

Investigation of inflammatory cytokines and new biomarkers in ischemic heart diseases

Investigation de l'intérêt des cytokines inflammatoires et de nouveaux biomarqueurs dans les cardiopathies ischémiques

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ABSTRACT

Introduction-Aim: Ischemic heart disease is a leading cause of global mortality and morbidity, resulting from reduced blood supply to the heart. Biomarkers are pivotal in diagnosing and understanding the disease. This study explores the utility of new biomarkers (cytokines, PLA2 (phospholipase A2), hs-CRP (high-sensitivity C-reactive protein), PCT (procalcitonin), H-FABP (Heart-type fatty acid-binding protein)) for diagnosing ischemic heart diseases.

Methods: Conducted at Nasiriyah Heart Hospital, Iraq, from October 2021 to October 2022, the study included 86 stable angina patients, 50 acute myocardial infarctions (MI) (myocardial infarction) patients, and healthy controls. Serum levels of biomarkers and inflammatory cytokines (TNF- α (tumor necrosis factor alpha), IL-1 β (interleukin-1 beta), IL-6 (interleukin-6), IL-9 (interleukin-9)) were measured using ELISA (enzyme-linked immunosorbent assay) and immunoassays.

Results: In stable angina, the study showed that the level of troponin I didn't significantly change in patients with stable angina. However, compared with healthy controls, patients showed a significant increase in serum levels of CK-MB (creatinine kinase-MB), myoglobin, hs-CRP, H-FABP, Lp-PLA2 (lipoprotein-associated phospholipase A2), and PCT. Significantly elevated levels of serum IL-6, IL-1 β , IL-9, and TNF- α were also recorded in patients with stable angina compared to healthy controls. In IMI group, the study revealed that serum troponin I level was insignificantly changed. However, compared with controls, a significant increase in the level of myoglobin, CK-MB, hs-CRP, Lp-PLA2, PCT, and H-FABP were recorded. Significantly elevated levels of serum TNF- α , IL-9, IL-6, and IL-1 β were also noted in the patients in comparison with healthy controls.

Conclusion: Novel biomarkers such as H-FABP, PCT, Lp-PLA2, and cytokines are valuable diagnostic tools for ischemic heart disease, alongside traditional markers like Troponin I, CK-MB, and myoglobin. Targeting cytokines with monoclonal antibodies could enhance therapeutic strategies.

Keywords: Biomarqueurs ; Cytokines; Cardiopathie ischémique; angor stable

RÉSUMÉ

Introduction-Objectif: La cardiopathie ischémique est l'une des principales causes de mortalité et de morbidité dans le monde, résultant d'une réduction de l'apport sanguin au myocarde. Les biomarqueurs sont essentiels dans le diagnostic et la compréhension de la maladie. Cette étude explore l'utilité de nouveaux biomarqueurs (cytokines, PLA2 (phospholipase A2), hs-CRP (protéine C-réactive hautement sensible), PCT (procalcitonine), H-FABP (protéine de liaison aux acides gras de type cardiaque)) pour diagnostiquer les cardiopathies ischémiques.

Méthodes: L'étude a été menée à l'hôpital cardiaque de Nasiriyah, en Irak, d'octobre 2021 à octobre 2022. Elle a inclus 86 patients ayant un angor stable, 50 patients ayant un infarctus du myocarde (IDM) et des témoins sains. Les niveaux sériques des biomarqueurs et des cytokines inflammatoires (TNF- α (facteur de nécrose tumorale alpha), IL-1 β (interleukine-1 bêta), IL-6 (interleukine-6), IL-9 (interleukine-9)) ont été mesurés par ELISA (essai immuno-enzymatique) et des immunoessais.

Résultats: Dans le groupe d'angor stable, l'étude a montré que le niveau de troponine I ne changeait pas de manière significative chez les patients. Cependant, par rapport aux témoins sains, les patients ont montré une augmentation significative des niveaux sériques de CK-MB (créatine kinase-MB), de myoglobine, de hs-CRP, de H-FABP, de Lp-PLA2 (phospholipase A2 liée aux lipoprotéines) et de PCT. Des niveaux significativement élevés d'IL-6, IL-1 β , IL-9 et TNF- α ont également été observés chez les patients par rapport aux témoins sains. Dans le groupe des patients ayant un-IDM, l'étude a révélé que le niveau de troponine I ne changeait pas de manière significative. Cependant, comparés aux témoins, les patients ont montré une augmentation significative des niveaux de myoglobine, CK-MB, hs-CRP, Lp-PLA2, PCT et H-FABP. Des niveaux significativement élevés de sérum TNF- α , IL-9, IL-6 et IL-1 β ont également été notés chez les patients par rapport aux témoins sains.

Conclusion: De nouveaux biomarqueurs tels que H-FABP, PCT, Lp-PLA2 et les cytokines sont des outils diagnostiques précieux pour les cardiopathies ischémiques, en plus des marqueurs traditionnels comme les troponines I, CK-MB et la myoglobine. La cible des cytokines avec des anticorps monoclonaux pourrait améliorer les stratégies thérapeutiques.

Mots clés: Biomarkers ; Cytokines; Ischemic heart disease; stable angina

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What is known?

Ischemic heart disease is a leading cause of mortality and morbidity globally, characterized by a reduction in blood supply to the heart muscle. Biomarkers, including traditional markers such as troponin, CK-MB, and myoglobin, are essential for diagnosing ischemic heart disease.

Inflammatory cytokines and additional biomarkers have shown potential in enhancing diagnostic accuracy.

