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Serum Homocysteine and Methionine as Complementary Biomarkers in the Early Detection of Myocardial Infarction

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Annotation: This paper tries to establish a concept about the role of homocysteine and methionine in early diagnosis of patients with myocardial infraction. this research aims to make a comparison between the concentration of homocysteine and methionine along with lipids spectrum. A case control study required 80 of recruitments (40 diagnosed with myocardial infraction as patients and 40 healthy people as control group). Homocysteine and methionine concentration were assessed by high performance liquid chromatography (HPLC) and lipid profile parameters were measured by Cobas C 111 analyzer. According to the result taken has found that myocardial infraction patients show highly significant amount of homocysteine, cholesterol, triglyceride and LDL, but low values of methionine and HDL values when compared to control group. In hence homocysteine could be present as a strong diagnostic biomarker to early diagnose MI cases, moreover these findings are just altered amino acid and lipid metabolism in patients with myocardial infraction. the integration of these biomarkers in specific homocysteine could be a suitable diagnostic marker and may improve early detections of MI. furthermore the ways of understanding the mechanism of methionine role in methylation pathway could open a new avenue for

therapeutic intervention targeting metabolic and vascular health and high-risk individuals.

Keywords: Homocysteine, Methionine, lipid spectrum, Myocardial infraction.

Introduction

Myocardial infarction (MI), or a heart attack, is still a major threat to human health and life. Ischemic heart disease with MI accounts for about 16% of the global mortality. Atherogenic lipid profile, inflammation, oxidative stress, and endothelial dysfunction are few etiologies of MI. Out of all the different sulfur amino acids, homocysteine and methionine gained popularity as their extreme relevance has been documented in cardiovascular physiology and pathology. Homocysteine is a methionine metabolic intermediate, and hyperhomocysteinemia, its elevation, is known to be associated with vascular damage, brought about by oxidative stress, endothelial injury and procoagulant status. [1,2]. In contrast, methionine, the precursor of homocysteine, is essential for methylation and cellular metabolism. Anomalous fluctuations in this cycle may be indicative of underlying cardiovascular disease.

Several recent reports have identified increased homocysteine as one of the independent risk factors for CAD including MI[3,4]. Likewise, changes in methionine levels may also be associated with metabolic perturbations and oxidative stress in heart [5]. However, there is little clinical data on the evaluation of the two biomarkers in combination in MI.

Our objective was to determine and compare serum interferential levels of homocysteine and methionine in MI cases as well as in healthy controls. The database consists of 80 people (40 MI patients and 40 healthy controls) where even cholesterol, TG, LDL and HDL cholesterol etc were estimated to study the risk of cardiovascular disease profile. A preliminary analysis showed that homocysteine concentrations were significantly increased in MI than in control subjects (mean ± SD: 12.71 2.03 μmol/L and 8.43 ± 1.21 μmol/L) in controls, respectively) and that methionine levels were slightly lower in the disease. These results add to an increasing body of literature supporting a role of homocysteine and methionine as candidate biomarkers in the risk stratification for cardiovascular disease, and may help further to improve prediction models that are useful in early diagnosis and prevention of myocardial infarction.

Materials and Methods

A case-control study was carried out in Iraq – Baghdad from 14th of April to the 7th of September 2024. Patient samples were obtained from Sheikh Zayed and Ibn Al-Nafis Hospital in Iraq. serum levels of methionine and homocysteine, along with lipid profile used to detect early diagnose in cases with myocardial infarction (MI). about 80 participants were included, of which 40 with MI and 40 were recruited as healthy controls.. The diagnosis of patients was made by cardiologists based on clinical history, ECG findings, and biochemical markers.

A 5ml syringe and torniqute were used to collect blood from all the subjects into geltubes. samples were centrifuged at 3000 rpm for 10 minutes to obtain serum and stored at −20°C until tests procedures done.

Serum lipid profile of total cholesterol (T-CHO), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined by the Roche – Cobas c111 auto analyzer (Germany Analyzer) machine using enzymatic colorimetric method based on the manufacturer's manual.

For detections the levels of methionine and homocysteine, A High-Performance Liquid Chromatography (HPLC) were used. Deproteinization and reverse-phase C18 column treatment were done for the samples, with UV detection. Methionine was detected at 214 nm and

homocysteine, following derivatization, at 385 nm. Accuracy was done through standard calibration curves.

All the information were statistically managed using SPSS 27, a t sample test carried out by chi sequare method with pearson correlation, ROC estimated for determining the sensetivity and specificity of tests at which disease diagnosis was carried out, all information are an indication of set significance at $p < 0.05$.

