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Research Article

The Role of Interleukin-6 and Myeloperoxidase in Cardiovascular Diseases

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RKAH carried out the experimental work, performed the data analysis, and drafted the manuscript. SKH supervised the study and critically revised the manuscript. Both authors approved the final version of the manuscript.

Keywords
IL-6, myeloperoxidase, Cardiovascular diseases, Inflammation biomarkers, ELISA, Diagnostic correlation

Abstract | Cardiovascular diseases (CVDs) are a set of conditions that affect the heart and the blood vessels, which include heart attacks, strokes, high blood pressure and heart failure. Mostly, these complications are seen from the accumulation of fats into arteries and stop the blood flowing regularly. The goal of the current work was to include the relationship between IL-6 and myeloperoxidase with cardiovascular diseases. One hundred (100) blood samples are collected from patients (60) with cardiovascular diseases and (40) are collected from healthy individuals. Blood was drawn from patients before the operation for people who needed a catheterization procedure. The serum was prepared by centrifuging of the samples. Myeloperoxidase and interleukin-6 are determined by sandwich ELISA techniques. The results showed that the distribution of gender in the control group in males and females was 20 and 20 respectively, while in the patient's group was 35 and 25 respectively. The distribution of age categories in the control group (48-60), (61-73), and (74-86) were 14, 18, and 8 respectively, the age groups of 48–60, 61–73, and 74–86 years were selected dependent on the natural range of participant ages in the research group and to reflect clinically relevant stages of cardiovascular risk. These brackets roughly correspond to middle-aged adults, early elderly, and advanced elderly populations each with distinct inflammatory and cardiovascular profiles. This stratification allows for more meaningful interpretation of biomarker trends in relation to aging and disease progression. While in the patient's group were 37, 20, and 3 respectively. Levels of IL-6 in patients and controls were (0.169) and (0.167) respectively. There was no significant difference between the two groups in IL-6 level, while the Myeloperoxidase level in the patients group showed a higher as compared with the control group. Levels of myeloperoxidase were distributed in many age groups (48–60, 61–73, and 74–86 years) in control and patient groups. In the 48–60 age group, MPO levels were significantly higher in the patient group compared to the control group. In the (61–73) age group, MPO levels was elevated in patients compared to controls. In the (74–86) age group, MPO levels in patients continued to be higher than in controls, though with slightly reduced statistical significance. There is no significant difference in IL-6 levels between the two groups in the age categories (48-60) and (61-73). while IL-6 level showed a negative significant difference in the Patients group as compared with the control group in (74–86) age. The Pearson correlation coefficients between three parameters: MPO, IL-6, and Age. The correlation showed a negative correlation (-0.82) between MPO and IL-6 levels. The strongest relationship observed was the negative correlation between MPO and IL-6 (R = -0.82), suggesting a possible inverse biological relationship. Age does not show a meaningful correlation with either MPO or IL-6. ROC curve was conducted to evaluate the diagnostic performance of MPO and IL-6.

Novelty Statement | This study identifies a strong, age-independent inverse relationship between myeloperoxidase (MPO) and interleukin-6 (IL-6) in cardiovascular disease and shows that MPO, unlike IL-6, discriminates cases from controls across clinically stratified age groups.

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Introduction

According to the World Health Organization, cardiovascular diseases (CVDs) remain the single

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largest killer in the world to this day, taking approximately 17.9 million lives each year (WHO, 2023). Among the various risk factors and pathophysiological mechanisms implicated in CVDs, inflammation has emerged as a central player in disease initiation and progression (Libby, 2021). Inflammatory biomarkers not only provide insight into disease processes but also serve as potential therapeutic targets. Two such biomarkers of interest are interleukin-6 (IL-6) and myeloperoxidase (MPO), both of which have shown significant associations with the development and severity of CVDs (Ridker *et al.*, 2017; Nicholls *et al.*, 2018).

IL-6 is produced by many types of cells such as adipocytes, macrophages, and endothelial cells after the tissue injury or infections or chronic inflammation (Tanaka *et al.*, 2014). IL-6 contributes to endothelial dysfunction, increase the adhesion molecules expression (Schuett *et al.*, 2009). High level of IL-6 asociated with the cardiovascular risk such as heart failure and coronary syndromes (Mossmann *et al.*, 2022).

MPO is a heme-containing peroxidase produce in monocytes and neutrophils. MPO have great ability to host defense by generating ROS, which are involved in bacterial cidal (Arnhold, 2020). However, excessive or chronic MPO activity has been implicated in oxidative damage to vascular tissues, promoting lipid peroxidation, endothelial dysfunction, and plaque instability (Zhang *et al.*, 2019). High level of MPO associated with cardiovascular diseases, also it predictor of cardiac diseases. The both IL-6 and MPO synergistic effects have role in oxidative stress and vascular inflammation that contributing to the pathophysiology of CVDs. Studies examining how biomarkers TNF-α and hs-CRP work mechanistically could develop diagnostic methods with suitable treatments to decrease cardiovascular deformities and associated fatality rates (Tang *et al.*, 2006).