What this article adds

This study explores the role of novel biomarkers (cytokines, PLA2, hs-CRP, PCT, and H-FABP) in diagnosing ischemic heart disease, particularly stable angina and myocardial infarction. It highlights the significant elevation of these biomarkers in patients compared to healthy controls, suggesting their value in diagnosing ischemic heart disease. The study also underscores the potential of targeting cytokines with monoclonal antibodies to improve therapeutic strategies.

INTRODUCTION

Ischemic heart disease has become by far one of the chronic conditions associated with significant mortality and morbidity. It increasingly affects younger populations and women, imposing a substantial social and economic burden. The underlying basis of this disease is atherosclerosis, a systemic disorder resulting from the cumulative effect of multiple interconnected cardiovascular risk factors.(1-5)

Inflammation is a central driver in the initiation and progression of atherosclerosis.

Biomarkers play a crucial role in both the diagnosis and the mechanistic understanding of the disease. As well known that Cardiac troponin I (cTn-I) and creatine kinase MB (CK-MB) remain the standard markers for detecting myocardial injury there is other biomarkers recognized as a marker of systemic inflammation in cardiovascular disease such as C-reactive protein (CRP) (6-7) , Procalcitonin (PCT) has been shown to correlate with atherosclerotic burden and to predict the prognosis of coronary artery disease (8,9). Similarly, heart-type fatty acid-binding protein (H-FABP) has been identified as a sensitive marker of early myocardial injury and a predictor of adverse long-term outcomes (10).

Cytokines, including interleukin-6 (IL-6), interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF- α), are key mediators in cardiovascular pathology, exerting their effects through paracrine, autocrine, and endocrine signaling (11,12). Investigating these additional biomarkers may strengthen diagnostic accuracy and improve prognostic assessment in IHD.

This study aims to investigate the role of additional biomarkers in the diagnosis and management of ischemic heart disease .

METHODS

We conducted a comparative cross-sectional study in Nasiriyah Heart Hospital Irak ; the study was carried

out between October 2021 to october 2022 The study involved two patient groups diagnosed with IHD: stable angina and acute myocardial infarction (MI). Each patient group was compared with a corresponding healthy control group.

The inclusion criteria for the study included patients diagnosed with ischemic heart diseases (stable angina or acute MI) and who were admitted to Nasiriyah Heart Hospital during the study period. Exclusion criteria included patients with other types of heart disease, a history of autoimmune or inflammatory conditions, or those who were receiving immunosuppressive treatments.

Ethical approval for the study was obtained from the Research Committee of the Dhi Qar Health Department (Research project number 2022161), under the Ministry of Health in Iraq, and the ethical committee at the Ibn El Jazzar Faculty of Medicine, University of Sousse, Tunisia. All participants provided written informed consent before enrollment. No procedures that posed risks to the participants were performed during the study.

Blood samples were collected by venipuncture (5 ml) from all participants. The samples were allowed to clot, then centrifuged at 5000 RPM for 5 minutes. The obtained serum was stored for analysis. Several biomarkers were evaluated in this study, including cytokines such as TNF- α , IL-6, IL-9, and IL-1 β , which were quantified using enzyme-linked immunosorbent assay (ELISA) (13). Additionally, biomarkers like troponin I, H-FABP, LP-PLA2, CK-MB, PCT, HS-CRP, and myoglobin were analyzed using electrochemiluminescence immunoassay (ECLIA) (14,15). ELISA is a widely used technique for detecting cytokines and other proteins in biological samples, known for its high sensitivity and specificity. In this study, the ELISA method was employed to measure the levels of various cytokines in patient and control sera. The process involves the use of enzyme-linked conjugates and colorimetric changes to identify the presence and concentration of specific antigens (13).

ECLIA is a highly sensitive method that involves electrochemical reactions to produce light for detection. ECLIA is advantageous due to its low detection limits, high stability, and broad dynamic range. The technique utilizes electrochemical compounds that emit light when oxidized or reduced, providing a sensitive and efficient detection system for multiple biomarkers (15).

For cytokine analysis, standard curves were generated using known concentrations of the cytokine in question. The optical density was measured at 450 nm using a microplate reader.

The results were analyzed by comparing the optical density values of the patient and control samples to the standard curve, allowing for the calculation of cytokine concentrations in the serum samples (13).

The descriptive study and comparison of means were done using IBM SPSS 24 Windows version software. We calculated absolute and relative frequencies (percentages) for the qualitative variables.

Means, medians and extreme values were calculated for the quantitative variables. Comparisons of the means of the quantitative variables were made using Student's

t-test after checking the distribution of the variable studied. Comparisons of percentages of qualitative variables were made using the Chi-square test. The links between 2 quantitative variables were studied by the Pearson or Spearman correlation coefficient according to the distribution of the variable studied. Bivariate correlation and binary logistic regression were used to search for the different correlations. Results were presented in tabular or graphical form. A p-value of less than 0.05 was considered the threshold of statistical significance for all tests.

RESULTS

Stable Angina

86 patients with stable angina and 86 healthy individuals are involved in this study. The patients' average age was 44.0 ± 10.9 yrs and the control was 41.9 ± 10.1 yrs. From the patients were, females were 39 (45.35%) while males 47 (54.65%), and in the control, 13 (15.12%) were females and 73 (84.88 %) were males ($p < 0.001$ both). (Table 1)

Table 1. Features of patients with stable angina and the control group.