Other quality control practices were implemented at all levels of biochemical testing, including the use of internal standards and replicate assays to confirm the accuracy of assay. Hemolyzed samples were rejected to prevent interference in tests. Demographic and clinical information like age, gender, history of smoking, and co-existing diseases were documented by standardized questionnaires. Institutional review board approval of ethics was obtained, and patient confidentiality was maintained during research. This method ensured reliability, reproducibility, and clinical relevance of the findings.

Results and Discussion

Sex according to groups

A Chi-square test revealed no significant association between gender and group (MI vs. Control), $\chi2(1) = 0.25$, $p = 0.617$. The study included 58 males and 22 females. Males slightly predominated in both the control (28) and disease (30) groups.

Table 1: Distribution of disease according to Sex

		Groups				
		Disease	**Control**	Total	p value	χ^2
Sex	**Male**	30	28	58	0.617	0.25
	Female	10	12	22		
	Total	40	40	80		

Parameters according to Sex

Gender-based comparison indicated significant differences in some biochemical indices. On average, the males (mean ± SD) had higher levels for homocysteine (10.83±2.89 µmol/L) and methionine (0.19±0.07 µmol/L) compared to their female counterparts (9.88±2.12 µmol/L and 0.17±0.04 µmol/L respectively). In spite of the distribution of cholesterol and LDL levels being approximately the same between sex, the average HDL value was ever so slightly higher in female (45.18±8.17 mg/dL) than male (42.29±8.04 mg/dL) subjects, which is consistent with literature that reports the sex-related pattern of lipid metabolism.

Moreover, females showed higher levels of triglycerides (185.56±21.47 mg/dL vs 173.68±30.34 mg/dL), which could be influenced by hormones or diet. These findings emphasize gender as a possible modulator of cardiovascular risk and the inclusion of sex evaluations in the genotyping panel used in the clinic. While no statistical comparisons are made for these subgroup analyses, the trends we observed are comparable to data from larger epidemiological studies which indicate that male patients have a higher likelihood of having raised homocysteine and LDL, the former of which is associated with atherogenesis and endothelial dysfunction.

These sex-specific patterns may potentially justify sex-based analysis strategies for personalized cardiovascular risk assessment and can serve as foundation for future studies on mechanistic explanations in regard to the sexual dimorphism of both homocysteine and lipid metabolism [6].

Table 2: Studied Parameters according to Sex

Tests	***Sex***	N	Min	Max	Mean ± Std.
Methionine	**Male**	58	0.05	0.37	0.19 ± 0.07
	Female	22	0.14	0.28	0.17 ± 0.04

Homocysteine	**Male**	58	6.2	15.3	10.83 ± 2.89
	Female	22	7.4	14.8	9.88 ± 2.12
Cholesterol	**Male**	58	132	270	182.69 ± 33.81
	Female	22	164.5	224.4	184.77 ± 19.13
TG	**Male**	58	110.3	235	173.68 ± 30.34
	Female	22	158.9	215.3	185.56 ± 21.47
HDL	**Male**	58	24.7	52.4	42.29 ± 8.04
	Female	22	32.3	54.3	45.18 ± 8.17
LDL	**Male**	58	47.6	198.3	106.89 ± 36.22
	Female	22	78.14	151.1	103.58 ± 21.28

Correlations between parameters

Homocysteine is positively related to LDL (r = 0.54) and cholesterol (r = 0.52). Methionine demonstrates low inverse correlation with homocysteine, confirming the role of disrupted metabolism of homocysteine in MI and HDL shows an inverse relation with all atherogenic factors.

These results are consistent with the concept of homocysteine-driven dyslipidemia in MI development [7]. This correlative physiology suggests a synergistic association between HCY and several lipid disturbances, particularly LDL and cholesterol, extending the notion of atherogenic risk being multifactorial. The inverse relationship found between HDL with homocysteine and LDL underscores even more its protective function. These interconnections are indicative of the metabolic perturbation in MI, and are consistent with emerging findings linking homocysteine to oxidative stress, endothelial dysfunction, and lipid peroxidation [8,9]. From such findings here would appear to be potential therapeutic value in the targeting of homocysteine to mediate both a thrombogenicity as well as a lipid-related atherogenesis. [7]

Table 3: Pearson correlation of the Parameters

Parameters	***pearson***	***Methionine***	***Hemosystine***	***Cholesterol***	***HDL***	***TG***	***LDL***
Methionine	r	1	-0.21	-0.17	0.23	-0.19	-0.21
	p		0.063	0.129	0.042	0.093	0.062
Homocysteine	r	-0.21	1	0.52	-0.6	0.38	0.54
	p	0.063		<.001	<.001	<.001	<.001
Cholesterol	r	-0.17	0.52	1	-0.78	0.61	0.93
	p	0.129	<.001		<.001	<.001	<.001
HDL	r	0.23	-0.6	-0.78	1	-0.54	-0.81
	p	0.042	<.001	<.001		<.001	<.001
TG	r	-0.19	0.38	0.61	-0.54	1	0.45
	p	0.093	<.001	<.001	<.001		<.001
LDL	r	-0.21	0.54	0.93	-0.81	0.45	1
	p	0.062	<.001	<.001	<.001	<.001	

To compare the serum levels of methionine, homocysteine, and lipid profile parameters between myocardial infarction (MI) patients and normal controls, t-test test was used in this study. A p-value < 0.05 suggests the differences observed are not likely to be chance.

