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Atherosclerosis-associated *MARCO* and *APOE* Gene Expression, PTX3, Lp-PLA2, and KIM-1 Biomarkers in Pre- and Post-Catheterization Patients

Abbas Oudah Lazim[1,2*], Sahar Saadi Karieb[1]
[1]*Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq*
[2]*Ministry of Education, Baghdad, Iraq*

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Abstract

Atherosclerosis (AS) is a chronic inflammatory disorder causing cardiovascular illnesses, responsible for rising death rates worldwide. This study aimed to investigate the diagnostic potential of Pentraxin 3 (PTX3), Lipoprotein-associated phospholipase A2 (Lp-PLA2), and Kidney Injury Molecule-1 (KIM-1) biomarkers, alongside *MARCO* and *APOE* gene expression. In this study, 90 participants were enrolled, aged 20 to 75, including 60 AS patients divided into two groups: 30 pre-catheterization (Pre-Cath) and 30 post-catheterization (Post-Cath) patients, along with 30 healthy controls. Results indicated markedly increased levels of PTX3, Lp-PLA2, and KIM-1 in AS patients compared to controls. Gene expression analysis using RT-qPCR showed a significant upregulation of *MARCO* in Pre-Cath patients. In contrast, *APOE* expression was notably increased in the Post-Cath patient. The higher the waist-to-hip ratio (WHR), the more significantly correlated with increased levels of Lp-PLA2, reinforcing the association between central obesity and vascular inflammation. Notably, KIM-1 levels were higher in patients with a WHR > 0.9, emphasizing its role in endothelial dysfunction in obese individuals. Correlation analysis demonstrated that there were many correlations between the parameters, such as association between KIM-1 and Age, highlighting their role in vascular inflammation. Higher PTX3 levels were observed in patients (≤50 years), particularly in the Post-Cath patient group. In contrast, KIM-1 levels were markedly higher in patients (>50 years), particularly in Pre-Cath cases, highlighting its relevance linking aging to sustained inflammation. In conclusion, this is the first Iraqi study to investigate these biomarkers and their relationship to AS, which is crucial for diagnosing, monitoring the onset, and assessing the progression of AS, and may aid in developing early diagnostic and therapeutic approaches to prevent its progression.

Keywords: *MARCO, APOE,* PTX3, KIM-1, Atherosclerosis

*Email: abbas.udah2202@ihcoedu.uobaghdad.edu.iq

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التعبير الجيني لجيني *MARCO* و*APOE*، والمؤشرات الحيوية PTX3 و Lp-PLA2 و KIM-1 المرتبطة بتصلب الشرايين لدى المرضى قبل وبعد القسطرة

عباس عوده لازم[1,2*] و سحر سعدي غريب[1]

[1]قسم علوم الحياة، كلية التربية للعلوم الصرفة (ابن الهيثم)، جامعة بغداد، بغداد، العراق

[2]وزارة التربية، بغداد، العراق

الخلاصة

يُعد تصلب الشرايين (AS) اضطرابًا التهابيًا مزمنًا يُسبب أمراضًا قلبية وعائية، وهو مسؤول عن ارتفاع معدلات الوفيات عالميًا .هدفت هذه الدراسة إلى دراسة الإمكانات التشخيصية للمؤشرات الحيوية PTX3 و Lp-PLA2 و KIM-1، إلى جانب التعبير الجيني لجينات *MARCO* و *APOE* في هذه الدراسة، تم تسجيل 90 مشاركًا تتراوح أعمارهم بين 20 و75 عامًا، من بينهم 60 مريضًا بتصلب الشرايين، مُقسمين إلى مجموعتين: 30 مريضًا قبل القسطرة (Pre-Cath) و30 مريضًا بعد القسطرة (Post-Cath) ، بالإضافة إلى 30 مريضًا سليمًا .أظهرت النتائج ارتفاعًا ملحوظًا في مستويات PTX3 و Lp-PLA2 و KIM-1 لدى مرضى تصلب الشرايين مقارنةً بالمجموعة الضابطة .أظهر تحليل التعبير الجيني باستخدام RT-qPCR ارتفاعًا ملحوظًا في التعبير الجيني لجين *MARCO* لدى مرضى ما قبل القسطرة .في المقابل، زاد التعبير الجيني لجين *APOE* بشكل ملحوظ لدى مرضى ما بعد القسطرة .ارتبط ارتفاع معدل WHR بشكل ملحوظ بزيادة مستويات Lp-PLA2 ، مما يعزز العلاقة بين السمنة المركزية والالتهاب الوعائي .والجدير بالذكر أن مستويات KIM-1 كانت أعلى لدى المرضى الذين لديهم معدل WHR > 0.9، مما يؤكد دوره في خلل وظائف بطانة الأوعية الدموية لدى الأفراد المصابين بالسمنة .أظهر تحليل الارتباط وجود العديد من الارتباطات بين المعايير، مثل الارتباط بين KIM-1 والعمر، مما يسلط الضوء على دوره في الالتهاب الوعائي .لوحظت مستويات أعلى من PTX3 لدى المرضى (≥ 50 عامًا)، وخاصةً في مجموعة مرضى ما بعد القسطرة .في المقابل، كانت مستويات KIM-1 أعلى بشكل ملحوظ لدى المرضى (≥ 50 عامًا)، وخاصةً في مجموعة مرضى ما قبل القسطرة، مما يبرز أهميتها في ربط الشيخوخة بالالتهاب المستمر. في الختام، هذه هي أول دراسة عراقية لهذه المؤشرات الحيوية وعلاقتها بتصلب الشرايين، وهو أمر مهم لتشخيص وظهور وتطور المرض، وقد يساعد في تطوير مناهج تشخيصية وعلاجية مبكرة لمنع تطورها.

