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Cardiac and Biochemical Biomarkers in Acute Myocardial Infarction: Association of Fetuin-A, Endothelin-1 and Lipoxygenase-1 with Cardiac Injury, Lipid Disturbance and Electrolyte Imbalance

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ABSTRACT: Acute myocardial infarction (AMI) is a condition of acute myocardial damage due to inadequate coronary blood flow. It is characterised by inflammation, oxidative stress, endothelial dysfunction, lipid abnormalities and electrolyte imbalance. The present investigation was performed to evaluate the blood levels of Fetuin-A, Endothelin-1 (ET-1), Lipoxygenase-1 (LOX-1), Troponin, lipid profile parameters, potassium and magnesium in patients of AMI and healthy controls. A total of 120 subjects were examined, including 80 patients with AMI and 40 healthy controls. Blood was collected (approx 5 mL), serum separated, and stored at −20 °C. Biochemical indices and biomarkers were analysed by validated assays. LOX-1 was partially purified by ammonium-sulphate precipitation, dialysis, DEAE-cellulose ion-exchange chromatography and Sephadex G-100 gel filtration. Statistical analysis was performed using SPSS version 26. Significant biochemical changes were observed in AMI patients with increased LOX-1 and ET-1 and decreased Fetuin-A. The results suggest that they may be involved in oxidative, inflammatory and endothelial processes in AMI. Prior to clinical implementation further multicenter and independent validation investigations are necessary.

1. INTRODUCTION:

Acute myocardial infarction (AMI) is a major cardiovascular disease caused by disruption or significant reduction of coronary blood flow, resulting in inadequate oxygen delivery to the myocardium, myocardial ischemia, and subsequent necrosis [1]. AMI is a complex pathological process involving interactions among inflammation, oxidative stress, endothelial dysfunction, lipid abnormalities, and electrolyte imbalance. These interconnected mechanisms contribute to the development and progression of coronary artery disease and influence myocardial injury and clinical outcomes [2, 3]. Atherosclerosis is a major underlying cause of coronary artery disease and AMI. It is a chronic inflammatory process characterized by accumulation of lipids, particularly low-density lipoprotein (LDL), within the arterial wall, accompanied by inflammatory-cell infiltration and vascular remodeling. Oxidation of LDL increases its atherogenicity, while oxidized LDL is recognized by scavenger receptors, including SR-A1, CD36, and lectin-like oxidized LDL receptor-1 (LOX-1), promoting lipid accumulation and foam-cell formation. Progressive plaque development may lead to plaque instability, coronary thrombosis, and AMI. Hypertension, diabetes mellitus, smoking, obesity, and elevated cholesterol are important risk factors for atherosclerotic cardiovascular disease[4-6].

Cardiac troponin is an established biomarker of myocardial injury and is widely used in the diagnosis of AMI [7]. However, additional biomarkers may provide information about vascular, inflammatory, oxidative, and metabolic alterations. Endothelin-1 (ET-1) is a potent 21-amino-acid vasoconstrictor produced mainly by vascular endothelial cells. Through ETA and ETB receptors, it regulates vascular tone, while increased ET-1 activity is associated with endothelial dysfunction, hypertension, atherosclerosis, and cardiovascular disease [8]. Fetuin-A, also known as alpha-2-Heremans–Schmid glycoprotein (AHSG), is a

liver-derived circulating glycoprotein involved in mineral metabolism, inflammation, insulin sensitivity, and vascular calcification. By binding calcium and phosphate and inhibiting hydroxyapatite crystallization, Fetuin-A may contribute to protection against vascular calcification. Altered Fetuin-A levels have been associated with cardiovascular and metabolic disorders [9].

Lipoxygenase-1 (LOX-1) is a non-heme iron-containing enzyme involved in the oxygenation of polyunsaturated fatty acids and production of lipid mediators. LOX-related pathways have been implicated in oxidative stress, inflammation, lipid metabolism, and cardiovascular disease. Electrolytes such as potassium and magnesium are also essential for myocardial contraction and cardiac electrical activity, and their disturbances may increase the risk of arrhythmias [1- 2, 10].

Therefore, the present study evaluated serum Fetuin-A, ET-1, LOX-1, Troponin, lipid-profile parameters, potassium, and magnesium in AMI patients and apparently healthy controls. LOX-1 was also partially purified using ammonium-sulfate precipitation, dialysis, DEAE-cellulose ion-exchange chromatography, and Sephadex G-100 gel filtration, followed by characterization of selected biochemical and kinetic properties. This integrated approach aimed to clarify the potential association of LOX-1, Fetuin-A, and ET-1 with the inflammatory, oxidative, endothelial, lipid, and metabolic disturbances accompanying AMI.

2 MATERIALS AND METHODS

2.1 Design and Participants of the Study

The research recruited 120 individuals from November 2025 to January 2026. The patient group consisted of 80 subjects diagnosed with myocardial infarction while the control group consisted of 40 seemingly healthy subjects. The sick group was made up of 41 males and 39 females whereas the control group was composed of 21 males and 19 females. Patients were admitted to critical care after diagnosis by specialised physicians. They were recruited from Ibn Al-Bitar Cardiac Center and Al-Yermouk Teaching Hospital in Baghdad. All individuals with myocardial infarction were positive for troponin, the source said.

The source cites patient ages between 30 to 75 years in the techniques section, yet the abstract and tables of data show the maximum patient age as 78 years. This paper does not seek to resolve that disparity and instead retains the published numbers. The mean age of patients was 49.98 ± 11.79 years and controls was 53.63 ± 12.45 years; no statistically significant difference was seen between groups.

2.2 Blood Collection and Serum Preparation

Venous blood (approx. 5 mL) was taken from each participant in tubes without anticoagulant. Samples were spun at 3500 rpm for at least 15 min after clotting at room temperature. The serum was separated and placed into plain plastic tubes, split among Eppendorf tubes and kept at −20 °C until analysis.

2.3 Biochemical Assays

Cardiac Troponin I was tested by latex enhanced immunoturbidimetric technique utilising Biorex Monarch 240 automated analyser. The test was based on antibody-mediated agglutination of latex particles, with turbidity proportional to the quantity of troponin. was determined by immunoinhibition and the measured B-subunit activity was multiplied by two to estimate activity. ET-1 was quantified by sandwich ELISA employing biotinylated antibody, avidin-HRP and TMB substrate. Absorbance was read at 450 nm. Concentration was measured using a sandwich ELISA according to the kit method. Total cholesterol was assessed using the enzymatic CHOD-POD technique; triglycerides were quantified by GPO-POD method; HDL-C and LDL-C were assayed by the diagnostic methods given; VLDL-C was calculated as stated in the source. Potassium and magnesium were measured using the relevant diagnostic kits. The source specifies the makers and sources of the major kits, including ELK Biotechnology for ET-1 and Fetuin-A, Elabscience for the LOX test, Longford Diagnostics for lipid assays, Agappe Diagnostics for potassium, and AMS for magnesium.