This study evaluates the functions of IL-6 and MPO in cardiovascular diseases, examines their usefulness for prognosis and diagnosis and assesses existing therapeutic methods as well as future possibilities related to these molecules.

Materials and Methods

Study design

One hundred blood as a total sample was collected from patients (60) with cardiovascular diseases and samples (40) which collected from healthy individuals and then stored it in tubes. Blood was drawn from patients before the operation for people who needed a catheterization procedure. The sample size of 60 patients and 40 control individuals was determined based on practical constraints, including patient availability during the study period and feasibility across the involved hospitals. Although a priori power analysis was not performed, the post hoc statistical results particularly for MPO, which showed highly significant differences ($P < 0.0001$) indicate that the sample size was sufficient to detect true biological differences. However, the lack of significance in IL-6 levels suggests either smaller effect sizes or biological variability rather than insufficient sample size. The samples were centrifuged to obtain the serum used in the research that were taken from Ibn al-Bitar Hospital in Baghdad, Diwaniyah General Hospital, and Qastra Heart Hospital in Diwaniyah. As for the procedures. Myeloperoxidase (enzyme) and interleukin-6 (antibody) are tested by sandwich ELISA techniques in the patient's group and healthy group with documentation of the individual's data (age, duration of illness, type of treatment, smoking duration, and other diseases of the patient suffers from). Control individuals were screened through self-reported medical histories and basic clinical assessments to ensure the absence of cardiovascular diseases, diabetes, chronic inflammatory conditions, or recent infections. This approach aimed to minimize confounding variables that might influence IL-6 or MPO levels.

MPO ELISA kits (myeloperoxidase activity assay)

Myeloperoxidase (MPO) catalyzes the reduction of hydrogen peroxide into a reactive intermediate. This intermediate then reacts with *o*-dianisidine, which serves as a hydrogen donor, resulting in the formation of a yellow-colored product. This product exhibits a peak absorbance at 460 nm, allowing for indirect quantification of MPO activity by measuring the optical density (OD) at that wavelength.

Experimental procedure

- Mix 45 µL of the sample with 45 µL of Powder A solution thoroughly. Add 10 µL of Powder B solution and incubate at 37°C for 15 minutes.
- Control tube: Combine 350 µL of double-distilled water, 20 µL of sample, and 20 µL of clarifying agent in a 1.5 mL Eppendorf tube.
- Sample tube: Mix 20 µL of the sample with 20 µL of clarifying agent and 350 µL of chromogenic reagent in a 1.5 mL Eppendorf tube.
- Vortex all tubes thoroughly and incubate at 37°C for 30 minutes.
- Add 5 µL of acid reagent to each tube, vortex again, and incubate at 60°C for 10 minutes.
- Centrifuge the tubes at 3000×g for 10 minutes. Transfer 300 µL of the supernatant for analysis.
- Measure the absorbance at 460 nm using a microplate reader.

Human IL-6R (Interleukin-6 Receptor) ELISA Kit: This kit operates based on the Sandwich ELISA method. The wells of the provided microplate are pre-coated with antibodies specific to human IL-6R. When the sample

is added, IL-6R in the sample binds to these antibodies. Subsequently, a biotin-labeled detection antibody specific to IL-6R and an avidin-conjugated horseradish peroxidase (HRP) are added sequentially, forming a complex for detection.

Assay protocol

- Dispense 100 µL of each standard, blank, and sample into the designated wells. Seal the plate.
- Incubate for 90 minutes at 37°C.
- Without washing, discard the liquid from each well. Add 100 µL of the biotinylated detection antibody to each well, reseal, and incubate for 1 hour at 37°C.
- Wash each well with 350 µL of wash buffer. Let it soak for 1 minute, discard the liquid, and blot the plate dry on absorbent paper. Repeat this wash step three times.
- Add 100 µL of the HRP conjugate working solution to each well. Cover and incubate at 37°C for 30 minutes.
- Wash the wells five times as described in step 4.
- Add 90 µL of the substrate reagent to each well, cover the plate, and incubate for 15 minutes at 37°C.
- Add 50 µL of stop solution to each well to halt the reaction.
- Immediately measure the OD at 450 nm using a microplate reader.

MPO activity was measured using a colorimetric Myeloperoxidase Activity Assay Kit (Abcam, ab111749, Lot # GR3265930-1), and IL-6 concentration was determined using a human IL-6 ELISA kit (Elabscience, E-EL-H0102, Lot # L20240120). Assays were performed following the manufacturers' protocols.