1. Introduction

Atherosclerosis (AS) represents a chronic inflammatory process driven by the deposition of cholesterol-rich lipoproteins and inflammatory activation in the vessel wall to produce atheromatous plaques, which cause arteries to thicken and narrow, which can trigger dangerous cardiovascular incidents such as heart attacks and strokes [1]. The development of AS starts with damage to endothelial cells, followed by the accumulation of lipoproteins and an inflammatory process that leads to fibrous plaques forming on the arterial wall [2, 3]. Risk factors for AS include age, hypertension, high cholesterol, smoking, diabetes, obesity, and chronic inflammation due to other conditions [4, 5]. Chronic hypertension is a significant cause of several cardiovascular diseases (CVD) [6]. Diagnosing AS involves different techniques and strategies; these techniques ranging from biochemical markers and imaging variants to clinical assessments.

Cytokines are essential pushers of AS, the leading cause of CVD. These signaling proteins play roles in different steps of AS, from early endothelial dysfunction to the destabilization of AS plaques [1]. Interferon-gamma (INF-γ) and Tumor Necrosis Factor-alpha (TNF-α) are the most crucial pro-inflammatory cytokines triggering the thinning of the fibrous cap of

atherosclerotic plaque, therefore increasing the risk of their rupture [7,8]. Among the several inflammatory mediators and cellular components involved in AS development, macrophages are essential in coordinating immune responses inside atherosclerotic lesions [1].

The Macrophage Receptor with Collagenous Structure (*MACRO*) gene is involved in the pathogenesis of AS [9]. The *MARCO* gene encodes the protein macrophage receptor with collagenous structure, a scavenger receptor found on the surface of macrophages, the central executive cell type of the inflammatory response in AS, these cells lead to instability and progression of plaques through lipid accumulation and secretion of immunological cytokines [10]. Monocytes capacity to phagocytose oxidized lipids and dead cells rises with high expression of the *MARCO* gene, it also enhances the inflammatory response within the arterial walls, which promotes disease progression [11]. Furthermore, the Apolipoprotein E *(APOE*) gene has an important role in fat metabolism, cholesterol transport in the body, and the distribution of fats and their transport to cells. *APOE* action in atherogenesis mainly depends on its lipid-interaction based vascular effects [12]. These effects can be influenced by genetic variations within the APOE gene itself. This polymorphism consists of three common alleles (ε2, ε3, and ε4) that have different phenotypic effects with opposing actions on cardiovascular risk [13].

Pentraxin 3 (PTX3) is an important biomarker for CVD and a modulator of inflammatory processes related to AS. PTX3, an acute-phase protein induced by inflammatory signals, participates in several cellular mechanisms that contribute to atherogenesis [14]. Increased PTX3 levels are present in response to acute myocardial infarction and heart failure and correlate with the level of tissue damage or inflammation [15].

Moreover, Lipoprotein-associated phospholipase A2 (Lp-PLA2) significantly contributes to the AS process, which is a common cause of CVD [16]. Lp-PLA2 hydrolyzes oxidized phospholipids, compounds linked to oxidative stress and inflammation in AS lesions [17]. Lp-PLA2 is produced by inflammatory cells, including monocytes, and its levels correlate strongly with the number of monocytes [18]. In addition to that, Kidney Injury Molecule-1 (KIM-1) is part of the T-cell immunoglobulin and mucin domain family, involved in immune response regulation and expressed by both immune and epithelial cells [19]. AS causes constriction of the renal blood arteries, hence lowering of blood flow to the kidneys (renal ischemia) and hence gradual renal tubular damage, ischemia raises the expression of KIM-1, an early indicator of renal tubular damage, therefore helping to forecast future renal failure by inducing oxidative stress and inflammation within the kidney [20]. Due to the lack of studies on these biomarkers (PTX3, LP-PLA2, and KIM-1), the study aimed to gain a deeper understanding of AS pathogenicity by exploring the roles of these biomarkers and role some genes such as, *MARCO* and *APOE,* in the AS initiation and progression, which may help in developing early diagnostic and therapeutic approaches to prevent disease progression.

2. Materials and methods

2.1 *Subject collection*

A total of 90 individuals were enrolled in this study. Among them, 60 patients diagnosed with AS were randomly selected while attending the Ibn Al-Bitar Specialized Cardiac Surgery Center/Baghdad/Iraq. These patients were divided into two groups: the first group included 30 Pre-Cath patients, and the second group included 30 Post-Cath patients. In addition, 30 apparently healthy individuals participated in this study. The study extended the period from August 2024 to February 2025. The age of AS patients and healthy individuals ranged from 20 to 75 years. All the studied patients were given a questionnaire that asked about sex, age, weight, length, waist circumference, hip circumference, smoking, duration of illness, whether they had chronic hypertension, family history, type of treatment used, and

whether the patient had undergone catheterization and open-heart surgery or not, People with chronic other diseases were excluded from the study. Participants were categorized for subgroup analysis based on age into two groups: ≤50 years and >50 years. According to standard cutoff values, patients were categorized based on their waist-to-hip ratio (WHR) into two groups: WHR ≤ 0.9 and WHR > 0.9, thereby evaluating the impact of age and central obesity on biomarker levels.

2.2 *The waist-to-hip ratio measurement*

The waist-to-hip ratio (WHR) measures the body fat distribution around the waist and hip regions. The calculation is based on the hips, which represent the largest portion of the buttocks, and the waist circumference. The formula is expressed as WHR ¼ waist circumference/hip circumference. A desirable WHR is below 0.9 in males but below 0.8 in females. It is noteworthy that patients with AS had an elevated WHR of 0.98 [21].

2.3 *Blood samples collection*

Venous blood samples were taken from the patients and control groups, and 6 mL of blood was obtained from each subject by venipuncture. Four mL was slowly injected into the disposable serum tubes containing the separation gel. 0.5 mL of blood was added to 0.5 mL of TRIzol™ Reagent mixed in a 1.5 mL Eppendorf tube for subsequent genetic testing (RT-qPCR) and stored in deep refrigeration at -20°C. Blood in gel-containing tubes was allowed to clot at room temperature for 10-15 minutes and then centrifuged at 3000 rpm for 10 minutes. The serum was then distributed into several Eppendorf tubes in equal amounts and stored at -20°C until used in serological tests (ELISA).