2.4 Quantification of Proteins:

The protein content was determined in the purification by a Folin based technique using as reference the bovine serum albumin. Alkaline reagent was added to the protein sample and it was then incubated with diluted Folin–Ciocalteu reagent. Absorbance was read at 750 nm after incubation and a standard curve was prepared to quantify protein content in serum and chromatographic fractions.

2.5 Partial Purification of Lipoxygenase-1

The purification approach was based on variations in protein solubility, charge and size. The following sequence was used: crude serum extract, ammonium-sulfate precipitation, dialysis, DEAE-cellulose ion-exchange chromatography, Sephadex G-100 gel filtration. At each phase the protein content, recovery and purification fold were determined specific activity, total activity. Purification fold was calculated as a ratio of specific activity after purification to the crude extract and recovery as the percentage of total activity retained.
Precipitation was carried out using ammonium sulphate. To 5 ml serum was added around 0-7 g ammonium sulphate. Precipitation was carried out at 40-60% saturation. The precipitates were then recovered by centrifugation at 5,000 rpm for 15 min and redissolved in 100 mmol/L buffer, pH 7.0. Dialysis was performed overnight at 4 °C with periodic buffer changes. DEAE-cellulose was produced, washed and equilibrated with sodium phosphate buffer and elution was accomplished with sodium chloride solutions. A 2 × 50 cm glass column was filled with Sephadex G-100 to about 40 cm and equilibrated with 100 mmol/L phosphate buffer (pH 7.0) and fractions of about 2.5 mL were recovered by gel filtration.

2.6 Kinetics Characterisation

The purified LOX-1 was assayed with linoleic acid at concentrations of 6.25, 12.5, 25, 50, 100 and 200 μM . The reaction rate increased with increasing substrate concentration and reached a plateau. The first kinetic description yielded Vmax = 1.942. The Lineweaver–Burk calculation gave Vmax = 1.924 U/mg protein with Km = 71.45. The latter is reported as the computed kinetic result and is taken as the main value in this publication, while the difference is kept open and not adjusted implicitly. The influence of temperature was studied in the temperature range of 5 °C to 60 °C . Activity rose across the lower-temperature range and reached a peak at around 34°C, followed by a fall at 40°C and more noticeably at 50-60°C. The effect of pH was investigated in the range pH 2-12. The activity was enhanced with alkaline pH and was maximal at pH 8 and reduced at pH 10-12 .

Estimating molecular weight

2.7 Molecular-Weight Determination:

Pure LOX-1 molecular weight was determined by HPSEC-HPLC. Proteins are separated on the basis of hydrodynamic size, with larger molecules eluting before smaller molecules. Calibration curve was made using standards of known molecular weight. The main peak was observed at 23.21 min and represented 75.57% of the total peak area, corresponding to a molecular mass of an estimated 50.48 kDa. Further, two peaks at 28.32 and 29.26 min were associated with the estimated molecular masses of 32.92 and 30.77 kDa, respectively, and were taken into account in the source as possible contaminants, degraded forms or different protein complexes. R^2 of calibration curve = 0.9933.

2.8 Statistical Analysis

The statistical analysis was conducted using SPSS version 26. Data were summarised as mean, standard deviation, median, minimum, maximum, range, frequency and percentage. Shapiro–Wilk test was used to check normality of distribution. Independent samples t Comparison of two groups was performed by Student's t-test (independent groups). Hedges' g was used to evaluate the effect size and interpreted as insignificant (<0.20), small (0.20–0.49), moderate (0.50–0.79) or high (≥0.80). The association between quantitative factors was assessed using Pearson correlation and simple linear regression; diagnostic performance was tested using ROC analysis. HPLC results were analysed on the basis of retention duration, peak area, peak height, percentage peak area and apparent molecular weight. Statistical significance was established at $P \leq 0.05$.

3. RESULTS

3.1 Cardiac and Emerging Biomarkers

All five principal biomarkers differed very highly significantly between groups. Fetuin-A decreased by approximately 53.3%, while ET-1 increased by 164.3%, LOX-1 by 108.9%, Troponin by 5210.8% and CK by 788.7% according to the source's percentage-change analysis. The source interprets this collective pattern as evidence of myocardial injury accompanied by endothelial dysfunction, oxidative and inflammatory activation, and altered vascular biology (Table 1 fig. 1).

Table 1. Comparative analysis of Cardiac and Emerging Biomarkers

Biomarker	Controls (n=40)	AMI patients (n=80)	P value	Significance
Fetuin-A (µg/mL)	16.760 ± 1.253	7.827 ± 1.147	<0.001	*** Highly Sig
Endothelin-1 (pg/mL)	13.07 ± 0.89	34.54 ± 3.41	<0.001	*** Highly Sig
Lipoxygenase (ng/mL)	29.69 ± 2.10	62.02 ± 7.36	<0.001	*** Highly Sig
Troponin (ng/mL)	0.46 ± 0.08	24.24 ± 1.95	<0.001	*** Highly Sig

The asterisk (*) indicates high statistical significance when the p-value is < 0.001; n: number of study participants.

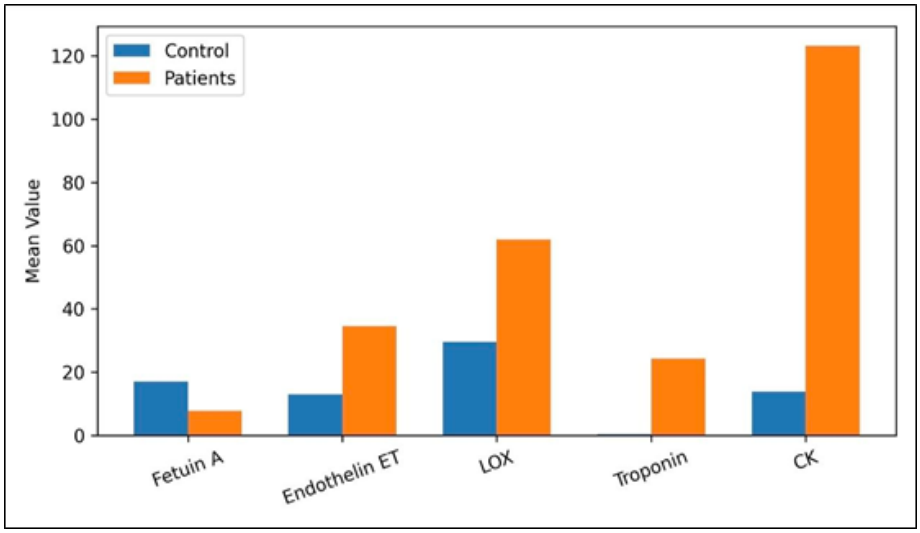

Figure (1): Levels of cardiac biomarkers in the serum of patients and the control group.

3.1.1 Lipid Profile

All lipid variables were reported as significantly higher in the patient group. LDL-C showed the largest reported effect size in the ROC/effect-size analysis (24.63). The source interprets the lipid findings as evidence of metabolic disturbance accompanying AMI and discusses the role of LDL oxidation and LOX-1-mediated recognition in atherosclerotic processes. (Table 2 figure 2).

