SERUM MMP-9 DIAGNOSTICS AND ITS ASSOCIATIONS BETWEEN ELECTROLYTES, UREA AND CREATININE IN ACUTE MYOCARDIAL INFARCTION

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ABSTRACT:

Introduction: Atherosclerosis is a chronic inflammatory condition affecting the arteries. Matrix metalloproteinases (MMPs) contribute to the destabilization of atherosclerotic plaques by breaking down the extracellular matrix (ECM), particularly in the shoulder regions of the plaques. This process can result in plaque rupture, potentially leading to acute coronary syndrome (ACS), which can be fatal. The production of MMPs is increased by inflammatory and oxidative factors. Matrix metalloproteinase-9 (MMP-9), also referred to as gelatinase B, is an enzyme that primarily breaks down type IV collagen and elastin. It is secreted by various cell types, including neutrophils, macrophages, endothelial cells, and smooth muscle cells. The activity of MMP-9 is regulated by its interactions with specific tissue inhibitors of matrix metalloproteinases (TIMPs). The purpose of the study was to examine the diagnostics value of MMP-9 and its associations between serum electrolytes, urea and creatinine. ***Methods:*** AMI Subjects with symptom onset within ≤24 hours were enrolled. Patients with predominant lung and kidney diseases were excluded from the study. 5 ml of peripheral venous blood was collected, centrifuged, and subjected to standard biochemical

analysis. Serum Electrolytes, Urea and creatinine was estimated by standard appropriate method, while MMP-9 levels were quantified using an immunoassay ELISA kit, $p \leq 0.05$ was considered statistically significant. ***Results:*** Serum MMP-9 levels were significantly elevated in AMI subjects. These levels were positively correlated with , potassium, urea, and creatinine, but showed no association in sodium and chloride. ***Conclusion*****:** MMP-9 could be used as a supportive and standard biomarker in AMI subjects.

Keywords: Acute Myocardial infarction, ST-elevation acute myocardial infarction, non-ST-elevation acute myocardial infarction, Matrix mettalloproteinase-9.

Introduction:

Myocardial infarction (MI) occurs when there is extended ischemia caused by the blockage of coronary arteries, leading to irreversible death of cardiomyocytes(1). ECM constituents are crucial in all stages of cardiac repair following myocardial infarction (MI). Matrix metalloproteinases (MMPs), a group of 25 proteolytic enzymes, work together to degrade various components of the extracellular matrix (ECM)(2–4). This can result in plaque rupture, potentially causing a fatal acute coronary syndrome (ACS) event. Inflammatory and oxidative mediators elevate the levels of matrix metalloproteinases (MMPs)(5). Matrix metalloproteinases (MMPs) facilitate the migration of leukocytes and inflammatory mediators through tissues, thereby speeding up the formation of pathogenic atherosclerotic plaques(6). Matrix metalloproteinase-9 (MMP-9), also called gelatinase B, is an enzyme primarily responsible for breaking down type IV collagen and elastin. It is produced by various cell types, including neutrophils, macrophages, endothelial cells, and smooth muscle cells. The activity of MMP-9 is regulated by its interactions with specific tissue inhibitors of matrix metalloproteinases (TIMPs)(7–9). The inactive, latent pro-form of MMP-9 can be activated by reactive oxygen species (ROS), trypsin, chymotrypsin, or bacterial proteases(10). Additionally, MMP-9 can be chemically activated in vitro using APMA (p-amino phenyl mercuric acetate)(11). Significantly increased plasma MMP-9 levels have been previously observed in patients with acute coronary syndrome (ACS)(12–14). Higher serum MMP-9 concentrations were linked to plaque rupture, as compared to patients with stable angina pectoris(15). Electrolytes are essential for intermediate metabolism and all aspects of cellular activity, such as electrical gradients and enzyme activity.

This study aims to detect the serum MMP-9 diagnostics and its association with electrolytes, urea and creatinine levels.

Materials and methods:

Study group comprised 80 subjects with 50 AMI subjects between the age group of 35-60 years who were visiting the emergency department will be considered. Diagnosis and selection of 50 cases were made on the basis of recent onset of chest pain, abnormal ECG pattern and by cardiac biochemical markers. The control group included 30 normal healthy subjects visiting hospital for routine check-up without any history of chest pain or previous incidence of AMI, hypertension, diabetes, alcoholism and smoking. Under aseptic conditions about 5 ml of venous blood was drawn and collected in plain vacutainer, after taking informed consent and study was approved by the ethical committee of the institution. Serum was separated by centrifugation at 3000 rpm for 10 minutes at room temperature and the following parameters were estimated. Estimation of serum cardiac Troponin I (cTnI) was done by enzyme-linked fluorescent assay (ELFA) method with the miniVIDAS system (Biomerieux). Serum urea was done by Diacetyl Monoxime using sysmex BX 3010 analyzer and serum creatinine modified Jaffe's method was done by sysmex BX 3010 analyzer. Serum sodium, potassium and chloride were determined by direct ion selective electrode methods by using EasyLyte electrolyte analyser.