Parameters	Patients group	Control group	P value
Patient	86	86	
Age	44.0 ± 10.9	41.9 ± 10.1	NS
Gender			
Females	13 (15.12%)	39 (45.35%)	< 0.001
Males	73 (84.88%)	47 (54.65%)	
Hypertension	27. (31.40%)	1. (1.16%)	<0.001
typical weight (18.5 – 24.9) (Kg/m ²)	17 (19.77%)	30 (34.88%)	
Overweight (25.0 – 29.9) (Kg/m ²)	41 (47.67%)	37 (43.02%)	NS
Obese ≥ 30 Kg/m ²	28. (32.56%)	19 (22.10%)	
Diabetic	39. (45.35%)	1 (1.16%)	<0.001

NS: non-significant.

According to this study, there was no significant difference in the level of troponin I between patients with stable angina and the control group. However, a elevations in serum myoglobin (62.02 ± 8.40 vs 49.40 ± 6.00 ng/ml, $P < 0.01$), CK- MB level, (3.02 ± 1.46 vs 2.15 ± 1.91 ng/ml, $P < 0.001$), hsCRP, (28.90 ± 5.50 vs 7.35 ± 3.51 nmol/l, $P < 0.01$), H- FABP (6.59 ± 2.71 vs 4.90 ± 1.43 , ng/ml, $P < 0.001$), PCT (0.056 ± 0.05 vs 0.026 ± 0.02 ng/ml, $P < 0.01$) and Lp-PLA2, (127.6 ± 19.2 vs 105.0 ± 22.7 ng/ml, $P < 0.01$), were noted in patients with stable angina compared with control group (Table 2).

When comparing the serum levels of unstable angina patients to the control group, there were significant increases in TNF- α (5.2 ± 4.6 vs 2.6 ± 1.7 ng/ml, $P < 0.001$), IL-9 (3.7 ± 2.5 vs 2.5 ± 1.6 pg/ml, $P < 0.01$), IL 6 (7.9 ± 6.8 vs 4.3 ± 2.2 Pg/ml, $P < 0.001$), and IL1 β (11.5 ± 3.6 vs 4.6 ± 3.2 nmol/l, $P < 0.001$) in patients with unstable angina. (Table 2).

Table 2. The levels of serum biochemical markers and cytokines in Stable Angina patients compared to control group.

Parameters	Patients group	Control group	P value
Trop I (ng/ml)	0.0210 ± 0.0034	0.0200 ± 0.0038	(NS)
MYO (ng/ml)	62.02 ± 8.40	49.40 ± 6.00	< 0.01
CK-MB (ng/ml)	3.02 ± 1.46	2.15 ± 1.91	<0.001
Lp-PLA2 (ng/ml)	127.6 ± 19.2	105.0 ± 22.7	< 0.01
HsCRP (nmol/l)	28.90 ± 5.50	7.35 ± 3.51	< 0.01
H- FABP (ng/ml)	6.59 ± 2.71	4.90 ± 1.43	<0.001
PCT (ng/ml)	0.056 ± 0.05	0.026 ± 0.02	<0.01
TNF α (ng/ml)	5.2 ± 4.6	2.6 ± 1.7	<0.001
IL 9 (Pg/ml)	3.7 ± 2.5	2.5 ± 1.6	<0.01
IL 6 (Pg/ml)	7.9 ± 6.8	4.3 ± 2.2	<0.001
IL1 β (nmol/l)	11.5 ± 3.6	4.6 ± 3.2	<0.001

CK-MB: Creatine Kinase-MB, H-FABP: Heart-Type Fatty Acid Binding Protein, hsCRP: High-Sensitivity C-Reactive Protein, IL-1 β : Interleukin-1 Beta, IL-6: Interleukin-6, IL-9: Interleukin-9, Lp-PLA2: Lipoprotein-Associated Phospholipase A2, NS: Not Significant, PCT: Procalcitonin, TNF- α : Tumor Necrosis Factor Alpha, Trope I: Troponin I.

Acute myocardial infraction

The research was performed on 50 MI patients and 30 healthy age matched participants to serve as control. The patients' mean age was 29.1 ± 4.1 years, while the healthy control group's mean age was 30.2 ± 3.2 years (non-significant difference) From the patients, 34 (68.00%) were males and 16 (32.00%) females, and in healthy ,20 (66.67%) were males and 10 (33.33%) females. (Table 3).

Table 3. Characteristics of MI patients' group and controls group.

Parameter	Patients group	control group	P value
Patients	50	30	
Age (yrs.)	29.1 ± 4.1	30.2 ± 3.2	NS
Gender			
Females	10 (33.33%)	16 (32.00%)	NS
Males	20 (66.67%)	34 (68.00%)	

NS: non-significant.

The serum levels of Trop I (0.36 ± 0.002 vs. 0.02 ± 0.00 ng/ml, $P < 0.001$), MYO (68.2 ± 6.1 vs. 59.4 ± 8.4 ng/ml, $P < 0.05$), CK-MB (2.67 ± 0.09 vs. 1.8 ± 0.06 ng/ml, $P < 0.001$), hs-CRP (92.7 ± 4.2 vs. 15.1 ± 1.1 nmol/l, $P < 0.001$), PCT (0.048 ± 0.013 vs. ± 0.004 ng/ml, $P < 0.001$), and H-FABP (7.16 ± 1.3 vs. 4.9 ± 0.6 ng/ml, $P < 0.01$). In contrast to the control group, MI patients' serum LpPLA2 level did not significantly differ (132.9 ± 6.2 vs. 128.2 ± 5.4). (Table 4)