Statistical analysis

Data were extracted in a digital data base form. Prior to performing t-tests and ANOVA, assumptions of normality and homogeneity of variance were assessed. The Shapiro–Wilk test was used to evaluate the normality of continuous variables, while Levene's test was applied to verify the equality of variances across groups. These tests confirmed that the data met the necessary assumptions for valid parametric testing. Statistical analyses were conducted using SPSS software version 27 and Microsoft Excel 2026. Descriptive statistics, including frequency distributions, percentages, and means ± standard error (SE), were initially computed. Inferential analyses were then performed using the t-test, one-way ANOVA, and Chi-square test, as appropriate. Furthermore, the area under the curve (AUC) and cutoff points for myeloperoxidase and IL-6 were established. To account for the increased risk of Type I error due to multiple comparisons in the ANOVA analyses, Tukey's Honest Significant Difference (HSD) post hoc test was applied where appropriate. This adjustment ensured more reliable identification of significant differences between age groups and biomarker levels. The correlation between two parameters was assessed using the correlation coefficient. A p-value of ≤ 0.05 was considered statistically significant (Bennett *et al.*, 2022).

Results

According to our results, the Distribution of gender in the control group in males and females was 20(50%) and 20(50%) respectively, while in the patient's group were 35(58.88%) and 25(41.66%) respectively. Distribution of age categories in the control group (48-60), (61-73), and (74-86) were 14(35%), 18(45%), and 8(20%) respectively, while in the patient's group was 37(61.66%), 20(33.33%) and 3(5%) respectively, as showed in the Table 1 and Figure 1.

Table 1: Distribution of gender and age in control and patients.

Variable	Category	Control		Patients	
		No.	%	No.	%
Gender	Male	20	50	35	58.88
	Female	20	50	25	41.66
Age	48-60	14	35	37	61.66
	61-73	18	45	20	33.33
	74-86	8	20	3	5

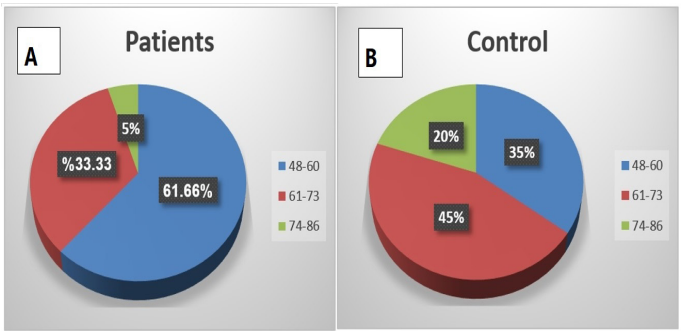

Figure 1: Age distribution among control and patient groups. Panel A shows the age distribution of patients; Panel B shows controls. Age is measured in years.

The present work found that the serum levels of IL-6 in patients and controls were (0.169±0.011) and (0.167±0.009) respectively at P<0.01. There is no significant difference between the patient group and control group in the level of IL-6 as shown in Table 2 and Figure 2.

Based on our results, The serum level of Myeloperoxidase (MPO) in the control group was (535.96±207.9) at a significant level (P<0.0001), while The serum level of Myeloperoxidase (MPO) in the patients group was (978.92±365.3) at a significant level

(P<0.0001). The patients group showed a higher level of Myeloperoxidase (MPO) as compared with the control group, as shown in Table 3 and Figure 3.

Table 2: The serum level of IL-6 in patients and control.

Groups	IL-6
Control	0.167±0.009
Patients	0.169±0.011
T value	0.851
P value	0.397*

* No significant difference at P<0.05, ** Highly significant difference at P<0.01

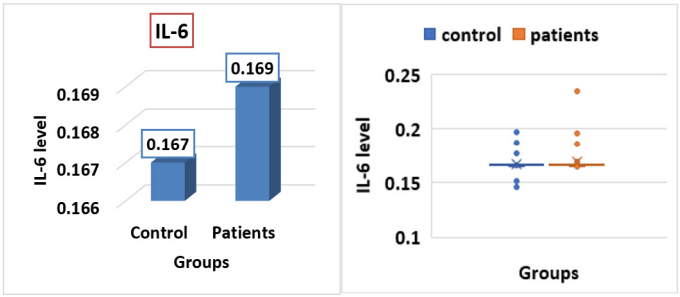

Figure 2: Serum IL-6 levels in patients and control group. IL-6 concentrations are expressed in pg/ml. No significant difference observed (p > 0.05).