2.4 *RNA extraction, reverse transcription, and measurement of RT-qPCR.*

Total RNA was extracted using the TRIzol™ Reagent kit (Thermo Scientific, USA), according to the manufacturer's protocol. The quality and quantity of the extracted RNA were assessed using a quantum fluorometer to ensure its suitability for downstream applications. Lyophilized primers (Table 1) were dissolved in nuclease-free water to give a final concentration of 100 pmol/µL as a stock solution. A working solution of these primers was prepared by adding 10 µL of primer stock solution (stored at freezer -20 °C) to 90 µL of nuclease-free water to obtain a working primer solution of 10 pmol/µL. The primers utilized in this study were designed by using the NCBI Primer-BLAST tool, informed by the reference sequence of the target gene. These primers were supplied by Macrogen Company (Korea) in a lyophilized form.

Table 1. Primers used in RT-PCR for cDNA amplification in the second step

Primer Name	Sequence (5′ → 3′)	Tm. (°C)	Product size
β-Globin-F	ACACAACTGTGTTCACTAGC	65	123bp
β-Globin-R	CAACTTCATCCACGTTCACC		
*APOE*_exp-F	GGGTCGCTTTTGGGATTACCTG	60	124 bp
*APOE*_exp-R	CAACTCCTTCATGGTCTCGTCC		
*MARCO*_exp-F	GGACAATTTGCGATGACGAGTGG	60	137 bp
*MARCO*_exp-R	CCGACACTGAACATTATCCAGCC		

For the analysis of gene expression, a two-step qPCR method was followed in this study. It consists of two broad steps: reverse transcription of RNA into complementary DNA (cDNA) and then qPCR amplification and quantification of the target genes. RNA was extracted from biological samples according to a standard protocol to ensure high purity and integrity. For setting up the first reaction, 4 µL of total RNA was added to 1 µL of random primers (the

final volume was adjusted to 5 µL with nuclease-free water in the tube). A thermal cycling program was performed to denature samples at 70°C for 5 min to fully uncoil the secondary structures that RNA would make, and then cooled to 4°C for 10 min to stabilize the reaction. Samples were then immediately placed on ice and centrifuged to obtain a homogenous solution for further use.

A master mix containing 2 µL of 5X Goscript Reaction Buffer, 1 µL of $MgCl_2$, 0.5 µL of dNTPs, 0.25 µL of RNasin, and 0.25 µL of GoScript Reverse Transcriptase mixed in 3.5 µL of nuclease-free water, bringing the total volume to 7.5 µL. The mixture was distributed into multiple tubes, with 7.5 µL of the master mix added to each tube containing 5 µL of previously treated RNA. The final reaction volume was 12.5 µL. Reverse transcription reaction was performed on a thermal cycler, using the following conditions: annealing, 25°C, 5 minutes to assist with primer binding; and cDNA synthesis at 42°C for 60 minutes. The reaction was stopped through incubation at 70°C for 15 minutes, and samples were kept at 4°C for 10 minutes before the qPCR step. Primers and probes were utilized in reactions after a reaction master mix was prepared (10-µL reaction) for qPCR amplification. The same type of mixture contains 5 µL of 2X Master Mix, 0.3 µL forward primer (10 µM), 0.3 µL reverse primer (10 µM), 3.4 µL nuclease-free water, and 1 µL of cDNA as a template. A reaction volume of 10 µL was made for each tube, with 9 µL of master mix and 1 µL of cDNA. qPCR reactions were analyzed on a RT-PCR system under the following thermal cycling conditions: initial denaturation at 95 °C 5 min (1 cycle), followed by 40 cycles of 95 °C 20 s for denaturation, annealing of primers to the template at 60 °C for 20 s for *APOE*, and *MARCO*, 65 °C for *β-Globin* with fluorescent signal acquisition; and extension at 72 °C for 20 s. Analysis of the levels of gene expression was performed using the Livak Method.

ΔCT =CT target gene - CT House Keeping gene

$\Delta\Delta CT$ =ΔCT Target gene - ΔCT Control

Folding =$2^{-\Delta\Delta CT}$

The kits and molecular compounds utilized in this study, together with their suppliers, are presented in Table 2.

Table 2. The molecular kits and chemical compounds utilized in the present study

Kits	Company/ Origin
Chloroform	LiChrosolv, Germany
GoScript Reverse Transcription System	Promega, USA
$MgCL_2$	Promega, USA
Nuclease Free Water	Promega, USA
Quantifluor RNA System	Promega, USA
Isopropanol	ROMIL pure chemistry, UK
70% Ethanol	ROMIL pure chemistry, UK
Primers	Macrogen, Korea
TRIzol Reagent	Thermo Scientific, USA

2.5 *Enzyme-linked immunosorbent assay (ELISA)*

This test employs the "Double Antibody Sandwich" method (ELISA Kits, ELK Biotechnology, USA). It quantifies the levels in the samples of (PTX3, KIM-1, Lp-PLA2). This is done by taking the optical density (O.D.) of the samples and comparing them against

a calculated standard curve. All standards, samples, and reagents were prepared as per the test preparation instructions in the kit leaflet to ensure accuracy and consistency.

2.6 *Statistical analysis*

This study analyzed the results of collected data using the Statistical Package of Social Science (SPSS) version 23 program, with One-Way ANOVA applied, and using Pearson's correlation coefficient. Data were expressed as mean ± S.E, and the differences were significant at $p \leq 0.05$. In addition, the Receiver Operating Characteristic (ROC) curve test was performed to calculate sensitivity and specificity and Area under the curve (AUC) for each studied parameter to measure the accuracy of them in the diagnosis of the disease.