Data were expressed as means ± SD and the comparison of normally distributed continuous data was conducted using the Student's T-test, while the Mann-Whitney test was applied for non-normally distributed continuous data. Pearson's / spearman's correlation was done to find the association between electrolytes, Urea, Creatinine in patients with AMI subjects. A receiver operating characteristic (ROC) curve was designed to determine the cut-off point of MMP-9 level in AMI patients. A *p*-value equal or less than 0.05 was considered statistically significant, p Value < 0.001 was considered as highly significance. Data were analysed by SPSS version 23 software.

Results:

Fifty AMI subjects with thirty control groups were enrolled in this study. In **Table 1** Urea and creatinine was significantly higher (44.80±35.1, p value <0.001*, 1.7±1.2, p value <0.001*) in AMI groups than in the control group (18.07±4.8, 0.94±0.13), electrolytes such as sodium, potassium showed significant difference in AMI groups (136.38±4.17, p value <0.001*, 4.458±0.48, p value 0.044) than in the control groups (142.37±6.105, 4.320±0.27) But, Chloride levels didn't show any difference in both the groups.

In **Table 2** the cardiac biomarkers such as CPK NAC and Troponin I was significantly was (1530.24±1970.9, p value <0.001*, 9057.4±15767.6 p value <0.001*) higher in AMI groups than in healthy control groups (102.83±13.8, 0.30±0.610) similarly MMP-9 shows the significant difference (86.30±25.50, p value <0.001*) in AMI groups than in the control groups (53.27±16.38).

Table 1: Shows the Biochemical parameters of AMI and Control

BIOCHEMICAL PARAMETERS MEAN ± SD	AMI N= 50	CONTROL N=30	P VALUE
Urea (mg/dl)	44.80±35.1	18.07±4.8	<0.001*
Creatinine (mg/dl)	1.7±1.2	0.94±0.13	<0.001*
Sodium (mg/dl)	136.38±4.17	142.37±6.105	<0.001*
Potassium (mg/dl)	4.458±0.48	4.320±0.27	0.044
Chloride(mg/dl)	99.84±5.97	102.60±3.2	0.063

*p value < 0.05 indicates statistically significant, *p value < 0.001 indicates highly significant All biochemical parameters were significantly higher in AMI subjects compared to control groups

Table 2: Shows the Cardiac Biomarkers of AMI and Control

CARDIAC BIOMARKER MEAN ± SD	AMI N= 50	CONTROL N=30	P VALUE
CPK-NAC	1530.24±1970.9	102.83±13.8	<0.001*
Troponin I (ng/dl)	9057.4±15767.6	0.30±0.610	<0.001*
MMP-9	86.30±25.50	53.27±16.38	<0.001*

*p value < 0.001 indicates highly significant. MMP-9 levels were significantly higher in AMI subjects compared to control groups

Table 3: shows the correlation coefficient of Urea, Creatinine and electrolytes with Matrix metalloproteinase 9:

VARIABLES	COEFFICIENT CORRELATION	P VALUE
Urea	**0.306**	**0.006**
Creatinine	**0.277**	**0.013**
Sodium	**-0.257**	**0.023**
Potassium	**0.307**	**0.006**
Chloride	**-0.059**	**0.602**

Among the variables, serum matrix metalloproteinase-9 level was correlated with serum urea level (correlation coefficient of 0.306, p value < 0.01), creatinine level (correlation coefficient of 0.277, p value 0.013) and potassium level (correlation coefficient of 0.307, p value <0.01), Other variables, i.e., sodium (correlation coefficient of -0.257) and chloride (correlation coefficient of -0.059) negatively correlated with serum MMP-9 levels (Table 3).

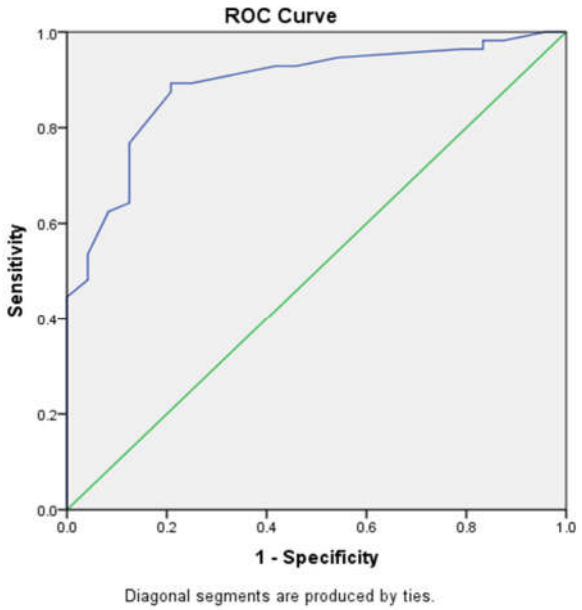

Figure 1: The ROC curve to determine the accuracy of MMP-9 (AUC 0.88), cut-off value 44ng/ml (sensitivity 96%, specificity 79%)

The ROC curve indicated that serum MMP-9 level has a predictive value for acute myocardial infarction with area under the curve of 0.88. Based on the ROC curve, the MMP-9 level of 44 ng/mL had a sensitivity of 96% and specificity of 79% to accurately predict AMI patients.