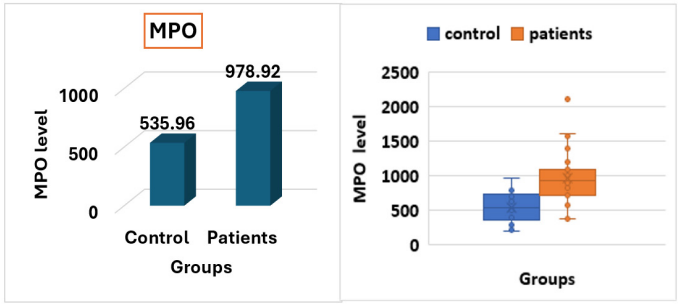

Figure 3: Serum MPO levels in patients and control group. MPO concentrations are expressed in ng/ml. A statistically significant increase was observed in patients (p < 0.01).

The levels of myeloperoxidase (MPO) disterbuted on many age groups (48–60, 61–73, and 74–86 years) in control and patient group as mean ± standard deviation (SD). In the 48–60 age group, MPO levels were significantly higher in the patient group (1028.4 ± 360.3) compared to the control group (570.51 ± 210.9). In (61–73) age group, MPO levels remained elevated in patients (901.7 ± 390.6) compared to controls (521.48 ± 221.1). In (74–86) age group, MPO levels in patients (882.7 ± 178.5) continued to be higher than in controls (508.07 ± 189), though with slightly reduced statistical significance, as shown in Table 4 and Figure 4.

These findings indicate that myeloperoxidase (MPO) levels are consistently higher in patients compared to controls across all age groups, with statistical significance diminishing in older age groups. This trend suggests a potential role of MPO in disease pathology that persists across aging but with varying degrees of statistical strength, as shown in Table 4 and Figure 4.

Table 3: The serum level of Myeloperoxidase (MPO) in patients and control group.

Groups	MPO
Control	535.96±207.9
Patients	978.92±365.3
T value	7.705
P value	<0.0001*

* Highly significant difference at P<0.01

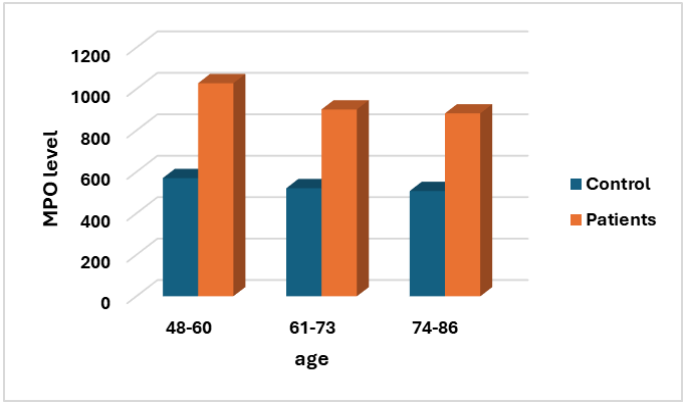

Figure 4: Distribution of MPO levels across age groups in patients and controls. Data are stratified by age brackets (48–60, 61–73, 74–86 years). MPO measured in ng/ml.

Table 4: Disterbution of the Myeloperoxidase (MPO) in the age.

Groups	Age (year)			F value	P value
	48-60	61-73	74-86		
Control	570.51±210.9	521.48±221.1	508.07±189	0.887	0.417*
Patients	1028.4±360.3	901.7±390.6	882.7±178.5	0.298	0.744*
T value	4.75	3.63	2.96		
P value	<0.0001***	0.001***	0.016**		

* No significant difference at P<0.05, ** Significant difference at P<0.05; ***Highly significant difference at P<0.01

According to our results, there was no significant difference of IL-6 level between the control group and patients group in the age catagories (48-60) and (61-73) at level P<0.05. while IL-6 level showed nagative significant difference of Patients group as compared with the control group in (74–86) age at at (P,0.01), as shown in Table 5 and Figure 5.

Based on the results, This table shows the Pearson correlation coefficients (R values) between three parameters: MPO (myeloperoxidase), IL-6 (interleukin-6), and age. The correlation (R) showed nagetive correlation (-0.82) between MPO and IL-6 level. The strongest relationship

Table 5: IL-6 level disterbution based on the age catagories.

Groups	Age (year)			F value	P value
	48-60	61-73	74-86		
Control	0.162±0.001	0.165±0.011	0.179±0.007	14.43	<0.0001**
Patients	0.167±0.006	0.172±0.016	0.165±0.007	1.23	0.299*
T value	0.923	1.18	8.34		
P value	0.274*	0.162*	0.001**		

* No significant difference at P<0.05, Significant difference at P,0.01

observed is the negative correlation between MPO and IL-6 (R = -0.82), suggesting a possible inverse biological relationship. Age does not show a meaningful correlation with either MPO or IL-6 in this dataset as (-0.100) and (0.037), respectievly, as shown in Table 6.