Table 2. Comparative analysis parameters of lipid profile

Variable	Controls (n=40)	AMI patients (n=80)	P value	Significance
Total cholesterol (mg/dL)	84.20 ± 9.41	337.54 ± 37.18	<0.001	*** Highly Sig
Triglycerides (mg/dL)	102.25 ± 9.29	248.65 ± 29.66	<0.001	*** Highly Sig
LDL-C (mg/dL)	55.59 ± 8.22	244.86 ± 7.41	<0.001	*** Highly Sig
HDL-C (mg/dL)	31.23 ± 3.16	101.16 ± 10.06	<0.001	*** Highly Sig
VLDL-C (mg/dL)	23.153 ± 2.995	57.911 ± 4.594	<0.001	*** Highly Sig

The asterisk (*) indicates high statistical significance when the p-value is < 0.001; n: number of study participants

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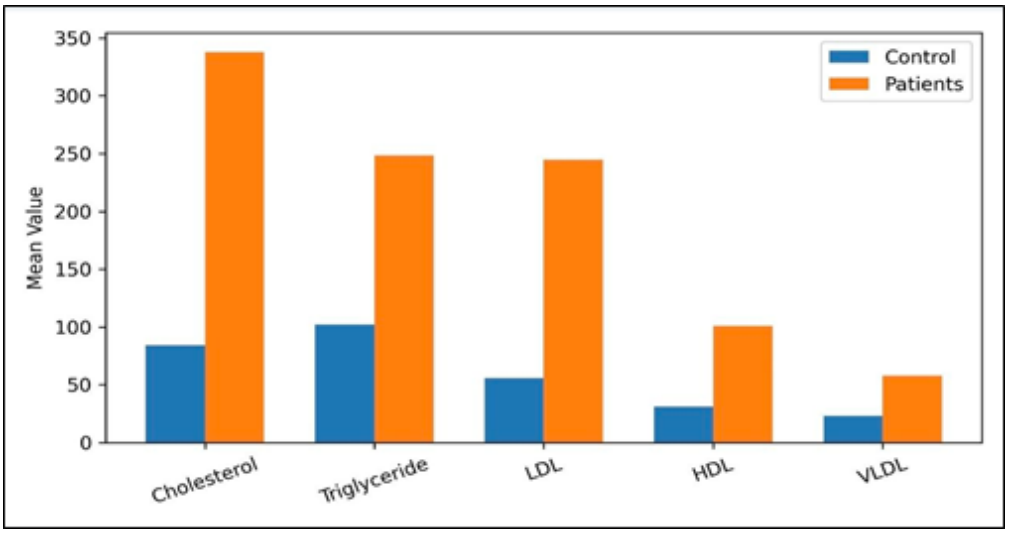

Figure (2): Levels of biochemical lipid variables in the serum of patients and the control group.

3.1.2 Electrolytes:

In AMI group both potassium and magnesium were significantly decreased. These abnormalities are associated with acute ischaemia and disturbed electrolyte homeostasis, and are relevant to cardiac electrical activity and arrhythmia risk, the source said. It refers to previous studies linking low magnesium to ventricular arrhythmias, QTc prolongation and worse clinical condition.(Table 3 figure 3).

Table 3. Analysis of electrolyte in samples

Variable	**Controls (n=40)**	**AMI patients (n=80)**	**P value**	Significance
Potassium (mmol/L)	4.17 ± 0.13	1.45 ± 0.41	<0.001	*** Highly Sig
Magnesium (mmol/L)	1.86 ± 0.10	0.92 ± 0.09	<0.001	*** Highly Sig

n: Number of study participants

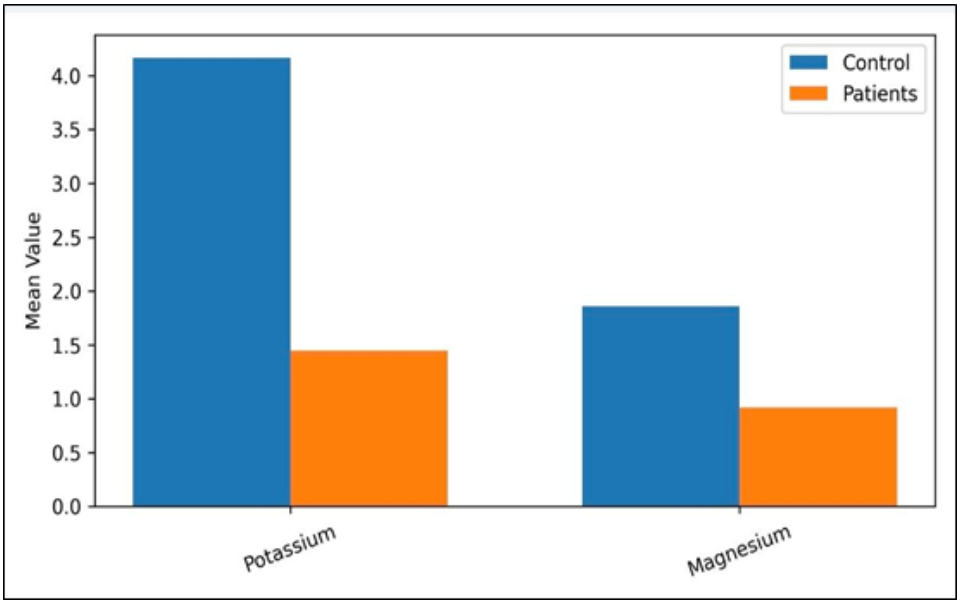

Figure (3): Level of electrolyte changes in the serum of patients and the control group.

3.1.3 Sex and age:

The mean age of the patients and of the controls did not differ significantly. In the three age categories (<40, 40-55 and >55 years) there were no significant age-related differences in the AMI group for Fetuin-A, ET-1, LOX-1, Troponin, CK, lipid variables, potassium or magnesium. Correlation study did not show significant relationship between age and most of the factors. CK exhibited weak negative correlation with age by Pearson coefficient ($r = -0.241$, $P = 0.031$), but not by Spearman association. The source warns against reading too much into it.