In comparison to the control group, MI patients had significantly higher serum levels of TNF α (17.97 ± 2.82 vs. 8.19 ± 1.82 ng/ml, $P < 0.01$), IL-9 (5.21 ± 0.84 vs. 1.98 ± 0.42 Pg/ml, $P < 0.05$), IL-6 (7.255 ± 3.81 vs. 4.48 ± 1.98 Pg/mL, $P < 0.05$), and IL1 β (20.11 ± 3.53 vs. 11.71 ± 2.11 nmol/l, $P < 0.01$). (Table 4)

Table 4. The levels of biomarkers and cytokines in MI patients compared with control group

Parameters	Patients Group	Control Group	P value
Trop I (ng/ml)	0.36 ± 0.002	0.020 ± 0.00	< 0.001
MYO (ng/ml)	68.2 ± 6.1	59.4 ± 8.4	< 0.05
CK-MB (ng/ml)	2.67 ± 0.09	1.8 ± 0.06	< 0.01
Lp-PLA2 (ng/ml)	128.2 ± 5.4	132.9 ± 6.2	NS
hsCRP (nmol/l)	92.7 ± 4.2	15.1 ± 1.1	< 0.001
H-FABP (ng/ml)	7.16 ± 1.3	4.9 ± 0.6	< 0.01
PCT (ng/ml)	0.048 ± 0.013	0.012 ± 0.004	< 0.01
TNFα (ng/ml)	8.19 ± 1.82	17.97 ± 2.82	< 0.01
IL 9 (Pg/ml)	1.98 ± 0.42	5.21 ± 0.84	< 0.05
IL 6 (Pg/ml)	4.48 ± 1.98	7.25 ± 3.81	< 0.05
IL 1β (nmol/l)	11.71 ± 2.11	20.11 ± 3.53	< 0.01

CK-MB: Creatine Kinase-MB, H-FABP: Heart-Type Fatty Acid Binding Protein, hsCRP: High-Sensitivity C-Reactive Protein, IL-1β: Interleukin-1 Beta, IL-6: Interleukin-6, IL-9: Interleukin-9, Lp-PLA2: Lipoprotein-Associated Phospholipase A2, NS: Not Significant, PCT: Procalcitonin, TNF-α: Tumor Necrosis Factor Alpha, Trope I: Troponin I.

DISCUSSION

Our main findings indicate a generalized elevation of both muscular and inflammatory biomarkers in patients with stable coronary artery disease, with the notable exception of troponin levels. Likewise, among patients with myocardial infarction, all assessed biomarkers were increased, except for Lp-PLA2, which did not show a significant elevation.

Coronary artery disease (CAD) is a leading global cause of mortality and morbidity. Its unpredictable course necessitates identifying additional predictive and diagnostic markers to improve patient outcomes (17). This study analyzed various biomarkers to explore their association with IHD and MI.

Our findings showed no significant differences in age between patients with stable angina and MI. Male patients (54.65%) outnumbered females (45.35%), aligning with prior evidence that women are protected by estrogen and progesterone until menopause, where these hormones not only regulate reproduction but also support vascular health (18).

The results demonstrated an insignificant elevation in troponin I levels among stable angina patients. Daněk et al. demonstrated that low levels of troponins can be observed in patients with stable coronary artery disease, reflecting chronic myocardial ischemia and potentially providing prognostic value for risk stratification in this population, whereas Maayah et al. emphasized that troponin testing enables the detection of minimal myocardial injury that was previously undetectable (19–21). Literature suggests that such increases are linked to the extent of atherosclerosis rather than cardiomyocyte necrosis, associating elevated troponin I with poor prognoses, including heart failure and sudden death (19–21). In MI cases, we observed considerably higher levels of troponin I, myoglobin, and CK-MB, which remain primary diagnostic markers per the European Society of Cardiology and the American College of Cardiology (22). Troponin high-sensitivity assays have

improved diagnostic accuracy, underscoring troponin's critical role in MI identification (23,24).

Motamed H et al showed that CK-MB levels showed significant elevations in stable angina patients, consistent with previous findings that such increases predict mortality risk and indicate severe myocardial injury. Popa DM et al demonstrated that Dynamic changes in CK-MB were predictive of adverse cardiac events, reinforcing its role in post-AMI risk stratification. In MI, CK-MB's diagnostic accuracy within the first 24 hours of symptom onset is well-documented.

However, CK-MB interpretation must consider potential elevations caused by skeletal muscle damage (23–29).

Myoglobin levels also increased in stable angina, although its primary utility lies in early MI exclusion. Our study confirmed significantly elevated myoglobin levels in MI patients, aligning with prior research on its rapid release and diagnostic significance in myocardial damage (30–32). While myoglobin's diagnostic specificity is limited, its rapid release makes it valuable for early MI detection when combined with other biomarkers (33).

Hs-CRP levels were significantly elevated in stable angina patients, reflecting the inflammatory processes underlying atherosclerosis. Sm and al showed that hs-CRP levels were correlated significantly with the degree of coronary stenosis ; Although levels were higher in MI patients, hs-CRP's correlation with lesion necrotic core length and atherogenesis severity is noteworthy (34,35). Best LG et al demonstrated that As a systemic inflammatory biomarker, Elevated CRP independently predicted cardiovascular events, even after adjusting for diabetes and traditional risk factors ; hs-CRP predicts MI and promotes endothelial cell proliferation and complement system activation, contributing to atherosclerotic pathophysiology (36–40).