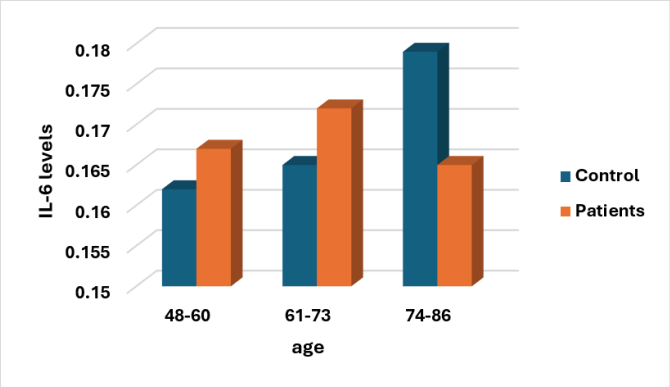

Figure 5: Distribution of IL-6 levels across age groups in patients and controls. Age brackets are shown on the x-axis; IL-6 is measured in pg/ml.

Table 6: Correlation/ patients.

Parameter	R and P value	MPO	IL-6	Age
MPO	R	1		
	P	<0.00001**		
IL-6	R	-0.82	1	
	P	0.535*	<0.00001**	
Age	R	-0.100	0.037	1
	P	0.446	0.781	<0.00001**

* No correlation at P<0.05, **Highly correlation at P<0.01

Receiver Operating Characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of MPO and IL-6. The area under the curve (AUC) for MPO was 0.872 with a standard error of 0.034, indicating a high diagnostic accuracy. The analysis yielded a sensitivity of 96.7% and a specificity of 60.0% at a cutoff value of 571.66, with a 95% confidence interval ranging from 0.805 to 0.939 (P < 0.001). Receiver Operating Characteristic (ROC) analysis was augmented by 95% confidence intervals (CI) of the area under the curve (AUC) to indicate diagnostic accuracy. Moreover, the Youden index (J = Sensitivity + Specificity − 1) was used to define the optimal cutoff point of each biomarker. This will facilitate a less subjective determination of optimal thresholds for maximising diagnostic performance. In contrast, IL-6 demonstrated a lower AUC of 0.722 with a standard error of 0.051, reflecting moderate diagnostic accuracy. IL-6 had a sensitivity of 78.3% and a specificity of 50.0% at a cutoff value of 0.1668, with a 95% confidence interval between 0.622 and 0.822 (P < 0.001). These findings suggest that MPO is a more reliable biomarker than IL-6 for distinguishing between the studied groups, as shown in Table 7 and Figures 6 and 7.

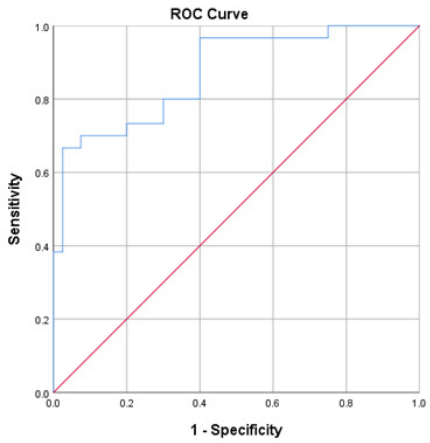

Figure 6: ROC curve for serum MPO levels in diagnosing cardiovascular disease. AUC, sensitivity, and specificity values are indicated. Units in ng/ml.

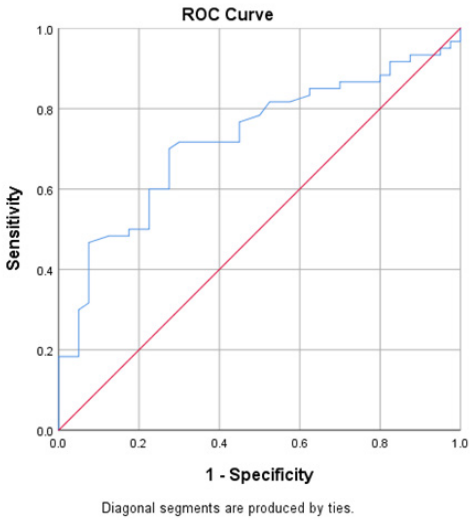

Figure 7: ROC curve for serum IL-6 levels in diagnosing cardiovascular disease. AUC and optimal cut-off values based on Youden index are provided. Units in pg/ml.

Table 7: Area under curve for myeloperoxidase between patients and control.