Most of the variables in the sex-related study showed no significant differences between male and female participants. In the AMI group, LOX-1 was significantly higher in females than males (64.003 ± 6.833 vs. 60.124 ± 7.415 ng/mL; $P = 0.010$). CK was significantly different in the AMI group (121.229 ± 8.400 vs. 125.251 ± 4.992 U/L; $P = 0.027$). Triglycerides were significantly different in the AMI group (253.585 ± 27.103 vs. 243.462 ± 31.642 mg/dL; $P = 0.038$). No significant sex differences were found for Fetuin-A, ET-1, Troponin, cholesterol, HDL-C, LDL-C, VLDL-C, potassium or magnesium according to the source. (figure 4)

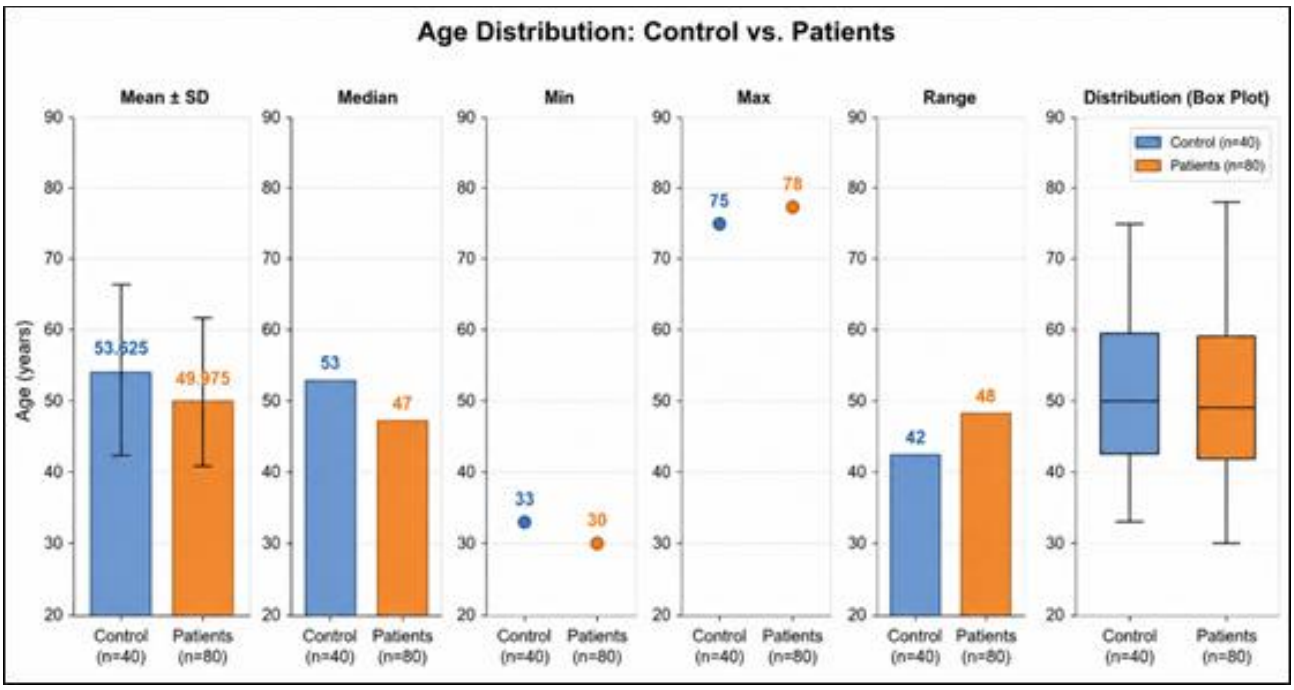

Figure (4): Age distribution of patients and the control group.

3.1.4 Diabetes, Hypertension and Smoking:

There were no significant changes between diabetic and nondiabetic AMI patients for the main biomarkers, lipid variables or electrolytes. Of the patients, 27 were diabetes and 53 were nondiabetic, the source said. In contrast, several key biomarker differences were associated with hypertension: increased ET-1 (36.98 ± 3.12 vs. 33.15 ± 2.84 pg/mL, $P < 0.001$, Hedges' $g = 1.28$), decreased Fetuin-A (7.31 ± 0.98 vs. 8.12 ± 1.15 µg/mL, $P = 0.002$), increased LOX-1 (65.34 ± 7.92 vs. 60.12 ± 6.85 ng/mL, $P = 0.004$), increased Troponin (24.92 ± 2.05 vs. 23.85 ± 1.92 ng/mL, $P = 0.018$) and increased LDL (247.2 ± 8.05 vs. 243.5 ± 7.12 mg/dL, $P = 0.042$).

LOX-1 (60.169 ± 7.175 vs. 64.270 ± 7.026 ng/mL; $P = 0.007$), CK (121.252 ± 8.551 vs. 125.558 ± 4.076 U/L; $P = 0.026$), and triglycerides (253.614 ± 26.335 vs. 242.583 ± 32.621 mg/dL; $P = 0.022$) were significantly different between smokers and nonsmokers. Other variables investigated were not substantially different. According to the source, all subjects in this research experienced AMI and acute illness effects may have confounded independent smoking effects.

3.1.5 Correlations Among Biomarkers:

The pattern of correlations gives a complete biochemical picture. The very strong association of Troponin is consistent with both indicators being markers of myocardial damage. Significant positive correlations of ET-1 with Troponin, LOX-1 and LDL

indicate the interlinkage of endothelial dysfunction, myocardial damage and lipid-oxidative pathways in AMI group. However, the negative associations of Fetuin-A with LDL, cholesterol, Troponin and ET-1 mean that the lower the Fetuin-A the more biochemical disruption. These are associations and do not necessarily imply causality (Table 4).

Table 4. Correlations in Biomarkers

Variable pair	r	P	Interpretation
Fetuin-A vs LDL	−0.82	<0.001	Strong negative
Fetuin-A vs Troponin	−0.79	<0.001	Strong negative
Fetuin-A vs cholesterol	−0.75	<0.001	Strong negative
Fetuin-A vs ET-1	−0.68	<0.001	Moderate negative
Fetuin-A vs magnesium	+0.65	0.002	Moderate positive
ET-1 vs Troponin	+0.88	<0.001	Strong positive
ET-1 vs LOX-1	+0.74	<0.001	Strong positive
ET-1 vs LDL	+0.71	<0.001	Strong positive
Troponin vs CK	+0.94	<0.001	Very strong positive
Troponin vs potassium	−0.58	0.004	Moderate negative
Cholesterol vs triglycerides	+0.91	<0.001	Very strong positive
Cholesterol vs LDL	+0.89	<0.001	Strong positive
LDL vs VLDL	+0.62	0.005	Moderate positive
LOX-1 vs Troponin	+0.69	<0.001	Moderate positive
Magnesium vs potassium	+0.52	0.012	Moderate positive

3.1.6 **Diagnostic Performance:**

ROC analysis showed AUC=1.000 in all tested variables with sensitivity and specificity 100% at the indicated cutoffs . These findings suggest a perfect separation of patient and control distributions in the research population. The source correctly points out the possible usefulness of combining traditional indicators with novel biomarkers. (Table 5. Figure 6)

Table 5. Diagnostic analysis of all studied biomarkers:

Variable	Change	AUC	Cutoff	Sensitivity	Specificity
Fetuin-A	−53.3%	1.000	9.958 μg/mL	100%	100%
Endothelin-1	+164.3%	1.000	30.086 pg/mL	100%	100%
Lipoxygenase	+108.9%	1.000	50.158 ng/mL	100%	100%
Troponin	+5210.8%	1.000	19.820 ng/mL	100%	100%
Total cholesterol	+300.9%	1.000	243.000 mg/dL	100%	100%
Triglycerides	+143.2%	1.000	200.000 mg/dL	100%	100%
HDL-C	+224.0%	1.000	86.000 mg/dL	100%	100%