3. Results

The results of the RT-qPCR findings show a significant upregulation of *MARCO* gene expression level (fold change) in the Pre-Cath patients with a significant difference (P =0.001), compared to the control group. In contrast, the Post-Cath patients exhibited a moderate increase in *MARCO* gene expression level (P = 0.002), which was significantly lower than that of the Pre-Cath patients but still higher than that of the control. While no significant change in the expression level of *APOE* was observed in the Pre-Cath patients with a significant difference (P=0.001) compared to the control group. Moreover, a significant increase was observed in the Post-Cath patients (P =0.001), where the expression level increased, showing a statistically significant difference compared to the control group. The study found that PTX3 levels were significantly elevated (p <0.001) in Pre-Cath patients in comparison to control individuals. However, in Post-Cath patients, PTX3 showed lower levels (p < 0.001) in compared with Pre-Cath patients. Similarly, the results showed the levels of Lp-PLA2 was substantially higher in Pre-Cath and Post-Cath patients (p <0.001) compared to the control group. Interestingly, this increase was not statistically significant in Post-Cath patients compared with Pre-Cath patients. Furthermore, results showed KIM-1 levels were dramatically elevated in both Pre- and Post-Cath patients (p <0.001) compared to controls, as shown in Table 2 and Figures 1 and 2.

Table 2. The gene expression of *MARCO* and *APOE*, and serum level of PTX3, Lp-PLA2, and KIM-1, in AS Pre-Cath, Post-Cath patients and the control group

Gene expression	Groups	Folding (Mean±S.E.)	P value
MARCO	Control	1.01±0.16 **a**	0.001*
	Pre-Cath AS patients	6.11±1.23 **b**	
	Post-Cath AS patients	1.85±0.19 **c**	
APOE	Control	1.09±0.13 **a**	<0.001**
	Pre-Cath AS patients	1.13±0.26 **b**	
	Post-Cath AS patients	4.13±0.45 **c**	
ELISA	**Groups**	**Levels (Mean±S.E.)**	**P value**
PTX3 (ng/mL)	Control	4.36±0.09 **a**	<0.001**
	Pre-Cath AS patients	9.27±1.07 **b**	
	Post-Cath AS patients	4.73±0.24 **a**	
Lp-PLA2 (ng/mL)	Control	7.10±0.71 **b**	<0.001**
	Pre-Cath AS patients	16.91±2.42 **a**	
	Post-Cath AS patients	13.64±1.34 **a**	
KIM-1 (ng/mL)	Control	88.67±7.35 **b**	<0.001**
	Pre-Cath AS patients	255.12±27.33 **a**	
	Post-Cath AS patients	320.71±42.59 **a**	

Different letters indicate differences between the groups, similar letters indicate non-significant differences.

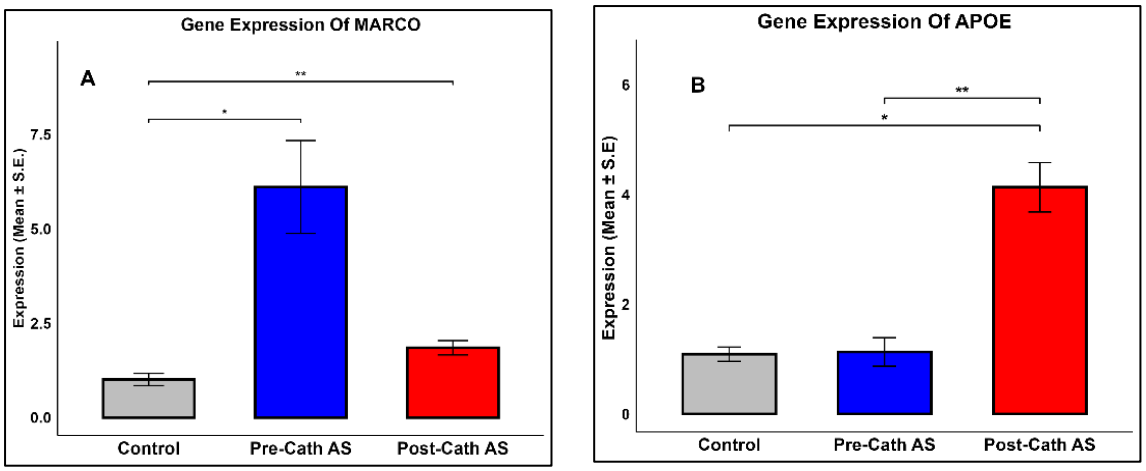

Figure 1: Gene expression levels of MARCO (A) and APOE (B) in Pre-Cath patients, Post-Cath patients, and control groups.