Our results also revealed elevated serum levels of H-FABP in stable angina, corroborating its prognostic value for adverse outcomes. kabekkodu SP et al found higher sensitivity of H-FABP in early AMI detection, especially in the first 6 hours after chest pain onset. In MI, H-FABP levels increased significantly within the first hour, surpassing other markers in early diagnostic sensitivity (41–43). Rezar R et al demonstrated that H-FABP shows potential for early detection, risk stratification, and prognosis in acute and chronic HF.

Lp-PLA2 is produced by macrophages within atherosclerotic plaques and contributes to lipid oxidation and inflammation. levels were significantly elevated in stable angina patients, reflecting its role in promoting atherosclerosis and endothelial dysfunction. However, no significant alterations were observed in MI patients, consistent with findings that Lp-PLA2 inhibitors provide limited benefit in MI prevention (44–45). Racherla S et al showed that Levels decrease with statins and certain lipid-lowering therapies, correlating with reduced cardiovascular risk (46). Its role as an inflammatory biomarker in stable angina underlines its diagnostic and prognostic potential. (47) PCT levels were elevated in stable angina and MI patients, suggesting its correlation with coronary artery disease severity (48–49). Zaki et al demonstrated that PCT showed moderate diagnostic

accuracy, improving early sepsis recognition but not sufficient alone it should be combined with clinical assessment and other biomarkers (50); As a biomarker initially used for sepsis detection, PCT's association with atherosclerotic lesions and inflammation highlights its predictive value for adverse cardiovascular outcome. Although non-specific, PCT offers additional insights into the inflammatory mechanisms of ischemic heart diseases (51-53).

IL-6 is a pro-inflammatory cytokine, contributes to ischemic heart disease through mechanisms such as increased blood viscosity, platelet activation, and fibrinogen deposition, levels were notably higher in stable angina and MI patients. Bouzidi et al showed that this biomarker was associated with complexity of coronary artery disease(54) . Elevated IL-6 levels correlate with larger infarct sizes and reduced left ventricular ejection fraction, emphasizing its potential as a therapeutic target (55–58).

Similarly, TNF- α levels were elevated in both stable angina and MI patients. TNF- α 's pro- atherosclerotic actions include reducing nitric oxide bioavailability, promoting oxidative stress, and inducing inflammatory cell infiltration, which exacerbate myocardial damage; Bialecka et al suggested that this biomarker as a potential marker of early cardiac remodeling specially in young CAD patients (59). The significant increase in TNF- α levels post-MI highlights its role as a biomarker for tracking heart failure and left ventricular dysfunction (60-62).

IL-1 β also showed significant elevation in both conditions. This cytokine mediates neutrophil infiltration and reactive oxygen species production, aggravating myocardial injury during ischemia and reperfusion (63,64). Moriya et al defended that Elevated IL-1 β levels post-MI have been associated with inflammatory responses and remodeling processes, underscoring its potential as a therapeutic target (65–67).

Lastly, IL-9 levels increased in stable angina patients, consistent with its role in inflammatory responses and cardiovascular risk Capuzzello et al demonstrated Elevated IL-9 levels in ischemic heart failure and Suggested it as a potential biomarker (68,69).

Limits of the study

This study has several limitations, including its single-center design, a relatively small sample size, the absence of randomization, lack of adjustment for potential confounding risk factors, and the possibility of selection and information biases.

CONCLUSION

Emerging biomarkers, including cytokines, Lp-PLA2, hs-CRP, PCT, and H-FABP, showed a potential in improving the diagnostic accuracy for ischemic heart diseases (IHD). The results highlight distinct patterns in biomarker levels between stable angina and acute myocardial infarction (MI), with significant increases in markers such as myoglobin, CK-MB, hs-CRP, PCT, and H-FABP in both conditions. Additionally, the elevation

of pro-inflammatory cytokines, including TNF- α , IL-6, IL-9, and IL-1 β , further supports their involvement in the pathophysiology of IHD. These findings provide valuable insights into the inflammatory and cellular mechanisms of IHD and suggest that these biomarkers. These biomarkers, particularly cytokines, may represent potential targets for monoclonal antibody therapies aimed at improving management strategies. could serve as important tools for early diagnosis and differentiation of ischemic heart conditions. Future research should explore the clinical utility and prognostic value of these biomarkers in larger, diverse populations. Further large-scale studies are required to assess the sensitivity, specificity, and predictive value of these biomarkers in order to determine their role in the management strategy of these conditions.

ABBREVIATIONS:

A2 (LpPLA2): Lipoprotein-associated phospholipase
CAD: Coronary artery disease
CK-MB: Creatine kinase – MB
CRP: C-reactive protein
cTn: Cardiac troponin
ELISA: Enzyme-linked immunosorbent assay
ECLIA: electrochemiluminescence immunoassay
h-FABP: Heart-type fatty acid-binding protein
IHD: Ischemic heart disease
IL-1 β : Interleukin-1 β
IL-6: Interleukin-6
IL-9: Interleukin-9
MI: Myocardial infarction
PCT: Procalcitonin
TNF α : Tumor necrosis factor α

