ORIGINAL ARTICLE

VALIDATION OF SERUM ADMA LEVELS AS A BIOMARKER FOR ASSESSING CARDIOVASCULAR RISK IN PATIENTS WITH CORONARY HEART DISEASE: A STUDY FROM A TERTIARY CARE HOSPITAL IN PAKISTAN

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Objectives: The diagnostic potential of serum asymmetric dimethylarginine (ADMA) levels as a biomarker for cardiovascular risk has been investigated in various populations, but no such study has been reported in Pakistan. This study aimed to assess the diagnostic efficiency of serum ADMA levels in detecting cardiovascular risk in patients with coronary heart disease (CHD).

Methodology: A cross-sectional study was conducted at the Chaudhry Pervaiz Elahi Institute of Cardiology (CPEIC), Wazirabad, Pakistan, using a consecutive sampling technique to ensure randomization. One hundred individuals were divided into two groups (CHD patients and healthy controls), and blood samples were collected between January 2022 and November 2022. Serum ADMA levels were measured using an enzyme-linked immunosorbent assay (ELISA), and statistical analysis was performed to determine the area under the receiver operating characteristic (ROC) curve (AUC).

Results: The mean age of CHD patients' serum samples was 58.6 ± 7.39 years ($p<0.001$) compared to 42.26 ± 14.4 years ($p<0.001$) in healthy controls. The mean ADMA concentration in the serum of CHD patients was determined as 1.37 ± 0.26 µmol/L ($p<0.001$) compared to 0.812 ± 0.207 µmol/L ($p<0.001$) in healthy controls. The AUC on the ROC curve was determined as 0.95, indicating high diagnostic accuracy. ADMA's sensitivity, specificity, and overall accuracy were determined as 82%, 88%, and 85%, respectively.

Conclusion: In conclusion, serum ADMA levels demonstrate a promising potential as a biomarker for assessing cardiovascular risk. The findings of this study suggest that ADMA measurements could be utilized as a diagnostic tool in CHD patients, aiding in the identification and management of cardiovascular risk in the Pakistani population. Further research is warranted to validate these findings and explore the utility of ADMA in more extensive and diverse cohorts.

Keywords: Pakistan, serum asymmetric dimethylarginine (ADMA), coronary heart disease, biomarker

Citation: Roomi FS, Abbas SQ, Raza MH. Validation of Serum ADMA Levels as a Biomarker for Assessing Cardiovascular Risk in Patients with Coronary Heart Disease: A Study from a Tertiary Care Hospital in Pakistan. Pak Heart J. 2023;56(02):152-156. DOI: https://doi.org/10.47144/phj.v56i2.2554

INTRODUCTION

The asymmetric isomer monomethyl-L-arginine (L-NMMA), the asymmetric dimethylarginine (ADMA), and the symmetric dimethylarginine (SDMA) are produced when L-arginine is methylated.[1] Methylated L-arginine is present in human urine; it is also found in animal neurons and human endothelial cells.[2]

Endothelial dysfunction is brought on by decreased nitric oxide (NO) bioavailability, which controls the circulatory system's operation.[3] The substrate for endothelial nitric oxide synthase is L-arginine (eNOS).[4] Vallance and his coworkers initially identified ADMA as an endogenous inhibitor of NO synthase in 1992.[5] The synthesis of methylated amino acids by the endothelium, such as ADMA, can lead to endothelial dysfunction. These methylated amino acids might challenge L-arginine for the position of the eNOS substrate.[5]

It has been demonstrated that ADMA, also known as asymmetric dimethylarginine, is an endogenous inhibitor of nitric oxide (NO) metabolism, leading to endothelium-dependent vasodilation.[6] Because NO controls the degree of vascular inflammation, vascular tone, cell proliferation, and the release of numerous growth factors, it is one of the crucial mediators that

control the function and vasodilation of the endothelium. Therefore, it is thought that NO is one of the most crucial mediators that control endothelial function and vasodilation.[7] ADMA has been widely utilised to evaluate and forecast the onset of cardiovascular disease events and early signs of endothelial dysfunction.[8] Patients with elevated serum ADMA had a higher risk for cardiovascular disease events, according to prospective research with 125 type II diabetic patients and a 2-year follow-up.[9] Cardiovascular disease patients showed significantly higher serum levels of ADMA (0.47 mol/L) than healthy people (0.42 mol/L) in research with more than 1,000 Taiwanese volunteers.[10]

A blood ADMA concentration over the normal range has been linked to a number of illnesses, including diabetes mellitus, atherosclerosis, hypertension, preeclampsia, stroke, and peripheral vascular disease.[1] In addition, our most recent study showed that high ADMA concentrations directly cause endothelial dysfunction by suppressing eNOS protein expression and upregulating superoxide anion generation in human internal thoracic arteries that had undergone coronary artery bypass grafting. These findings were acquired from human internal thoracic arteries obtained via coronary artery bypass grafting.[11]

Researchers have examined whether blood levels of ADMA are related to the likelihood of developing coronary artery disease (CAD).[12,13] However, different interpretations of the nature of this connection have been drawn from the considerable research carried out, partly because of the very small number of participants in each of these examinations. Moreover, there is only one study on ADMA serum levels completed in Pakistan on pre-menopausal women and post-menopausal women[14], and there is yet to be a study conducted here that concentrates on ADMA as a biomarker of cardiovascular risk. In this study, we have tried to determine the diagnostic accuracy of ADMA as a biomarker for the assessment of cardiovascular risk.