Parameter	AUC	Standard error	P value	Sensitivity	Specificity	95% confidence interval		Cutoff point
						Lower bound	Upper bound	
Myeloperoxidase	0.872	0.034	0	0.967	0.600	0.805	0.939	571.66
IL-6	0.722	0.051	0	0.783	0.500	0.622	0.822	0.1668

Discussion

The present work found that IL-6 level in patients and controls were (0.169 ng/mL) and (0.167 ng/mL), respectively. There is no significant difference between the patient group and control group in the level of IL-6, suggesting that IL-6 may not serve as a reliable standalone biomarker for CVD in this cohort. The lack of a statistically significant difference in IL-6 levels between the patient and control groups may be influenced by several factors. Firstly, blood samples were collected before catheterization procedures, potentially during a stable phase of cardiovascular disease, when systemic inflammation might be lower. Secondly, IL-6 is known to be influenced by various confounding factors such as age-related inflammation (inflammaging), obesity, and comorbid conditions, which may have also been present in control individuals. Finally, limitations related to assay sensitivity or timing of sample collection might have contributed to the observed results. These factors suggest that IL-6, while biologically relevant, may not always serve as a robust standalone biomarker in all clinical contexts.

A study by Li *et al.* (2021) demonstrated that while IL-6 levels are associated with inflammation, their elevation is not consistently predictive of cardiovascular events in healthy populations.

Contrary to these findings, a large body of literature supports the association of elevated IL-6 levels with increased cardiovascular risk. According to Khan *et al.* (2024) IL-6 performs a vital function in inflammation-caused atherogenesis while displaying independent links to myocardial infarction and stroke risks.

Scientific research based on more than forty prospective studies revealed that individuals with elevated IL-6 hormone levels developed cardiovascular disease events (Kaptoge *et al.*, 2014). IL-6 levels show no differences between clinical CVD patients and control subjects when evaluating early or stable phase of disease progression. IL-6 levels spanned a wide range among participants and their values connected to both aging patterns and obesity patterns and persistent inflammation status causing complications for IL-6 usage in cardiovascular pathology diagnosis (Müller and Di Benedetto, 2024).

The research results showed insufficient evidence of IL-6 variation between CVD patients and control subjects possibly due to testing timing variability and patient subgroup differences. Extensive evidence shows the role of IL-6 in cardiovascular pathology yet its sensitivity to detect disease varies among different populations thus research needs wider and more diverse samples (Kaptoge *et al.*, 2010).

Our experimental data revealed the patients group had elevated MPO levels when compared against the control group results. Multiple research studies confirm MPO association with oxidative stress together with endothelial dysfunction and plaque instability which are characteristic features of atherosclerosis.

MPO originates from neutrophil and monocyte activation to conduct lipid oxidation which fuels the developmental process of atherosclerosis. The measurement of MPO demonstrates its ability to forecast myocardial infarction development together with additional heart-related health complications. Research findings demonstrated elevated MPO levels in patients with unstable angina and acute myocardial infarction which confirms its role in plaque destabilization and cardiovascular risk assessment according to Zhang *et al.* (2001).

MPO demonstrates its potential as an effective pathophysiological biomarker for CD disease because it strongly links to vascular inflammation and oxidative stress processes. The ROS produced by MPO lead to lipid oxidation through its catalyst activity. Plaque development in atherosclerosis becomes more rapid due to oxidative damage which raises the chance of plaque rupture. Higher MPO measurements in the blood stream corresponded to more advanced coronary artery disease. When people have acute coronary syndromes their MPO levels are substantially elevated. MPO serves as a vital factor which enhances plaque susceptibility and leads to detrimental cardiovascular events while providing potential preclinical risk assessment for patients (Nicholls *et al.*, 2006).

MPO plays a critical role in the development of atherosclerosis and have role on oxidation. This oxidative activity contributes to endothelial dysfunction and accelerates lipid accumulation. The high level of MPO are associated with adverse cardiovascular outcomes. The patients with unstable angina and acute myocardial infarction had higher MPO level as compared to control. MPO's potential role as a biomarker for plaque instability

and its predictive value for major cardiac events. Thus, the elevation of MPO in cardiovascular disease is occure due to the inflammation (Zhang *et al*., 2001).

The patients with acute coronary syndromes had elevated MPO levels, and high MPO concentrations were predictive of future adverse cardiovascular events, independent of traditional risk factors (Kolodziej *et al*., 2019). MPO elevated levels are consistently associated with atherosclerotic plaque formation and instability (Trpkovic *et al*., 2015). Higher MPO plasma levels in patients undergoing elective coronary angiography were associated with a greater risk of major adverse cardiac events, suggesting MPO's role as both a marker and a mediator of CVD progression (Cavusoglu *et al*., 2007). Elevated MPO levels can also be observed in other inflammatory or infectious conditions, which limits its diagnostic precision. while MPO plays a role in inflammatory processes, its elevation alone is insufficient to distinguish between CVD and other systemic inflammatory responses, suggesting it should be interpreted in conjunction with other markers (Liu *et al*., 2023).