LDL-C	+340.5%	1.000	230.000 mg/dL	100%	100%
VLDL-C	+150.1%	1.000	44.900 mg/dL	100%	100%
Potassium	−65.3%	1.000	2.500 mmol/L	100%	100%
Magnesium	−50.4%	1.000	1.090 mmol/L	100%	100%

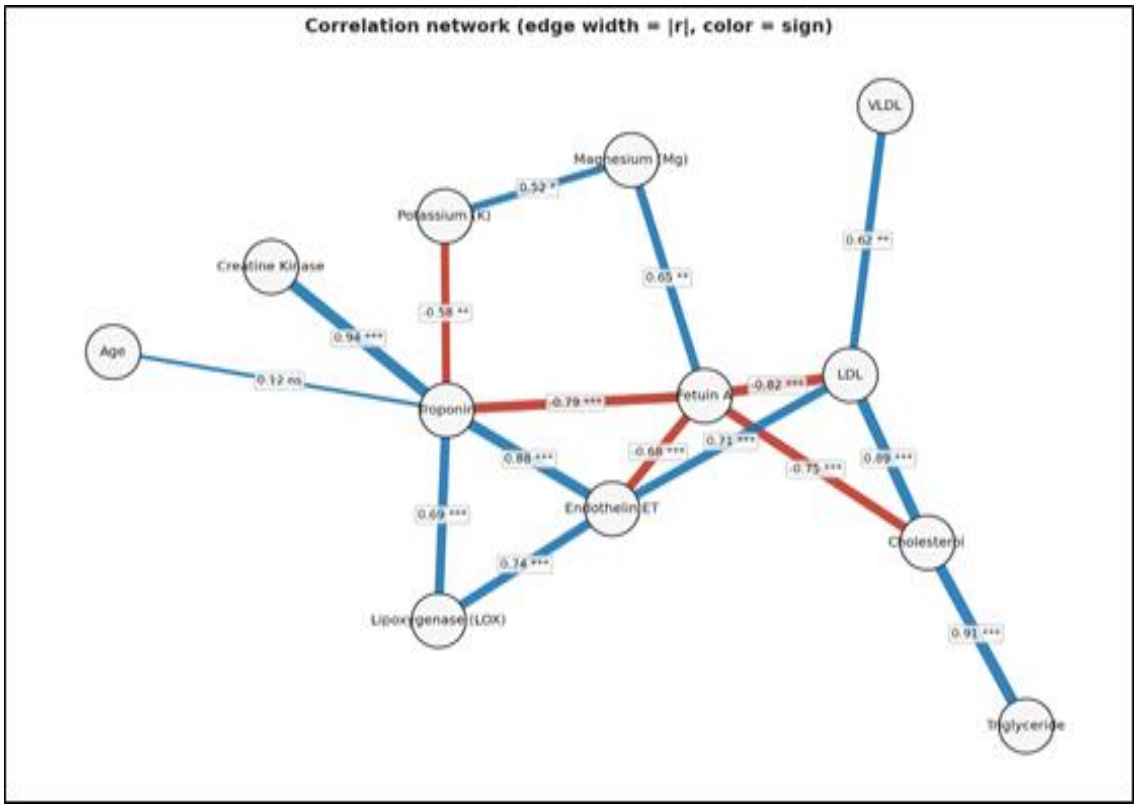

Figure (6): Relationship between cardiac and biochemical biomarkers in the patient group.

3.2 Purification of Serum Lipoxygenase-1:

The specific activity was raised step wise from 2.50 U/mg in crude extract to 4.19 U/mg following ammonium-sulfate precipitation, 6.222 U/mg after dialysis, 9.333 U/mg after ion exchange and 11.00 U/mg after gel filtration. The final yield was 68.10 % and the overall purification was 4.40 times. This pattern is interpreted as effective elimination of contaminating proteins with high residual enzyme activity (Table 6; Figure 7, 8).

Table 6. Purification profile of serum Lipoxygenase - 1:

Purification step	**Volume (mL)**	**Activity (IU/mL)**	**Total activity**	**Protein**	**Specific activity (U/mg)**	**Yield**	**Fold**
Crude extract	18	7.5	135.0	3.0	2.500	100.00%	1.00
Ammonium sulfate	15	8.8	132.0	2.1	4.190	97.78%	1.68
Dialysis	10	11.2	112.0	1.8	6.222	84.85%	2.49
DEAE-cellulose	6	14.0	84.0	1.5	9.333	75.00%	3.73
Gel filtration	4	14.3	57.2	1.3	11.000	68.10%	4.40

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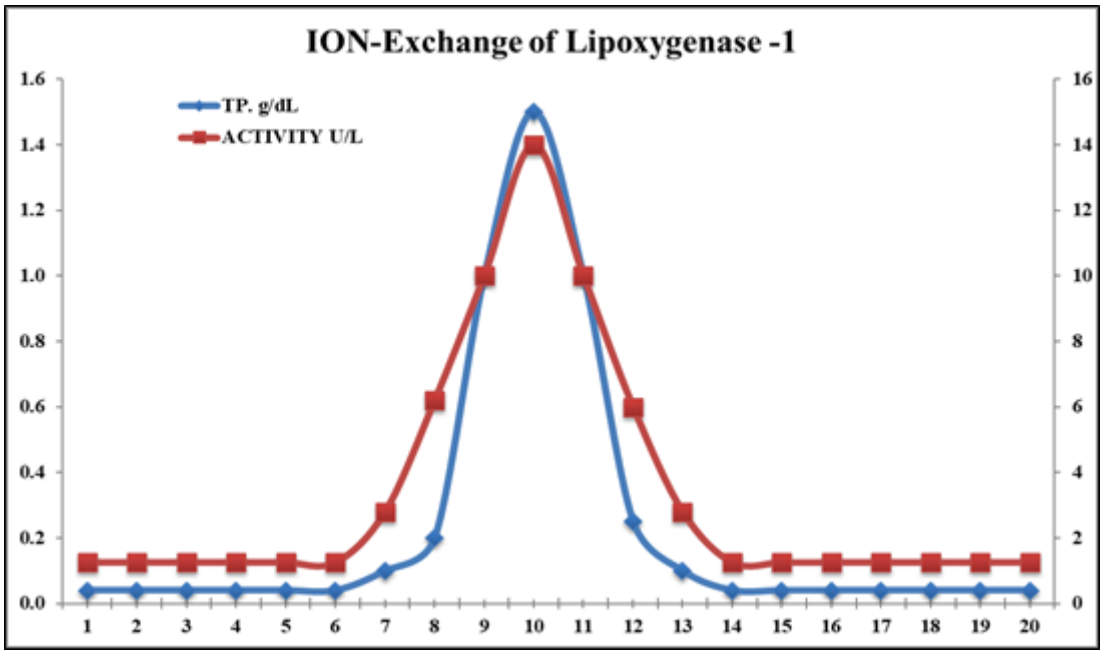

Figure (7): Purification of LOX-1 enzyme using ion-exchange chromatography. X-axis: Fraction number (1 to 21). Left Y-axis: Total protein concentration (TP, g/dL). Right Y-axis: Enzymatic activity (Activity, U/L).