REFERENCES

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol*. 2017 Jul 4;70(1):1–25.
2. Shiba HA, Galal SB, Tag El Din KA, Mohamed LA. A Case control study on ischemic heart disease and serum zinc at Al-Zahraa university hospital. *J Recent Adv Med*. 2022 Jul 26;3(2):108–18.
3. Severino P, D'Amato A, Pucci M, Infusino F, Adamo F, Birtolo LI, et al. Ischemic Heart Disease Pathophysiology Paradigms Overview: From Plaque Activation to Microvascular Dysfunction. *Int J Mol Sci*. 2020 Oct 30;21(21):8118.
4. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics–2015 update: a report from the American Heart Association. *Circulation*. 2015 Jan 27;131(4):e29–322.
5. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. *Card Fail Rev*. 2017 Apr;3(1):7–11.
6. Bularga A, Hung J, Daghem M, Stewart S, Taggart C, Wereski R, et al. Coronary Artery and Cardiac Disease in Patients With Type 2 Myocardial Infarction: A Prospective Cohort Study. *Circulation*. 2022 Apr 19;145(16):1188–200.
7. Sairam SG, Sola S, Barooah A, Javvaji SK, Jaipuria J, Venkateshan V, et al. The role of Lp-PLA2 and biochemistry parameters as potential biomarkers of coronary artery disease in Asian South-Indians: a case-control study. *Cardiovasc Diagn Ther*. 2017 Dec;7(6):589–97.
8. Potter JM, Hickman PE, Cullen L. Troponins in myocardial infarction and injury. *Aust Prescr*. 2022 Apr;45(2):53–7.
9. Fakanya WM, Tothill IE. Detection of the inflammation biomarker C-reactive protein in serum samples: towards an optimal biosensor formula. *Biosensors*. 2014 Dec;4(4):340–57.
10. Ibrahim MI, Mohamed YM, Taher MA, Abdelrahman MA. The Utility