MPO level disterbuted on many age groups (48–60, 61–73, and 74–86 years) in control and patient group. In the 48–60 age group, MPO levels were significantly higher in the patient group (1028.4) compared to the control group (570.51). In (61–73) age group, MPO levels remained elevated in patients (901.7) compared to controls (521.48). In (74–86) age group, MPO levels in patients (882.7) continued to be higher than in controls (508.07).

Studies found that MPO contributes to oxidative stress, lipid peroxidation, and endothelial dysfunction, which are age-independent processes involved in atherosclerosis and plaque instability. High MPO levels were predictive of adverse cardiovascular outcomes, when stratified by age, confirming its role as an marker of vascular inflammation (Kolodziej *et al*., 2019; Nicholls and Hazen, 2006).

MPO elevation can reflect systemic inflammation or comorbidities common in aging populations, such as chronic infections, autoimmune conditions, or even certain cancers. MPO lacks disease specificity, and elevated levels can be misleading in elderly individuals without CVD but with other inflammatory conditions (Liu *et al*., 2023). Moreover, MPO's predictive value diminishes in very elderly populations due to overlapping inflammatory signals from multiple aging-related disorders, thereby recommending that MPO be used alongside other biomarkers for more accurate CVD (Tang *et al*., 2006).

According to our results, There was no significant difference between the control group and patients group in the age catagories (48-60) and (61-73). while IL-6 level showed nagative significant difference of Patients group as compared with the control group in (74–86) age.

IL-6 is a key pro-inflammatory cytokine implicated in the pathogenesis of atherosclerosis and cardiovascular diseases. Some studies support elevated IL-6 levels in patients with CVD (Barcena *et al*., 2024). IL-6 increases with age, contributing to inflammaging a chronic, low-grade inflammation common in elderly populations (Ajoolabady *et al*., 2024). This could partially explain elevated IL-6 levels in control individuals of advanced age, which may mask the cytokine elevation normally associated with CVD in this age group.

Moreover, in very elderly populations, immune system remodeling and comorbidities may result in higher baseline IL-6 levels, independent of cardiovascular disease status (Singh and Newman, 2011). This may explain why the control group in the 74–86 age category had unexpectedly higher IL-6 levels than the patient group, leading to the observed negative difference.

Some reports also suggest that IL-6 as a marker loses specificity with age, especially beyond the age of 70, due to systemic factors like frailty, infections, or malignancy (Kelley *et al*., 2019). IL-6 elevation may no longer be a reliable standalone indicator for CVD in the elderly.

In contrast, studies on younger or middle-aged patients generally show IL-6 elevation associated with CVD and metabolic syndrome (Khan *et al*., 2024). The lack of difference in our younger groups might be due to limited sample size or pre-existing low inflammatory burden in the study population. The age-related declines in IL-6 sensitivity or feedback inhibition mechanisms might also affect circulating cytokine levels and their interpretability (Barcena *et al*., 2024).

The data analysis demonstrates that MPO and IL-6 levels share a negative correlation relationship, this strong negative correlation (r = -0.82) between MPO and IL-6 is indeed biologically counterintuitive, given that both are markers for inflammatory reactions. One such explanation may be, the discrepancy in their kinetics of expression in inflammation: MPO is early product of release from neutrophils in acute phases while IL-6 may not he maximal or may be suppressed in chronicity. The heterogeneity in immune responses between individuals, time points of samples collected, and underlying comorbidities might also play a role in the expression of these markers. This inhibitive relationship may as well describe complex regulatory loops in the inflammatory cascade. So, additional mechanistic research is required to better understand this association. MPO exhibits a negative connection to IL-6 amounts which may reflect an opposite biological impact between these markers. This dataset demonstrates that age does not show any relevant

statistical relationship with MPO or IL-6 measurements. The substantial negative relationship between MPO and IL-6 demonstrates an opposite biological interaction between these inflammatory markers. The age correlation analysis showed a weak result because age demonstrated no significant connection to these markers in this particular dataset.

It is important to note the negative relationship between MPO and IL-6 because these markers take part in inflammatory processes. The inflammatory protein MPO arises from activated neutrophils together with monocytes and research demonstrates its established connection to oxidative stress (Linton and Fazio, 2003). The immune system activation mechanism involves IL-6 as a cytokine substance that performs its functions during inflammatory processes (Kawasaki *et al*., 2013). Elevated MPO levels might establish an intricate regulation that results in decreased IL-6 synthesis or activity level. The immune-regulatory process between different inflammatory biomarkers becomes negatively correlated following modulation (Van der Meer *et al*., 2012).

Higher age fails to produce statistically important connections to these biomarkers because aging inflammation seems to be influenced predominantly by genetic characteristics and environmental components and medical conditions that exist beneath the surface (Gale *et al*., 2013).