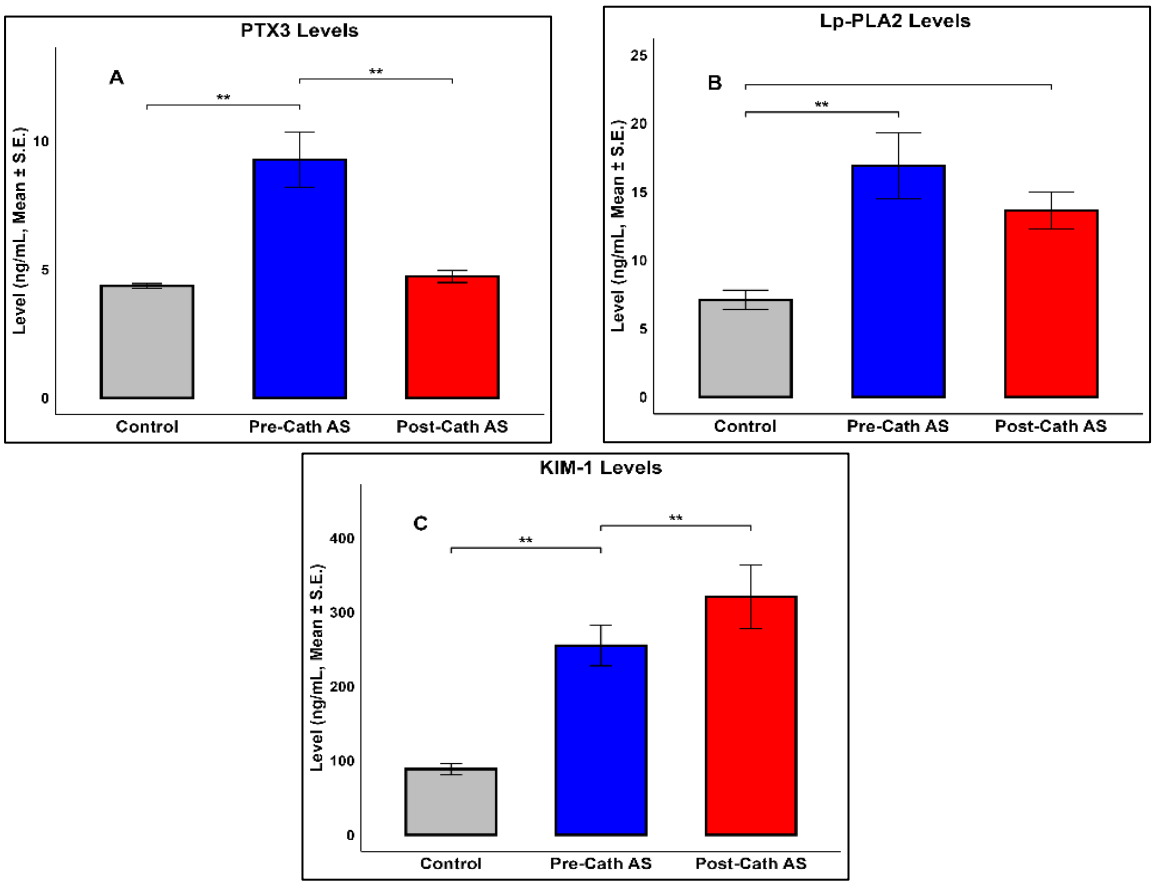

Figure 2: Serum levels of *PTX3* (A), *Lp-PLA2* (B), and *KIM-1* (C), in AS Pre-Cath patients, Post-Cath patients, and the control group.

In Table 3, analysis of ELISA results reveals significant differences in several biomarkers across different age groups. For PTX3, in Pre- and Post-Cath, patients groups in both age categories ≤50 and >50 years showed significantly higher levels ($P < 0.001$) compared to healthy individuals. Similarly, those who Pre-Cath patients had significantly higher PTX3 levels in the ≤ 50 year compared to those >50 years ($P = 0.01$), while Post-Cath patients showed no significant difference between age categories.

For Lp-PLA2, Pre- and Post-Cath, patient groups in both age categories ≤50 and >50 years also showed a significant increase (P = 0.001) compared to healthy controls. However, no significant differences were noted in Pre- and Post-Cath patients in both age categories. Finally, *KIM-1* levels were significantly higher in Pre- and Post-Cath, patient groups in both age groups (P < 0.001) compared to the control group. Although no significant differences were observed in the Pre-Cath patients between the two age categories, the Post-Cath patient group showed significantly higher *KIM-1* levels in the age categories ≤50 years (P = 0.005).

Table 3. The serum levels of PTX3, Lp-PLA2, and KIM-1 in AS Pre-Cath, Post-Cath patients, and the control group according to age

ELISA	Groups	Age groups (Yrs.)	Levels (Mean±S.E.)	P value
PTX3 (ng/mL)	Control	≤50	4.30±0.11 **a**	<0.001**
		>50	4.52±0.14 **a**	
	Pre-Cath AS patients	≤50	12.18±3.03 **b**	
		>50	8.39±1.02 **c**	
	Post-Cath AS patients	≤50	4.10±0.36 **a**	
		>50	4.89±0.27 **a**	
Lp-PLA2 (ng/mL)	Control	≤50	6.83±0.84 **a**	0.001**
		>50	7.81±1.38 **a**	
	Pre-Cath AS patients	≤50	14.44±5.66 **b**	
		>50	17.67±2.71 **b**	
	Post-Cath AS patients	≤50	14.95±3.08 **b**	
		>50	13.32±1.52 **b**	
KIM-1 (ng/mL)	Control	≤50	91.50±8.56 **a**	<0.001**
		>50	80.87±14.92 **a**	
	Pre-Cath AS patients	≤50	213.89±11.85 **b**	
		>50	267.67±35.24 **b**	
	Post-Cath AS patients	≤50	486.15±105.01 **c**	
		>50	279.35±43.45 **b**	

Different letters indicate differences between the groups, similar letters indicate non-significant differences.

The current investigation reveals that PTX3 levels in Pre-Cath and Post-Cath patients, across both WHR ≤0.9 and WHR >0.9 categories, showed a significant difference (p <0.001) when compared to the control group. Among Pre- and Post-Cath patients, there is an elevation in PTX3 levels for WHR ≤0.9 in comparison to those with WHR >0.9, although

these values are not statistically significant. In the context of Lp-PLA2, the current study has demonstrated that the Pre- and Post-Cath patients in both WHR categories have a significant difference in Lp-PLA2 levels (p=0.001) compared to the control group. Significantly, Pre-Cath patients demonstrate extremely high levels of Lp-PLA2, for WHR ≤0.9 compared to WHR >0.9; these contrasts are not statistically significant. In contrast, Post-Cath patients display significant differences in Lp-PLA2 levels, for WHR ≤0.9 (p =0.01) compared with WHR >0.9. KIM-1 levels in the Pre- and Post-Cath patients in both categories, WHR showed a significant difference (p<0.001) compared to the control group. For Pre-Cath patients, KIM-1 serum levels are elevated in WHR >0.9 compared to patients with WHR ≤0.9, but these differences are not significant, as shown in Table 4.