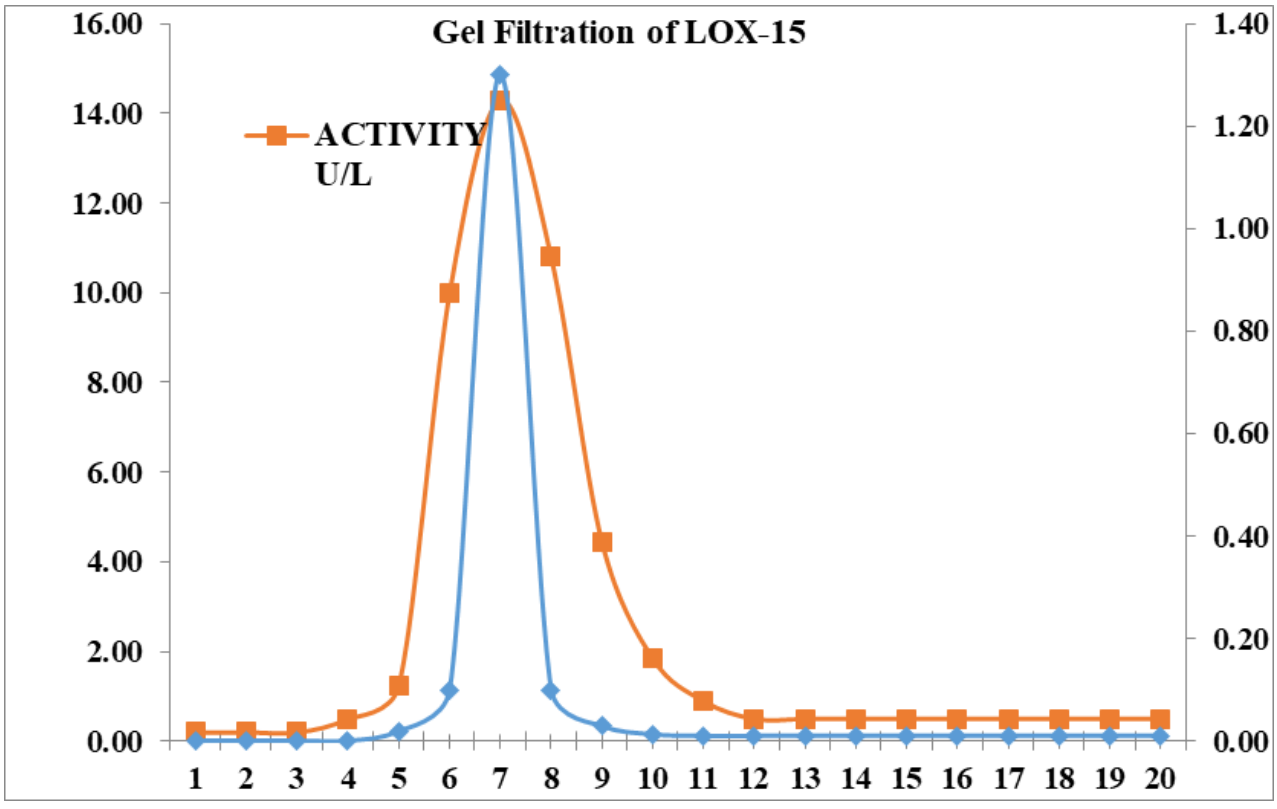

Figure (8): Purification of LOX-1 enzyme using gel filtration chromatography.** X-axis: Fraction Number; Left Y-axis: Enzyme activity (Activity U/L); Right Y-axis: Total protein concentration (TP, g/dL).

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3.3 Kinetic Parameters of Lipoxygenase – 1:

The isolated enzyme showed a hyperbolic dependence of reaction velocity on linoleic-acid concentration, with the highest substrate concentration tested (200 μM) nearly at the plateau of the reaction. The pattern is consistent with Michaelis-Menten kinetics, the source says. Lineweaver–Burk analysis was done and Vmax = 1.924 U/mg protein and Km = 71.45 were obtained and in the source they were considered as showing moderate affinity to linoleic acid under the experimental conditions.

Activity increased with increasing temperature from 5 °C through the lower range to an optimum of about 34 °C. Activity above 40 °C decreased with a sharp drop between 50 and 60 °C. The pH profile showed very low activity at pH 2 and 4, increased activity at pH 6, optimum activity at pH 8 and considerable inhibition at pH 10 and 12. The profiles are characterised by temperature-dependent structural stability and pH-dependent ionisation of active-site residues (Figure 10).

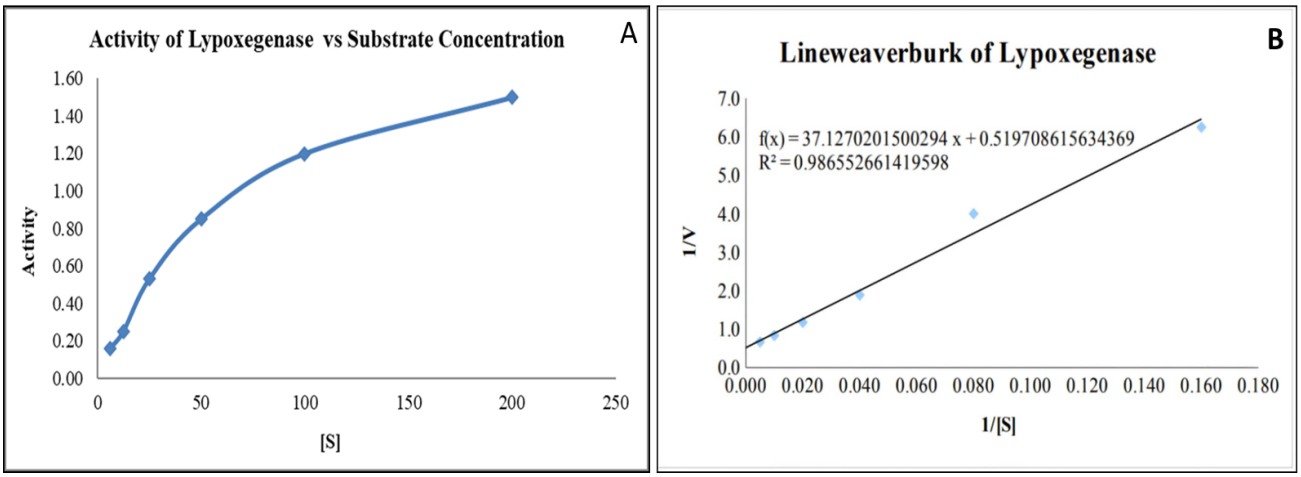


Figure (9): A) Effect of substrate (linoleic acid) concentration [S] on the activity of lipoxygenase purified from the serum of patients with myocardial infarction B) Lineweaver-Burk plot for the enzyme lipoxygenase to determine K_m and V_{max} values.**