METHODOLOGY

Study Design and Setting: This cross-sectional study was conducted at the Chaudhry Pervaiz Elahi Institute of Cardiology (CPEIC) in Wazirabad, Pakistan. The study utilized a consecutive sampling technique. Ethical approval was obtained from the institutional review committee, and informed consent was obtained from all participants before their inclusion in the study.

Sample Selection and Data Collection: Between January 2022 and November 2022, blood samples were collected from 100 individuals divided into two groups. The first group consisted of patients diagnosed with coronary heart disease (CHD), while the second group comprised healthy individuals. Selection criteria for CHD patients included: 1) Age greater than 20 years, 2) Diagnosis of cardiovascular disease (CVD), and 3) Greater than or equal to 50% stenosis of one coronary artery. Relevant information regarding diabetes and cardiovascular history was obtained from the patient's medical records.

Blood Sample Collection and Processing: Blood samples were collected from the patients using serum or gel tubes after an overnight fasting period. The samples were allowed to stand for 10 minutes and then centrifuged for 10 minutes at 2000 revolutions per minute (RPM). The serum was carefully extracted and stored at -80ºC in Eppendorf tubes. ADMA estimation was performed using commercial ADMA ELISA kits from Elabscience Biotechnology.

Data Analysis: The collected data were analyzed using SPSS version 28. Baseline characteristics of the patients were presented using frequency and percentages. To determine the difference in ADMA concentration between the serum samples from both groups, an independent sample t-test was employed. Furthermore, ROC curve analysis was conducted to evaluate the diagnostic efficacy of ADMA levels, as indicated by the area under the curve (AUC). A higher AUC value indicates greater diagnostic efficacy. The optimal cut-off value was determined by identifying the point on the ROC curve that yielded the highest sum of sensitivity and specificity. Using this cut-off value, the number of true positives, true negatives, false positives, and false negatives was determined. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated using a diagnostic calculator, such as the Medcalc Diagnostic calculator (https://www.medcalc.org/calc/diagnostic_test.php).

RESULTS

The study included 100 participants, comprising patients with coronary heart disease (CHD) and healthy controls. The mean age of CHD patients' serum samples was found to be 58.6 ± 7.39 ($p<0.001$), while the mean age of healthy controls' serum samples was 42.26 ± 14.4 ($p<0.001$), indicating a significant difference between the two groups.

Baseline characteristics of both CHD patients and controls were assessed, considering gender, age groups, presence of diabetes, and history of cardiovascular disease (CVD), as displayed in Table 1. In terms of gender, the majority of the population in

both CHD patients and healthy controls was male, although there were more men in the CHD patient group overall. Additionally, the majority of CHD patients fell within the 50-59 years age group, followed by the 60-69 years age group. Among the CHD patients, 15 individuals had a history of diabetes, and 14 individuals had a previous cardiovascular disease history.

Table 1: Baseline characteristics of the CHD patients enrolled in the study as compared to healthy controls

Characteristic	CHD patients (n=50)	Controls (n=50)
Gender (male/female)	41/9	32/18
Age Group		
20-29 n (%)	0 (0)	14(28)
30-39 n (%)	0(0)	13(26)
40-49 n (%)	3(6)	11(22)
50-59 n (%)	26(52)	4(8)
60-69 n (%)	15(30)	8(16)
70-80 n (%)	6(12)	0(0)
Diabetes n (%)	15(30)	0(0)
Cardiovascular history n (%)	14(28)	0(0)

The mean ADMA concentration in the serum of CHD patients was determined as 1.37 ± 0.26 µmol/L ($p<0.001$), whereas it was 0.812 ± 0.207 µmol/L ($p<0.001$) in healthy controls. This indicates a significantly higher mean ADMA concentration in CHD patients than in healthy controls.

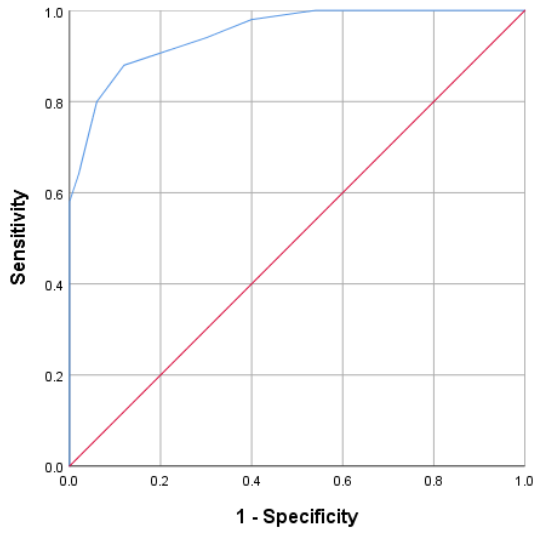

Figure 1: ROC curve analysis of serum ADMA levels in CHD patients and healthy controls

Furthermore, ROC curve analysis was performed using the ADMA concentrations obtained from CHD patients and healthy controls, as illustrated in Figure 1. The area under the curve (AUC) was calculated as 0.95, indicating a high diagnostic efficacy of ADMA levels in differentiating CHD patients from healthy individuals.