Table 4: The serum level of PTX3, Lp-PLA2, and KIM-1 in AS Pre-Cath, Post-Cath patients, and the control group according to WHR

ELISA	Groups	WHR	Levels (Mean±S.E.)	P value
PTX3 (ng/mL)	Control	≤0.9	4.33±0.13 **a**	<0.001**
		>0.9	4.39±0.11 **a**	
	Pre-Cath AS patients	≤0.9	12.51±2.80 **b**	
		>0.9	8.09±0.97 **b**	
	Post-Cath AS patients	≤0.9	5.15±0.89 **b**	
		>0.9	4.67±0.24 **b**	
Lp-PLA2 (ng/mL)	Control	≤0.9	6.89±0.78 **a**	0.001**
		>0.9	7.36±1.32 **a**	
	Pre-Cath AS patients	≤0.9	17.26±4.87 **b**	
		>0.9	16.79±2.86 **b**	
	Post-Cath AS patients	≤0.9	5.43±1.96 **c**	
		>0.9	14.91±1.37 **b**	
KIM-1 (ng/mL)	Control	≤0.9	96.25±11.28 **a**	<0.001**
		>0.9	78.76±8.04 **a**	
	Pre-Cath AS patients	≤0.9	212.44±5.48 **b**	
		>0.9	270.64±36.87 **b**	
	Post-Cath AS patients	≤0.9	473.14±163.49 **c**	
		>0.9	297.26±42.07 **b**	

Different letters indicate differences between the groups, similar letters indicate non-significant differences.

Variations in the diagnostic capacity of the ROC analysis for parameters and gene expression markers were observed. The *MARCO* gene scored an area under the curve (AUC) value of 0.86, with a sensitivity of 75% and a specificity of 93.3%. Regarding gene expression outcomes, the *MARCO* gene exhibited a P value of 0.001. Comparably, with a high AUC value of 0.91, sensitivity of 93.3%, and specificity of 90%, the *APOE* gene displayed a significant diagnostic function (P =0.001). PTX3 parameter scored AUC of 0.88, sensitivity of 68%, and specificity of 100% with Strong statistical significance (P < 0.001). By contrast, the Lp-PLA2 parameter had an AUC of 0.7, sensitivity of 53%, and specificity of 97% (P =0.05). On the other hand, KIM-1 had the best AUC value of 0.98, hence, it is the most trustworthy tool for separating unwell from healthy patients. It had a sensitivity of 93.3% and a specificity of 97%, with an extremely significant (P =0.001).

These results suggest that, considering its AUC values above 0.85, with great sensitivity and specificity, PTX3 showed outstanding diagnostic accuracy. Since their high AUC, sensitivity, and specificity, KIM-1 seems to be the most accurate indicator for differentiating between sick and healthy persons. Lp-PLA2, on the other hand, indicated limited applicability as a precise diagnosis marker with the lowest AUC value of 0.7 and somewhat low sensitivity. Regarding gene expression, *MARCO* and *APOE* provided encouraging findings that imply their possible function in illness diagnosis and progression determination. Table 5 and Figures 3 and 4 illustrate the results.

Table 5. ROC curve results for the studied parameters in AS patients and control groups.

Gene Expression	AUC	cut-off	Sensitivity	Specificity	Confidence Interval (95%)	P Value
MARCO	0.86	1.33	75%	93.3%	0.6-0.95	0.001**
APOE	0.91	2.23	93.3%	90%	0.8-1.03	<0.001**
ELISA	**AUC**	**Cut-off**	**Sensitivity**	**Specificity**	**Confidence Interval (95%)**	**P Value**
PTX3	0.88	5.28	68%	100%	0.79-0.97	<0.001**
Lp-PLA2	0.7	13.82	53%	97%	0.5-0.8	0.05*
KIM-1	0.98	164.58	93.3%	97%	0.95-1.01	<0.001**

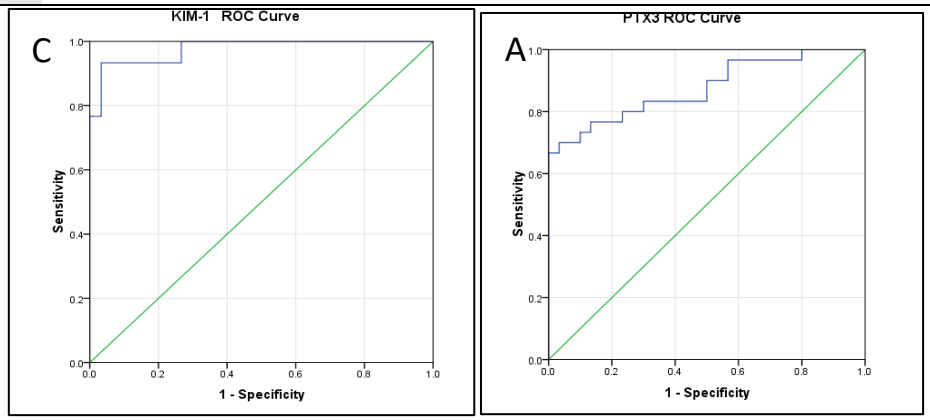

Figure 3. The ROC Curve for *MARCO* (A) and *APOE* (B) genes in Pre-Cath patients, Post-Cath patients, and controls.

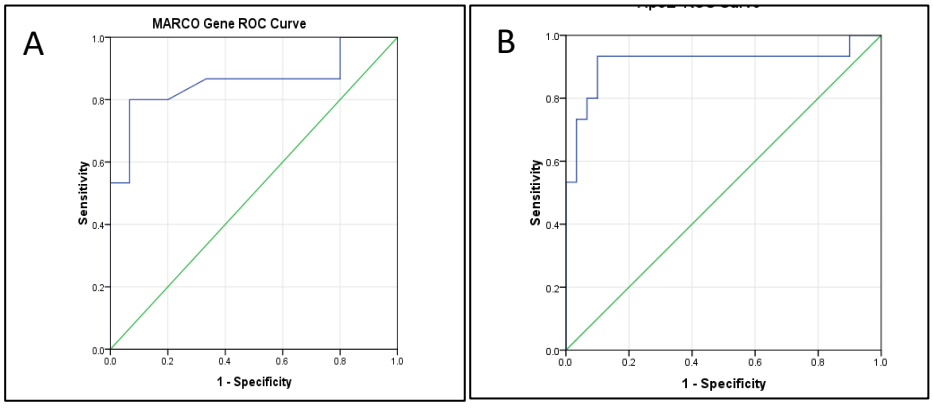

Figure 4: The ROC Curve of PTX3 (A), Lp-PL2 (B), and KIM-1 (C) biomarkers in AS patients and control groups.