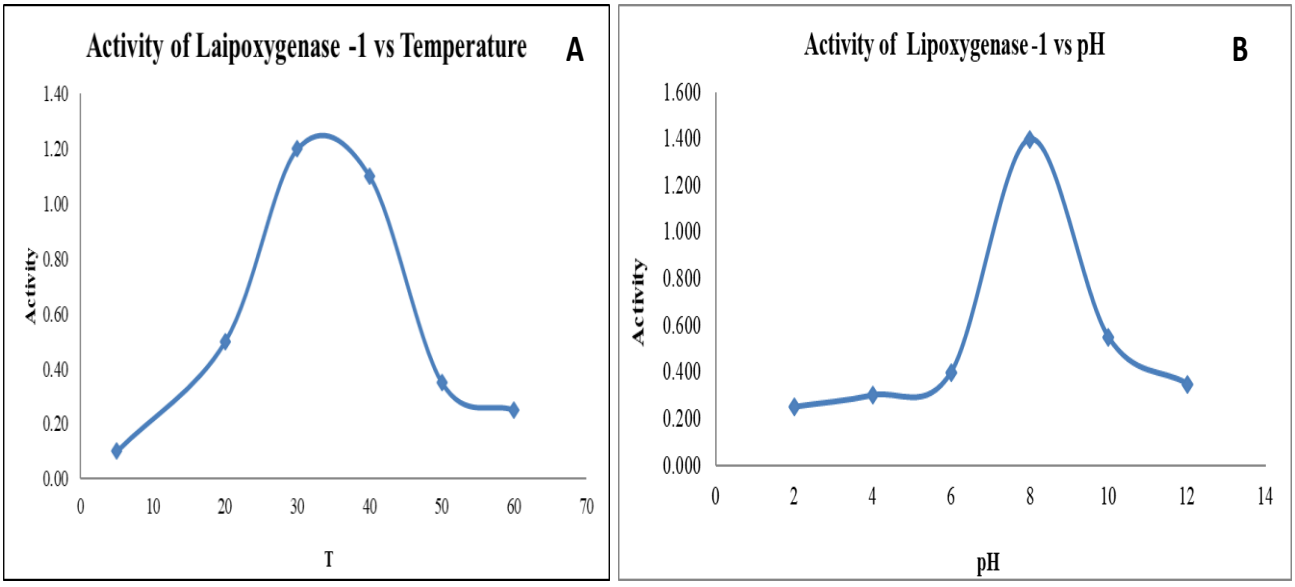
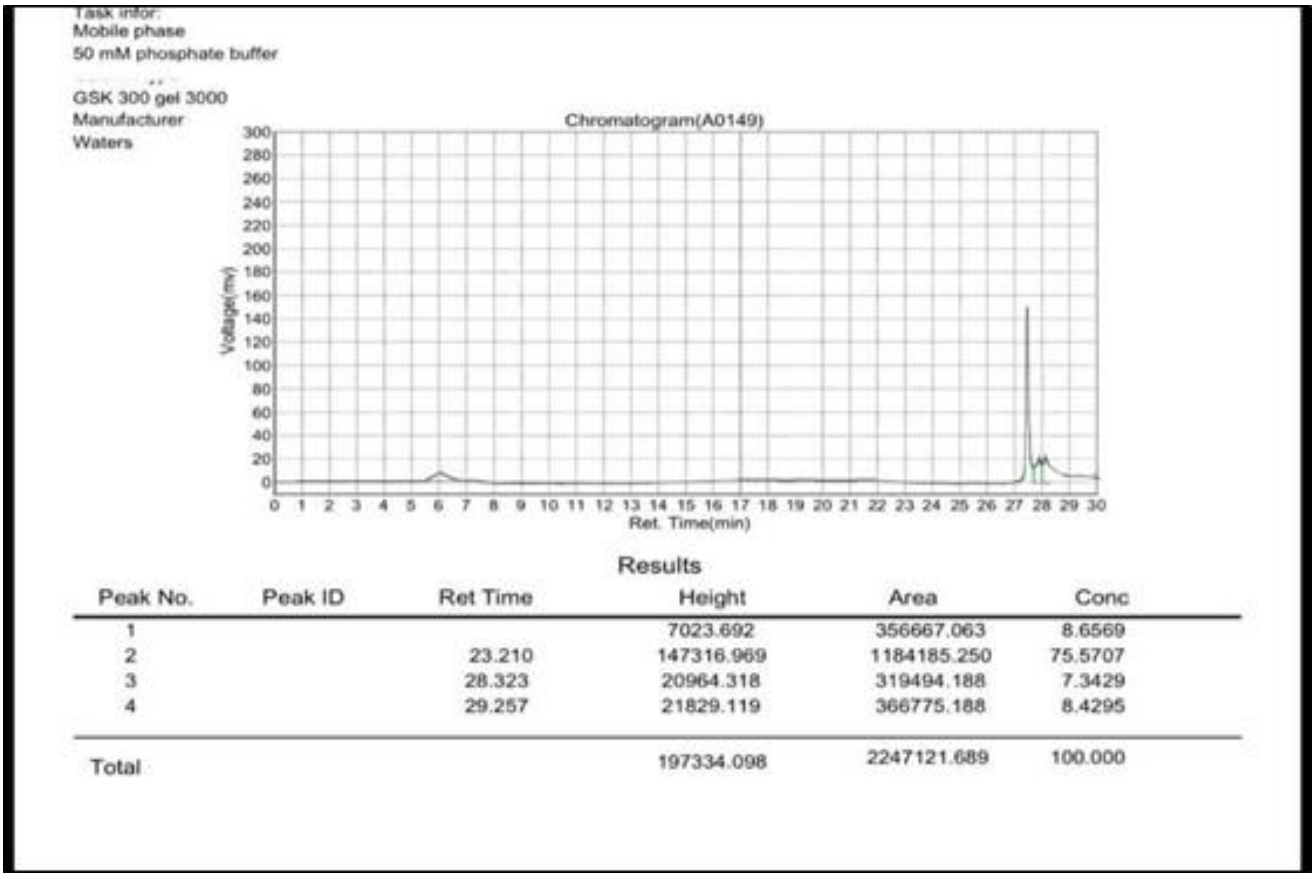


Figure 10: A) Effect of temperature on the activity of lipoxygenase purified from the serum of patients with myocardial infarction. B) Effect of pH on the activity of lipoxygenase purified from the serum of patients with myocardial infarction.

3.4 Molecular weight of purified Lipoxygenase – 1:

HPSEC-HPLC afforded four chromatographic peaks. The largest peak at 23.21 min represented 75.57% of the overall peak area. The apparent M.W. of its primary component was around 50.48 kDa. The other components were calculated to be 32.92

and 30.77 kDa. The calibration curve yielded a R 2 of 0.9933. The primary pure protein component is assumed to be the largest peak whereas the lesser peaks are potential impurities, breakdown products or alternative molecular assemblies (Figure 11).