Based on the ROC curve coordinates, the cut-off value for ADMA concentration was determined as 1.05 µmol/L, considering the maximum sum of sensitivity and specificity. Subsequently, the samples were categorized as true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN), as presented in Table 2.

Table 2: Determination of True Positives, True Negative, False positive and False negatives

Characteristic	Quantitation
True Positive	41
True Negative	44
False Positive	6
False Negative	9

In order to evaluate the diagnostic parameters of serum ADMA for detecting CHD, the TP, FP, TN, and FN values were utilized. The sensitivity of the test was determined to be 82%, the specificity was 88%, and the overall accuracy of the test was calculated as 85% (Table 3).

Table 3: Determination of diagnostic parameters of serum ADMA levels to detect CHD

Characteristic	Quantitation
Sensitivity	82%
Specificity	88%
Positive Predictive Value (PPV)	87.23%
Negative Predictive Value (NPV)	83.02%
Accuracy	85%

DISCUSSION

This study aimed to investigate the biomarker capability of serum ADMA levels for detecting coronary heart disease in patients in Pakistan. The results of this study indicate that serum ADMA possesses a strong diagnostic potential for the detection of coronary heart disease. The mean concentration of serum ADMA was significantly higher in CHD patients compared to healthy control samples. The ROC curve analysis also clearly marked the diagnostic potential with an AUC of 0.95.

Recent studies have linked high serum ADMA levels arising due to the inhibition of NO signaling, and this results in an increased risk of cardiovascular disease, specifically symptomatic peripheral arterial disease, and thus correspond to higher mortality rates.[16] In another study, similar reasoning of NO signaling

inhibition was employed to associate increased serum ADMA levels with carotid endothelial damage, thus highlighting the cardiovascular risk associated with higher serum ADMA levels.[17] The linkage between cardiovascular risk and NO signaling inhibition can be explained by resulting endothelial damage and albuminuria due to increasing collagen cross-linking and glycation.[11] One meta-analysis also linked the increased serum ADMA levels with coronary artery disease by analysis of 16 case-control studies.[1]

Nitric oxide is known to regulate cardiovascular functions in the body, and its inhibition is linked directly with endothelial dysfunction.[3] ADMA is known to inhibit nitric oxide synthesis by the inhibition of the NO-synthesizing enzyme eNOS. It inhibits the enzyme by acting as a competitor of the substrate of eNOS, i.e., L-arginine resulting in endothelial damage and, thus, increased cardiovascular risk.[4]

A recent study linked increased serum ADMA levels with CHD and periodontitis and found that both salivary and serum ADMA levels were increased in CHD and CP +CHD patients; however, they could not justify the increased salivary ADMA levels with CHD.[13]

In Pakistan, no study to date has been conducted on the biomarker potential of ADMA for cardiovascular health and coronary artery disease. Serum ADMA levels were investigated in pre-menopausal and post-menopausal women in Pakistan, and it was found that post-menopausal women had higher serum ADMA levels as compared to pre-menopausal due to decreased endogenous estrogens. They also justified the increased cardiovascular risk in post-menopausal women due to increased serum ADMA levels.[14]

The findings highlight the potential of ADMA as a valuable tool in clinical practice for identifying individuals at increased risk of cardiovascular events. Utilizing ADMA as a biomarker could aid in the early detection and management of cardiovascular disease, allowing for timely interventions and improved patient outcomes.

However, it is important to acknowledge the limitations of this study, such as its cross-sectional design and the relatively small sample size. Further research is necessary to validate the utility of ADMA as a biomarker in more extensive and diverse populations. Additionally, longitudinal studies are needed to assess the long-term predictive value of ADMA for cardiovascular outcomes.

CONCLUSION

This study investigated the biomarker potential of asymmetric dimethylarginine (ADMA) for assessing cardiovascular risk. The findings suggest that ADMA holds promise as a convenient blood-based biomarker for cardiovascular risk assessment, particularly among cardiac patients in Pakistan. The results demonstrate that ADMA exhibits relatively high sensitivity, specificity, and accuracy in differentiating patients with cardiovascular disease from healthy individuals.

AUTHORS' CONTRIBUTION

RU and JA: Concept and design, data acquisition, interpretation, drafting, final approval, and agree to be accountable for all aspects of the work. AB, DAJ, AR, and WS: Data acquisition, interpretation, drafting, final approval and agree to be accountable for all aspects of the work.

Conflict of interest: Authors declared no conflict of interest.

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