The Pearson correlation coefficient facilitates the examination of the relationships among PTX3, Lp-PLA2, KIM-1, age, and WHR. Aside from a few instances demonstrating statistically significant associations, the data indicated that the majority of correlations were

weak or non-significant. A weak number of correlations, despite their weak or non-significant character, were determined to be statistically significant. Lp-PLA2 and the WHR had a weak but noteworthy positive connection. KIM-1 displayed a weak association but significant connections with age and the WHR. Moreover, age and the WHR showed a modest positive association (P = 0.001). In summary, a few notable correlations were identified, despite most of the investigated interactions revealing weak or non-significant correlations. The most interesting results were the modest correlation between age and the WHR. Table 6 shows the findings.

Table 6: Correlation of studied parameters

Parameter	Pearson Correlation	P-value
PTX3 and Lp-PLA2 level	0.093	0.382
PTX3 and KIM-1 level	0.055	0.609
PTX3 and age	0.013	0.904
PTX3 and WHR	-0.007	0.945
Lp-PLA2 and KIM-1 level	0.167	0.115
Lp-PLA2 and age	0.139	0.192
Lp-PLA2 and WHR	0.214*	0.043
KIM-1 and age	0.250*	0.017
KIM-1 and WHR	0.215*	0.042
Age and WHR	0.418**	0.001

The* and **, as * significance at P < 0.05 and ** significance at P < 0.001

4. Discussion

The substantial elevation in *MARCO* gene expression in Pre-Cath patients compared to the control group suggests a possible regulatory role of *MARCO* in response to catheterization status and activation of the immune and inflammatory response, which supports the role of macrophages in the removal of fatty deposits and oxidized molecules in the blood vessel walls [22]. The significant decrease in Post-Cath patients may reflect the body's response after therapeutic intervention, as catheterization leads to changes in the inflammatory response, reducing the need for excessive macrophage activation [23]. A study explored the relationship between *MARCO* gene variations and CVD, including AS. Their *in-silico* analysis suggested that certain genetic variations in the *MARCO* gene could impact its expression and potentially contribute to the progression of AS [24]. Its upregulation in AS patients suggests a potential role in the disease's pathogenesis. The upregulation of *MARCO* could be a response to the chronic inflammatory circumstance and the presence of oxidized LDL in the arterial walls [22].

Additionally, the *APOE* gene plays an essential role in lipid metabolism, cholesterol transport, and cardiovascular health by facilitating the removal of fat from blood vessels, making its expression levels particularly relevant in the context of AS [12]. The present study showed that *APOE* gene expression was significantly increased in AS patients compared to the control group. This upregulation indicates an enhanced effort to clear cholesterol-rich lipoproteins and modulates inflammatory responses, which are critical in mitigating AS plaque formation [25]. In Post-Cath patients, a higher increase in *APOE* expression was observed. This suggests a sustained regulatory response, potentially due to the long-term impact of catheterization on lipid metabolism and cardiovascular health [13].

The lack of a significant difference in *APOE* gene expression between the control group and Pre-Cath patients suggests that changes in *APOE* gene expression may not be obvious during the early stages of AS [26]. The significant increase in Post-Cath patients may be due to increased lipid metabolism after the therapeutic intervention, as the catheter improves blood flow, which enhances the regulation of cholesterol and lipids by *APOE* [25]. A recent study has further elucidated the role of *APOE*, corroborating our observations of increased gene expression in patients with AS [26, 12]. Case in point, investigated the impact of *APOE* on lipoprotein metabolism and its relation with CVD. A review indicated that *APOE* polymorphisms, uniquely the E4 variant, are linked to increased cardiovascular risk and AS, their results indicate that *APOE* not only mediates lipoprotein clearance but also modulates inflammatory responses, which could demonstrate the upregulation observed in AS patients [27].

In contrast, the observed increase in PTX3, Lp-PLA2, and KIM-1, levels in AS patients is consistent with findings from recent studies, which underscores their roles as biomarkers of inflammation, oxidative stress, and endothelial dysfunction [16, 20]. Elevated levels of PTX3 in Pre-Cath patients indicate active inflammatory processes that contribute to disease progression [28]. Reduced PTX3 levels in Post-Cath patient may signify a post-interventional alteration of inflammatory responses, potentially resulting from vascular remodeling or therapy-induced resolution of acute inflammation [29]. PTX3 has been identified as a significant biomarker for inflammation and CVD, and is a key inflammatory marker secreted in response to vascular injury and endothelial dysfunction [28].

However, the Lp-PLA2, an enzyme implicated in the modification of lipoproteins, has also been related to CVD. Elevated levels in Pre-Cath patients reflect active plaque inflammation and instability, which are risk factors for CVD [30]. The non-significant reduction in Post-Cath patient suggests that the intervention did not drastically alter the inflammatory state or lipid metabolism within the plaques [29]. By influencing nitric oxide generation and encouraging the expression of adhesion molecules, Lp-PLA2 helps to cause endothelial dysfunction, a fundamental event in AS [16]. Furthermore, the significantly higher KIM-1 levels in Pre-Cath patients reflect endothelial damage and vascular stress [31]. The further increase in Post-Cath patients may indicate additional vascular injury induced by the procedure, requiring further investigation into its long-term implications [32]. A study found that KIM-1 is an early sign of proximal tubular damage in the kidneys, but its raised levels in AS could represent more general systemic inflammation and tissue damage [20]. Elevated KIM-1 indicates renal injury and impaired endothelial function, increased vascular permeability, which could indirectly affect kidney function and result in elevated KIM-1 levels [33].

Analysis of the ELISA results, as shown in Table 3, reveals significant variations in several biomarkers across different groups and age ranges. In both age categories of ≤50 and >50 years, PTX3 levels are raised in Pre- and Post-Cath patient groups compared to healthy controls, attributed to heightened systemic inflammation and endothelial dysfunction associated with aging [15]. Interestingly, those Pre-Cath patients had significantly higher PTX3 levels in the ≤50 years age category compared to those >50 years, suggesting early endothelial dysfunction or subclinical AS [28]. This pattern aligns with studies that show elevated PTX3 levels in younger patients with metabolic syndrome or obesity [34, 35]. For Post-Cath patients, the lack of significant difference across age categories might suggest a plateau effect, where the inflammatory response is equally elevated regardless of age, possibly due to the invasive nature of the procedure [29].

