86.8 | 0.4333 | 71.1 | <0.0001 |
| Fe | >54.58 | 83.33 | 57.78 | 66.4 | 77.6 | 0.4111 | 70.1 | <0.0001 |

**By MedCalc statistical software, *By ROC curve analysis

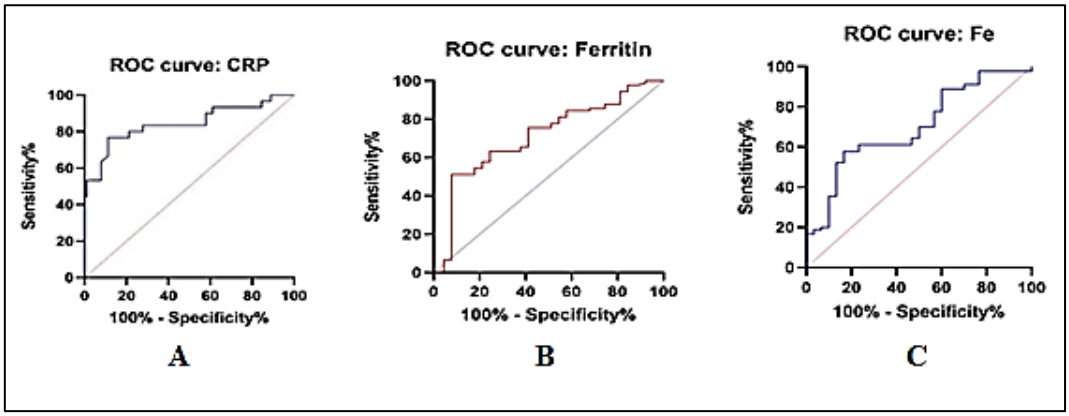


**Fig. 3: ROC curve analysis of CRP, Ferritin, and Fe.**

## CONCLUSIONS

According to the study's findings, having a higher-level iron, ferritin, and an inflammatory biomarker (CRP) increase the risk of cardiovascular disease. The findings of this investigation showed that a notably higher level of CRP is linked to elevated levels of body iron and ferritin, and results of this study illustrated that CRP has positive correlation with ferritin. Ferritin and CRP have good association with the possible CVD risk factors duration of menopause, MAP and BMI. While ferritin and CRP have positive significant correlation with duration of menopause.

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**Author contribution:** Authors contributed equally in the study.

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