- of Serum Procalcitonin Level as an Early Diagnostic Biomarker for COVID in Patients Presenting with ST-Elevation Myocardial Infarction Post Primary Percutaneous Coronary Intervention. *Egypt J Hosp Med*. 2023 Jan 1;90(1):843–51.
11. Polonschii C, Potara M, Iancu M, David S, Banciu RM, Vasilescu A, et al. Progress in the Optical Sensing of Cardiac Biomarkers. *Biosensors*. 2023 Jun 7;13(6):632.
 12. Ye XD, He Y, Wang S, Wong GT, Irwin MG, Xia Z. Heart-type fatty acid binding protein as a biomarker for acute myocardial injury and long-term post-ischemic prognosis. *Acta Pharmacol Sin*. 2018 Jul;39(7):1155–63.
 13. Hayrapetyan H, Tran T, Tellez-Corrales E, Madiraju C. Enzyme-Linked Immunosorbent Assay: Types and Applications. *Methods Mol Biol Clifton NJ*. 2023;2612:1–17.
 14. Mahadevarao Premnath S, Zubair M. Electrochemiluminescence Method. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 Nov 22]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK594228/>
 15. Wang C, Liu S, Ju H. Electrochemiluminescence nanoemitters for immunoassay of protein biomarkers. *Bioelectrochemistry Amst Neth*. 2023 Feb;149:108281.
 16. Kumari A, Mishra P, Chaulya SK, Prasad GM, Nadeem M, Kisku V, et al. Estimation of Soil Nutrients and Fertilizer Dosage Using Ion-Selective Electrodes for Efficient Soil Management. *Commun Soil Sci Plant Anal*. 2024;1–22.
 17. McCarthy CP, McEvoy JW, Januzzi JL. Biomarkers in stable coronary artery disease. *Am Heart J*. 2018 Feb;196:82–96.
 18. Majidi M, Eslami V, Ghorbani P, Foroughi M. Are women more susceptible to ischemic heart disease compared to men? A literature overview. *J Geriatr Cardiol JGC*. 2021 Apr 28;18(4):289–96.
 19. Daněš J, Hnátek T, Malý M, Táborský M, Běláček J, Škvaril J, et al. Troponin levels in patients with stable CAD. *Cor Vasa*. 2017 Jun 1;59(3):e229–34.
 20. W J, L A, K L, S J M, S E, C R R, et al. Necrotic cardiac myocytes skew macrophage polarization towards a classically activated phenotype. *PLoS One* [Internet]. 2023 Mar 30 [cited 2024 Nov 22];18(3). Available from: <https://pubmed.ncbi.nlm.nih.gov/36996254/>
 21. Maayah M, Grubman S, Allen S, Ye Z, Park DY, Vemmou E, et al. Clinical Interpretation of Serum Troponin in the Era of High-Sensitivity Testing. *Diagn Basel Switz*. 2024 Feb 26;14(5):503.
 22. Dugani SB, Ayala Melendez AP, Reka R, Hydoub YM, McCafferty SN, Murad MH, et al. Risk factors associated with premature myocardial infarction: a systematic review protocol. *BMJ Open*. 2019 Feb 11;9(2):e023647.
 23. Li X, Zhang F, Zhou H, Hu Y, Guo D, Fang X, et al. Interplay of TNF- α , soluble TNF receptors and oxidative stress in coronary chronic total occlusion of the oldest patients with coronary heart disease. *Cytokine*. 2020 Jan;125:154836.
 24. Daubert MA, Jeremias A. The utility of troponin measurement to detect myocardial infarction: review of the current findings. *Vasc Health Risk Manag*. 2010 Sep 7;6:691–9.
 25. Vikenes K, Melberg T, Farstad M, Nordrehaug JE. Elevated CK-MB values after routine angioplasty predicts worse long-term prognosis in low-risk patients. *Scand Cardiovasc J*. 2010 Apr;44(2):69–75.
 26. Wu P, Yu S, Wang J, Zou S, Yao DS, Xiaochen Y. Global burden, trends, and inequalities of ischemic heart disease among young adults from 1990 to 2019: a population-based study. *Front Cardiovasc Med*. 2023;10:1274663.
 27. Motamed H, Mohammadi M, Tayebi Z, Rafati Navaei A. The diagnostic utility of creatine kinase-MB versus total creatine phosphokinase ratio in patients with non-ST elevation myocardial infarction from unstable angina. *SAGE Open Med*. 2023;11:20503121221148609.
 28. Popa DM, Macovei L, Moscalu M, Sascău RA, Stătescu C. The Prognostic Value of Creatine Kinase-MB Dynamics after Primary Angioplasty in ST-Elevation Myocardial Infarctions. *Diagn Basel Switz*. 2023 Oct 6;13(19):3143.
 29. Xu LQ, Yang YM, Tong H, Xu CF. Early Diagnostic Performance of Heart-Type Fatty Acid Binding Protein in Suspected Acute Myocardial Infarction: Evidence From a Meta-Analysis of Contemporary Studies. *Heart Lung Circ*. 2018 Apr;27(4):503–12.
 30. Asl SK, Rahimzadegan M. The recent progress in the early diagnosis of acute myocardial infarction based on myoglobin biomarker: Nano-aptasensors approaches. *J Pharm Biomed Anal*. 2022 Mar 20;211:114624.
 31. Lanza GA, Careri G, Crea F. Mechanisms of coronary artery spasm. *Circulation*. 2011 Oct 18;124(16):1774–82.
 32. Wang Q, Wang Y, Xu D. Research progress on Th17 and T regulatory cells and their cytokines in regulating atherosclerosis. *Front Cardiovasc Med*. 2022;9:929078.
 33. Şahingöz E, Gökteş A, Martlı HF, Er S, Tez M, Şahingöz E, et al. Comparison of pathologies in patients operated for transverse colon tumor with embryogenic and anatomical insights. *J Compr Surg*. 2023 Nov 30;1(4):66–70.
 34. Sm S, F A, R D, H D. Relationship between high-sensitivity C-reactive protein serum levels and the severity of coronary artery stenosis in patients with coronary artery disease. *ARYA Atheroscler* [Internet]. 2016 Sep [cited 2024 Nov 22];12(5). Available from: <https://pubmed.ncbi.nlm.nih.gov/28458698/>
 35. Asakura K, Minami Y, Nagata T, Katamine M, Katsura A, Hashimoto T, et al. Impact of the eicosapentaenoic acid to arachidonic acid ratio on plaque characteristics in statin-treated patients with coronary artery disease. *J Clin Lipidol*. 2023;17(1):189–96.
 36. Best LG, Zhang Y, Lee ET, Yeh JL, Cowan L, Palmieri V, et al. C-reactive protein as a predictor of cardiovascular risk in a population with a high prevalence of diabetes: the Strong Heart Study. *Circulation*. 2005 Aug 30;112(9):1289–95.
 37. Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K, et al. High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? *J Am Coll Cardiol*. 2013 Jul 30;62(5):397–408.
 38. Devaraj S, Kumaresan PR, Jialal I. Effect of C-reactive protein on chemokine expression in human aortic endothelial cells. *J Mol Cell Cardiol*. 2004 Mar;36(3):405–10.
 39. Liu Y, Zhang D, Yin D. Pathophysiological Effects of Various Interleukins on Primary Cell Types in Common Heart Disease. *Int J Mol Sci*. 2023 Mar 30;24(7):6497.
 40. Ding Z, Wei Y, Peng J, Wang S, Chen G, Sun J. The Potential Role of C-Reactive Protein in Metabolic-Dysfunction-Associated Fatty Liver Disease and Aging. *Biomedicines*. 2023 Oct 5;11(10):2711.
 41. Rezar R, Jirak P, Gschwandtner M, Derler R, Felder TK, Haslinger M, et al. Heart-Type Fatty Acid-Binding Protein (H-FABP) and its Role as a Biomarker in Heart Failure: What Do We Know So Far? *J Clin Med*. 2020 Jan 7;9(1):164.
 42. Xu C, Zhang T, Zhu B, Cao Z. Diagnostic role of postmortem CK-MB in cardiac death: a systematic review and meta-analysis. *Forensic Sci Med Pathol*. 2020 Jun;16(2):287–94.
 43. Kabekkodu SP, Mananje SR, Saya RP. A Study on the Role of Heart Type Fatty Acid Binding Protein in the Diagnosis of Acute Myocardial Infarction. *J Clin Diagn Res JCDR*. 2016 Jan;10(1):OC07–10.
 44. Matsumoto S, Nakatani D, Sakata Y, Suna S, Shimizu M, Usami M, et al. Elevated serum heart-type fatty acid-binding protein in the convalescent stage predicts long-term outcome in patients surviving acute myocardial infarction. *Circ J Off J Jpn Circ Soc*. 2013;77(4):1026–32.
 45. L L, J T, Y S, Q X, B S, F X, et al. Lipoprotein-associated phospholipase A2 predicts cardiovascular death in patients on maintenance hemodialysis: a 7-year prospective cohort study. *Lipids Health Dis* [Internet]. 2024 Dec 1 [cited 2024 Nov 22];23(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/38216940/>
 46. Racherla S, Arora R. Utility of Lp-PLA2 in lipid-lowering therapy. *Am J Ther*. 2012 Mar;19(2):115–20.
 47. Dada N, Kim NW, Wolfert RL. Lp-PLA2: an emerging biomarker of coronary heart disease. *Expert Rev Mol Diagn*. 2002 Jan;2(1):17–22.
 48. Abbasi A, Vahabi P, Tabatabaei SA, Shahzad S, Mostafavi A, Nikdoust F. The Potential Role of Serum Procalcitonin and Coronary Angiographic Findings in Patients With Acute Coronary Syndromes Evaluated by the SYNTAX Score. *Iran Heart J*. 2023 Jul 1;24(3):77–84.