For Lp-PLA2, levels were significantly elevated in pre- and post-Cath patient groups in both age categories, especially in those>50 years, which may be linked to increased lipid peroxidation and plaque formation within the arteries as a result of aging and its impact on chronic inflammation [36]. In contrast, the absence of significant variations in Lp-PLA2 levels in the Pre- and Post-Cath patient groups suggests that Lp-PLA2 reflects a chronic inflammatory state rather than a rapidly responsive biomarker to interventional procedures [30]. Finally, KIM-1 levels were significantly higher in both pre- and post-Cath patient groups in both age groups, suggesting that KIM-1 may serve as a potential biomarker of kidney injury associated with AS or CVD [33]. Although no significant difference was observed in the Pre-Cath group alone, Post-Cath patients in the ≤50 years age group exhibited substantially higher KIM-1 levels. This finding may reflect renal stress induced by contrast agents used during the procedure, particularly in younger individuals who could exhibit a more pronounced inflammatory response. A previous study supports this, showing that exposure to contrast media during catheterization can lead to contrast-induced nephropathy [31, 32].
Overall, given their notable changes across patient groups and age ranges, the results point to PTX3, KIM-1, and Lp-PLA2 as generally highly sensitive indicators for AS and its therapy via catheterization.

WHR analysis of the studied biomarkers (PTX3, Lp-PLA2, and KIM-1) in AS patients with WHR ≤0.9 and >0.9 was used to further investigate the possible impact of central obesity on inflammation and AS by means of biomarker levels. The results of this study , as shown in Table 4, reveal that the PTX3 levels in Pre- and Post-Cath patients in both categories, WHR ≤0.9 and WHR >0.9, show a significant difference compared to the control group, supporting its role as an inflammatory marker in CVD. In the Pre- and Post-Cath patients with WHR ≤0.9 exhibit higher *PTX3* levels, suggesting a link between central obesity, systemic inflammation, and an increased risk of AS [37]. Additionally, this elevation may be associated with insulin resistance, further impairing endothelial function and exacerbating AS [28].

Among Pre-Cath patients, there is an elevation in PTX3 levels for WHR ≤0.9 in comparison to those with WHR >0.9, although these values are not statistically significant. For Post-Cath patients, the PTX3 levels do not show significant variations across different WHR groups, perhaps as the development of disease and systematic inflammation has more influence than central obesity [37].

In the context of Lp-PLA2, the current study has demonstrated a significant increase in Lp-PLA2 levels in the Pre- and Post-Cath patients in both categories of WHR compared to the control group, confirming its role as a key inflammatory and oxidative marker in disease progression. This enzyme is primarily secreted by macrophages within AS plaques, contributing to the hydrolysis of oxidized phospholipids, thereby promoting inflammation and plaque instability, which increases the risk of CVD [30]. In contrast, Lp-PLA2 is closely associated with plaque inflammation in AS, its elevation in individuals with WHR>0.9 suggests that central obesity may contribute to more severe vascular inflammation [36]. Furthermore, KIM-1 levels are elevated in Pre- and Post-Cath patients in both categories, WHR ≤0.9 and WHR >0.9 indicate the possibility of kidney damage, which may be related to AS and its effect on blood flow to the kidneys [20]. For Post-Cath patients, the KIM-1 levels remain elevated in WHR ≤0.9 patients, but are not statistically significant; high levels may be associated with an increased risk of insulin resistance and systemic inflammation [32]. While not all biomarkers showed significant differences based on WHR, Lp-PLA2 suggests that increased central adiposity may contribute to enhanced vascular inflammation [36]. This

study underscores the critical role of immune biomarkers in AS and their varying degrees of association with central obesity.

The analysis of the correlations in Table 6 between immune parameters and age, WHR in patients with AS reveals statistically significant associations, despite most of them being weak or moderate in strength. The modest but statistically significant link between Lp-PLA2 and the WHR is among the more interesting results here. This association implies that higher levels of Lp-PLA2 are linked to a greater WHR, consistent with recent research showing that visceral adiposity causes vascular inflammation and raised CVD risk [17].

Furthermore, the weak but statistically significant connection between KIM-1 and age suggests that KIM-1 levels may be linked to aging. This outcome is consistent with previous studies that have suggested that aging is linked to increased oxidative stress and chronic low-grade inflammation, which can accelerate renal failure already in progress [33]. Moreover, a weak but significant association between KIM-1 and WHR supports the hypothesis that central obesity may contribute to kidney damage through inflammatory and metabolic pathways [31].

Notably, age and the WHR showed a modest but statistically significant relationship, implying that aging is linked to a rise in visceral fat deposition [34]. These results highlight generally the importance of central obesity and aging as main causes linked with higher inflammatory markers and kidney damage, so guiding the development of AS.

Conclusion

The result of the current study recorded a significant elevation in the serum levels of the studied immunological biomarkers (PTX3, Lp-PLA2, and KIM-1) as well as an upregulation of both genes (*APOE* and *MARCO*) in patients with AS. Investigating the participation of the *MACRO* gene in these mechanisms is essential for devising specific treatments and enhancing diagnostic tools for AS. Additionally, *APOE* action in atherogenesis mainly depends on its lipid-interaction based vascular effects; therefore, these results may help in a deeper understanding of AS pathogenesis. The use of these biomarkers as a possible therapeutic target to reduce the severity of AS or perhaps cure it is arguably the most significant finding.

Declaration of Competing Interest

There are no stated conflicts of interest by the authors.

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Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki. The study was approved by the University of Baghdad Ethics Committee and the Baghdad Health Directorate (Date, September 23, 2024, NO:EC-40). Informed consent was obtained from all study participants. Furthermore, the writers fully adhered to ethical standards, including the avoidance of plagiarism, data manipulation, and double publication.

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