Discussion:

Over the past 30 years, numerous studies have consistently demonstrated a strong link between matrix metalloproteinase 9 (MMP-9) levels and mortality from myocardial infarction (MI), as well as left ventricular remodeling and dysfunction.(16) Matrix metalloproteinases (MMPs) play a key role in regulating the extracellular matrix (ECM) and are primarily introduced into the infarct area by neutrophils and macrophages, which begin infiltrating within minutes of ischemic injury. Besides leukocytes, MMPs are also produced by cardiomyocytes, fibroblasts, and endothelial cells(3,4,17). During the acute stage of ACS, serum MMP-9 values were higher, and they typically dropped as the patient recovered. The present study supports the findings of previous researchers, showing that MMP-9 levels were significantly elevated in AMI patients compared to the control group. Earlier studies have also suggested that elevated MMP-9 is associated with higher risk of death due to any cause(18) and with CVD risk factors and total cardiovascular risk in subjects without symptoms of CAD(19). The myocardium-specific markers CK-MB and troponin T showed a substantial connection with MMP-9, indicating that systemic MMP-9 following an ACS event may originate from ischaemic cardiac tissue. Myocardial damage can induce enhanced MMP expression and activation (20). MMP-9 plasma levels correlate with MI mortality, LV remodeling and dysfunction across a variety of species and in humans (21–23). Even after accounting for all other risk variables, Zhu and colleagues demonstrated that elevated plasma levels of MMP-9 predict in-hospital death in patients with acute MI. Somuncu and colleagues demonstrated that patients with myocardial infarction (MI) who had plasma MMP-9 levels above 12.92 ng/mL upon hospital admission faced 3.5 times greater odds of cardiovascular mortality and an elevated risk of developing advanced heart failure, compared to those with lower MMP-9 levels (22). High MMP-9 plasma levels during the first few hours of MI are associated with a lower ejection fraction and higher LV end-diastolic volume at discharge (24). Novel biomarkers are required for the prognosis of CVD, early detection of atherosclerotic plaque rupture or ischemia, and the identification of patients at risk for the disease. The measurement of serum MMP-9 is both sensitive and specific, making it a strong candidate as a biomarker in clinical practice. MMP-9 contributes to both the rupture of atherosclerotic plaques and tissue damage following a cardiac event. Moreover, analyzing MMP-9 activation potential could provide valuable new insights into cardiac diagnostics and prognostics. These findings suggest that serum MMP-9 could serve as a

valuable biomarker for evaluating both myocardial damage and associated renal and electrolyte disturbances in AMI patients.

The presence of cTnI in blood serum is widely recognized as an indicator of myocardial damage, making it a specific biochemical marker for acute myocardial infarction (AMI) (25). CK has also been utilized alongside cTnI in diagnosing AMI. In the present study, levels of both markers were significantly elevated in the AMI case groups. In the present study, serum urea and creatinine levels were significantly elevated in AMI subjects ($p < 0.001$) compared to the control group. Urea is the final product of protein metabolism, and an increase in serum urea levels was observed in AMI cases in this study. Few studies have investigated the association between urea and AMI, though some have reported that elevated urea levels are predictive of poor outcomes and higher mortality rates in AMI patients(26). Increased urea levels suggest a renal response to systemic hypoperfusion, often linked to reduced cardiac output in cases of decompensated heart failure(27). Similar findings were reported by Heraldo Guedis Lobo Filho et al., where experimental rats induced with myocardial infarction using isoproterenol showed elevated urea levels, likely associated with a low cardiac output state due to ventricular dysfunction (28).Additionally, serum sodium levels were notably reduced ($p < 0.001$) in AMI cases when compared to healthy controls. These findings align with the observations of Hadeel Rashid Faraj (29). In AMI, non-osmotic vasopressin release may occur due to acute left ventricular dysfunction triggered by pain and stress, or possibly due to the use of analgesics or diuretics, which can lead to lower sodium levels in the blood(30,31). However, further research, with larger sample sizes and longer follow-up periods, is necessary to confirm the diagnostic and prognostic utility of MMP-9 in clinical practice.

Conclusion: The best indicators for identifying an acute myocardial infarction (AMI) are CKP-NAC, cTnI. Furthermore, MMP-9 levels can be a major factor in determining whether patients are at high risk. Serum values of MMP-9, urea, creatinine, sodium, potassium, and chloride at admission may offer useful further information to help with treatment decisions and enhance the prognosis of AMI patients.

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Ethical oversight:

The study design was approved by IHEC.

Informed Consent Statement: The Consent was obtained from all patient's attender involved in the study.

Conflicts of Interest: The authors declared that there is no conflict of interest in the study.

Author Contributions: All authors have significantly contributed to the study's conception and design, as well as the analysis have read and agreed to the published version of the manuscript.

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