ROC curve analysis was used to determine the diagnostic capacities of MPO along with IL-6. The diagnostic accuracy for MPO was supported by its area under the curve measurement at 0.872. The evaluations using the ROC curve showed MPO achieved 96.7% sensitivity and 60% specificity throughout the designated confidence interval from 0.805 to 0.939. The diagnostic value of IL-6 proved to be moderate as assessed by its AUC value of 0.722. The sensitivity of IL-6 biomarker reached 78.3% while the specificity achieved 50.0% with confidence interval ranging from 0.622 to 0.822. The research shows MPO serves as a more effective biomarker than IL-6 for differentiating between the investigated patient groups.

The assessment of biomarker diagnostic accuracy relies heavily on ROC curve analysis since it enables measurement of sensitivity and specificity and calculation of area under the curve. The diagnostic accuracy determination of MPO biomarker was shown through its AUC value which reached 0.872. The high value of AUC indicates MPO effectively discriminates between the studied groups while maintaining high accuracy levels. The ability of MPO to detect CVD patients remains very precise even while maintaining enough accuracy for recognizing healthy control subjects. Evaluation using the 95% confidence interval (0.805–0.939) confirmed the solid base for researching MPO as the AUC results exceeded the threshold value of 0.5 for adequate performance (Zhang and Zheng, 2013).

The diagnostic accuracy of IL-6 testing showed a moderate to lower performance level with an AUC value of 0.722. While IL-6 demonstrates the capability to detect patients with cardiovascular disease it fails to match MPO's diagnostic effectiveness with a sensitivity of 78.3% along with specificity of 50.0%. The 0.622–0.822 confidence interval reveals that IL-6 measurement provides variable diagnostic performances and lower reliability for differentiating between patient and control groups according to (Dai *et al*. 2014). The assessment shows MPO demonstrates better promise than IL-6 as a diagnostic biomarker due to enhanced sensitivity and specificity which establishes it as a superior clinical testing method when used for patient-health control differentiation (Liu *et al*., 2009; Qin *et al*., 2025).

The chosen biomarkers emerge as vital elements in establishing disease identification processes for clinical applications. The moderate diagnostic capabilities of IL-6 for cardiovascular disease in this study imply it should be applied alongside other biomarkers as opposed to individual use as a diagnostic marker as documented in (Franceschi *et al*., 2007). Research by Khan *et al*. (2024) supports these findings which demonstrate that IL-6 evaluation alone fails to distinguish properly between cardiovascular disease patients and non-affected.

The performance of MPO surpasses IL-6 in terms of diagnostic accuracy for CVD since it reveals superior sensitivity and specificity along with higher AUC values. MPO shows promise as an effective cardiovascular disease biomarker but additional medical studies will help clinicians establish its ideal diagnostic use.

The lack of multivariate adjustment for confounders (i.e., smoking, BMI, hypertension, diabetes) is a limitation that will need further study in larger cohorts.

Clinical implications

The observed elevation of myeloperoxidase (MPO) levels among CVD patients, particularly in older age brackets, suggests a potential role for MPO as a clinically relevant biomarker. Given its strong discriminatory performance in ROC analysis, MPO could be integrated into existing cardiovascular risk assessment models alongside traditional markers such as hs-CRP and LDL. Incorporating MPO may enhance early detection of subclinical inflammation and vascular dysfunction, especially in populations with age-related risk factors. However, further large-scale prospective studies are needed to validate its predictive utility in clinical practice.

Future directions

While our findings suggest a strong association between MPO and cardiovascular risk, further longitudinal studies are warranted to validate these observations in larger, diverse populations. Future research should explore the integration of MPO into multiplex biomarker panels alongside IL-6, hs-CRP, and emerging inflammatory markers to improve early risk stratification. Prospective cohort studies with standardized follow-up and serial measurements of inflammatory mediators could provide insight into temporal changes and causal relationships. Additionally, mechanistic investigations are needed to elucidate the biological basis of the inverse MPO–IL-6 relationship observed in older patients, which may have implications for personalized cardiovascular risk prediction and targeted anti-inflammatory therapies.

Conclusion

MPO concentration were markedly increase in the cardiovascular conditions compared to healthy individuals across all the all age groups. MPO have high potential biomarker for cadiovascular diseases. In contrast, IL-6 did not show a significant difference between groups. A strong negative correlation was observed between MPO and IL-6 levels (inverse relationship). Age was not correlated with either biomarker. ROC analysis have the diagnostic value of MPO in CVD.

Declarations

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Ethical statement and IRB approval

IRB approval was obtained from the Department of Chemistry, College of Education, University of Al-Qadisiyah (Ref. 36-12/01/2025). The study complied with university ethical/biosafety policies and the Declaration of Helsinki; written informed consent was secured from all participants.

Declaration of generative AI and AI-assisted technologies in the writing process

No Generative AI and AI-assisted technologies wer used in the writing process.

Conflict of interest

The authors have declared no conflict of interest.

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