49. Schuetz P, Daniels LB, Kulkarni P, Anker SD, Mueller B. Procalcitonin: A new biomarker for the cardiologist. *Int J Cardiol.* 2016 Nov 15;223:390–7.
50. Zaki HA, Bensliman S, Bashir K, Iftikhar H, Fayed MH, Salem W, et al. Accuracy of procalcitonin for diagnosing sepsis in adult patients admitted to the emergency department: a systematic review and meta-analysis. *Syst Rev.* 2024 Jan 22;13(1):37.
51. Kelly D, Khan SQ, Dhillon O, Quinn P, Struck J, Squire IB, et al. Procalcitonin as a prognostic marker in patients with acute myocardial infarction. *Biomark Biochem Indic Expo Response Susceptibility Chem.* 2010 Jun;15(4):325–31.
52. Hashemipour SV, Pourhosseini H, Hosseinsabet A. Correlation between the serum procalcitonin level and the extension and severity of coronary artery disease in patients with non-ST-segment elevation myocardial infarction. *Cardiovasc Endocrinol Metab.* 2019 Jun;8(2):62–6.
53. Sultan RH, Abdallah M, Ali TM, Ahmed AE, Assal HH, Elesawy BH, et al. The Associations between Cytokine Levels, Kidney and Heart Function Biomarkers, and Expression Levels of Angiotensin-Converting Enzyme-2 and Neuropilin-1 in COVID-19 Patients. *Vaccines.* 2022 Jun 29;10(7):1045.
54. Bouzidi N, Gamra H. Relationship between serum interleukin-6 levels and severity of coronary artery disease undergoing percutaneous coronary intervention. *BMC Cardiovasc Disord.* 2023 Nov 28;23(1):586.
55. Tøllefsen IM, Shetelig C, Seljeflot I, Eritsland J, Hoffmann P, Andersen GØ. High levels of interleukin-6 are associated with final infarct size and adverse clinical events in patients with STEMI. *Open Heart.* 2021 Dec;8(2):e001869.
56. Su JH, Luo MY, Liang N, Gong SX, Chen W, Huang WQ, et al. Interleukin-6: A Novel Target for Cardio-Cerebrovascular Diseases. *Front Pharmacol.* 2021;12:745061.
57. Müller J, Gorressen S, Grandoch M, Feldmann K, Kretschmer I, Lehr S, et al. Interleukin-6-dependent phenotypic modulation of cardiac fibroblasts after acute myocardial infarction. *Basic Res Cardiol.* 2014;109(6):440.
58. Fernández-Ruiz I. Early inhibition of IL-6 signalling after an acute MI improves myocardial salvage. *Nat Rev Cardiol.* 2021 Jul;18(7):460.
59. Białecka M, Dziedziejko V, Safranow K, Krzystolik A, Marcinowska Z, Chlubek D, et al. Could Tumor Necrosis Factor Serve as a Marker for Cardiovascular Risk Factors and Left Ventricular Hypertrophy in Patients with Early-Onset Coronary Artery Disease? *Diagn Basel Switz.* 2024 Feb 18;14(4):449.
60. Fang C, Chen Z, Zhang J, Pan J, Jin X, Yang M, et al. The Value of Serum YKL-40 and TNF- α in the Diagnosis of Acute ST-Segment Elevation Myocardial Infarction. *Cardiol Res Pract.* 2022;2022:4905954.
61. Hinkley H, Counts DA, VonCanon E, Lacy M. T Cells in Atherosclerosis: Key Players in the Pathogenesis of Vascular Disease. *Cells.* 2023 Aug 26;12(17):2152.
62. Mahmoud AH, Taha NM, Zakhary M, Tadros MS. PTEN gene & TNF- α in acute myocardial infarction. *Int J Cardiol Heart Vasc.* 2019 Jun;23:100366.
63. Pluijmer NJ, Atsma DE, Quax PHA. Post-ischemic Myocardial Inflammatory Response: A Complex and Dynamic Process Susceptible to Immunomodulatory Therapies. *Front Cardiovasc Med.* 2021;8:647785.
64. Toldo S, Mauro AG, Cutter Z, Abbate A. Inflammasome, pyroptosis, and cytokines in myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol.* 2018 Dec 1;315(6):H1553–68.
65. Moriya J. Critical roles of inflammation in atherosclerosis. *J Cardiol.* 2019 Jan;73(1):22–7.
66. Richter J, Brouwer S, Schroder K, Walker MJ. Inflammasome activation and IL-1 β signalling in group A Streptococcus disease. *Cell Microbiol.* 2021 Sep;23(9):e13373.
67. Ridker PM, MacFadyen JG, Thuren T, Everett BM, Libby P, Glynn RJ, et al. Effect of interleukin-1 β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet Lond Engl.* 2017 Oct 21;390(10105):1833–42.
68. Cappuzzello C, Di Vito L, Melchionna R, Melillo G, Silvestri L, Cesareo E, et al. Increase of plasma IL-9 and decrease of plasma IL-5, IL-7, and IFN- γ in patients with chronic heart failure. *J Transl Med.* 2011 Mar 21;9:28.
69. Ormstad H, Aass HCD, Lund-Sørensen N, Amthor KF, Sandvik L. Serum levels of cytokines and C-reactive protein in acute ischemic stroke patients, and their relationship to stroke lateralization, type, and infarct volume. *J Neurol.* 2011 Apr;258(4):677–85.