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc
1			7023.692	356667.063	8.6569
2		23.210	147316.969	1184185.250	75.5707
3		28.323	20964.318	319494.188	7.3429
4		29.257	21829.119	366775.188	8.4295
Total			197334.098	2247121.689	100.000

Figure 12. High-performance liquid chromatography (HPLC) chromatogram of lipoxygenase-1 purified from the serum of patients with myocardial infarction.

4. DISCUSSION

4.1 Integrated Interpretation of the Biomarker Pattern:

The primary finding is a consistent change of all main investigated biomarkers in AMI. Troponin significantly increased suggesting acute myocardial damage. At the same time, ET-1 and LOX-1 were elevated while Fetuin-A decreased. This combination makes biological sense when considering the conceptual framework proposed in the source, where myocardial necrosis provides the classic signal of injury and endothelial dysfunction, oxidative stress, inflammation and altered lipid handling provide further information on the biological environment in which infarction takes place [11,12] .

The high positive correlation between ET-1 and Troponin (r = 0.88) suggests that in this group more damage to the myocardium was associated with higher endothelial vasoconstrictor activity [13, 14]. ET-1 was also correlated with LOX-1 (r = 0.74) and LDL (r = 0.71), linking the vascular dysfunction to the lipid-oxidative processes. LOX-1 associated to Troponin (r = 0.69) further supporting the association between LOX-related biology and acute myocardial damage. Activation of LOX in oxidative and inflammatory signalling after ischaemia or infraction.

Fetuin-A was inversely linked with LDL (r = −0.82), Troponin (r = −0.79), total-cholesterol (r = −0.75) and ET-1 (r = −0.68). Low Fetuin-A could be a marker for reduced protection against inflammation and vascular calcification (source). Since this is an observational research, these associations cannot confirms if reduced Fetuin-A is a cause of disease, a result of disease, or is driven by some other metabolic or clinical factor.

4.2 Lipid Disturbance and Lipoxygenase – 1:

The lipid results are consistent with the study's emphasis on LOX-1. Elevated LDL creates a substrate rich setting for oxidative modification and LOX-1 has been identified as a receptor mediating the recognition and uptake of oxidised LDL by macrophages and vascular cells [15, 16]. The close correlation of LDL-cholesterol and cholesterol-triglyceride values is consistent with a coordinated lipid disorder in the patient sample. The effect size for LDL-C is quite large, which highlights the significance of the difference between the groups in this dataset [17 – 20].

The upregulation of LOX-1 and the positive correlations with ET-1 and Troponin are consistent with the hypothesis that LOX-related pathways could be involved in the biochemical response to AMI. But the concentration of circulating LOX-1 is not always a direct reflection of the LOX enzymatic activity in the tissue or of a specific causative involvement in infarction. More molecular and functional studies are needed[19, 21- 23].

4.3 ET-1 and Endothelial Dysfunction:

ET-1 is a powerful endogenous vasoconstrictor and is involved in the source with hypertension, atherosclerosis and endothelial dysfunction. The remarkable rise in AMI patients is therefore commensurate with vascular stress in the setting of acute coronary illness [24]. This conclusion is further supported by subgroup analysis for hypertension, where ET-1 was considerably greater in hypertensive patients compared with normotensive individuals (huge Hedges' g of 1.28). This same group also showed increased levels of LOX-1 and Troponin and lower levels of Fetuin-A, indicating that hypertension may enhance numerous related biochemical processes [25- 27].

4.4 Electrolyte disturbances

The reductions in potassium and magnesium are of clinical significance as both these ions have a role in the electrical activity of the heart. Reference links low magnesium with higher arrhythmia risk. Says magnesium may fall early after infarction. Troponin showed a modest inverse relationship with potassium. Magnesium showed modest positive correlation with potassium. These data suggest an association of electrolyte abnormalities with the extent of biochemical myocardial damage and a possible interaction, although the direction of causality cannot be identified [28- 31]

4.5 Purification and Biochemical Characterisation of LOX-1

The purification sequence showed the predicted general trade-off as a function of purity and recovery. The specific activity was significantly increased with 97.78% of activity retained by precipitation with ammonium sulphate. Dialysis boosted specific activity but decreased the recovery. Further enhancement of specific activity was achieved using DEAE-cellulose ion-exchange and gel filtration that gave the final specific activity of 11.00 U/mg with 4.40-fold purification and 68.10% recovery. "DEAE-cellulose and gel filtration are particularly useful steps for separating proteins on the basis of charge and molecular size, respectively," the source adds. The obtained purification results showed similarity with previous findings of previously reported by Abdullah et al., (2023), Kamal and Hasan (2019) and Lateef et al., 2025) [32, 34]

The kinetic measurements give further evidence that appreciable catalytic activity was preserved by the isolated preparation. The hyperbolic substrate-response curve and Lineweaver-Burk analysis suggested Michaelis-Menten behaviour. Similar enzyme kinetics parameters were reported previously by Chrisnasari et al. (2022) [35] Whereas, An et al. (2021), [36] based on its kinetic parameters reported catalysis of Lipoxygenase with high affinity substrate unsaturated fatty acids. Maximum activity was seen under experimental settings of roughly 34 °C and pH 8, the optimal temperature and pH, respectivelyand this results were anticipated by the previously studied enzyme by An et al. (2021) [37- 38] . HPSEC-HPLC also detected a major component of around 50.48 kDa, although the existence of smaller peaks indicated that the preparation should still be deemed substantially, but not entirely, homogenous.

5. SUMMARY AND CONCLUSION:

This study shows a wide biochemical signature for acute myocardial infarction. Troponins were significantly raised showing significant damage to the heart muscle. LOX-1 and ET-1 were also elevated indicating correlations with endothelial dysfunction, oxidative stress, inflammation and altered lipid metabolism. Fetuin-A was significantly reduced and strongly inversely associated with LDL, cholesterol and Troponin, suggesting a potentially major association with the biochemical severity of AMI. Total cholesterol, triglycerides, LDL-C, HDL-C and VLDL-C were dramatically changed, potassium and magnesium were significantly reduced.

LOX-1 was partly purified from the serum of the patient by ammonium-sulfate precipitation, dialysis, DEAE-cellulose ion exchange and Sephadex G-100 gel filtration. The specific activity was enhanced from 2.50 to 11.00 U/mg, 4.40-fold purification of enzyme with 68.10% recovery. The purified product exhibited substrate-dependent Michaelis–Menten kinetics with reported Km of 71.45 and Vmax of 1.924 U/mg protein, optimal activity at around 34 °C and pH 8 and a major HPSEC-HPLC component estimated at 50.48 kDa.

Overall, the data support the hypothesis that LOX-1, Fetuin-A and ET-1 could contribute to the traditional cardiac biomarkers in the research of AMI-related pathophysiology. However, the study should be seen as an interesting exploratory study rather than conclusive proof for clinical biomarker application. Larger multi-centre investigations, standardised assays, longitudinal sampling and independent validation are necessary.

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