Crosses beside bar indicate the mean values that are significantly different with the data ($P < 0.05$). Serum homocysteine was similarly much elevated in the disease group (12.71 ± 2.03 vs 8.43 ± 1.21 µmol/L in controls, $p < 0.001$). This lends support to the hypothesis that plasma tHcy is an intense risk factor for cardiovascular events, based on its prothrombotic and endothelium-damaging effects [10,11].

Of note, methionine levels were significantly lower in MI patients (0.17 ± 0.05 µmol/L) compared to the controls (0.20 ± 0.07 µmol/, suggesting an increased catabolism or defected methylation cycles post-MI. Lipid profile specifically cholesterol, triglycerides (TG), low density lipoprotein (LDL) were highly increased among MI patients, on the other hand HDL was significantly decreased. These results are in accordance with the well-described dyslipidemia profile in the coronary artery disease. [12]

The strong statistical significance in all parameters further supports the clinical importance of these biomarkers for distinguishing MI patients from healthy individuals and potentially for therapeutic monitoring.

Table 4: Parameters comparison among the studied groups

Tests	*Groups*	*N*	*Min*	*Max*	*Mean ± Std.*	*p - value*
Methionine	Disease	40	0.05	0.25	0.17 ± 0.05	0.006
	Control	40	0.05	0.37	0.2 ± 0.07	
Homocysteine	Disease	40	8.4	15.3	12.71 ± 2.03	< 0.001
	Control	40	6.2	10.2	8.43 ± 1.21	
Cholesterol	Disease	40	154	270	203.12 ± 30.15	< 0.001
	Control	40	132	184	163.41 ± 12.3	
TG	Disease	40	110.3	235	190.78 ± 30.98	< 0.001
	Control	40	125.6	187.6	163.13 ± 17.32	
HDL	Disease	40	24.7	47	36.38 ± 5.98	< 0.001
	Control	40	46.5	54.3	49.8 ± 2.41	
LDL	Disease	40	83	198.3	127.38 ± 32.14	< 0.001
	Control	40	47.6	115.5	84.58 ± 13.89	

Homocysteine among the studied biomarkers had the best AUC (Area under the curve) (0.953) for discrimination of myocardial infarction (MI) patients and controls. This is consistent with the literature in which high levels of homocysteine are thought to be independent risk factors for cardiovascular events [13, 2].

HDL showed the strongest negative association (AUC= 0.995, close to AUC = 1) and hence HDL emerged as the most potent negative marker potentially because of its inverse correlation with atherogenesis. The evidences show that other lipid markers including LDL (AUC = 0.902) and cholesterol (AUC = 0.872) also have high predictive value of MI and play a role in MI risk assessment.

Methionine (AUC = 0.677) showed promising results with high sensitivity (90%) but low specificity (40%) and has some potential to be useful early screening marker. This suggests it may be useful when combined with more disease-specific markers such as homocysteine.

The results show a combined biomarker panel that can be used for the rapid, early and reliable assessment of MI [14, 15]. MI= Myocardial Infarction, AUC= Area Under the CurveHDL- High Density LipoproteinHDL was nearly perfect for discrimination (AUC = 0.995) and MI well above 0.5 (AUC = 0.953), establishing HDl to be a robust biomarker for MI. Methionine performed with a reasonably good AUC but exhibited high sensitivity. The paper, reflect the synergistic contribution of biosensors in clinical MI screening [14].

Table 5: ROC curve of Parameters to detect the sensitivity and specificity by groups

Parameter	*Groups*	*Area*	*Sensitivity*	*Specificity*	*Cutoff value*	*P – value*
Methionine	**Disease vs Control**	0.677	90%	40%	<0.229	0.0063
Homocysteine	**Disease vs Control**	0.953	85%	100%	>10.3	< 0.0001
Cholesterol	**Disease vs Control**	0.872	80%	100%	>195.7	< 0.0001
TG	**Disease vs Control**	0.877	75%	95%	>161.6	< 0.0001
HDL	**Disease vs Control**	0.995	95%	100%	<45	< 0.0001
LDL	**Disease vs Control**	0.902	80%	90%	>108	< 0.0001

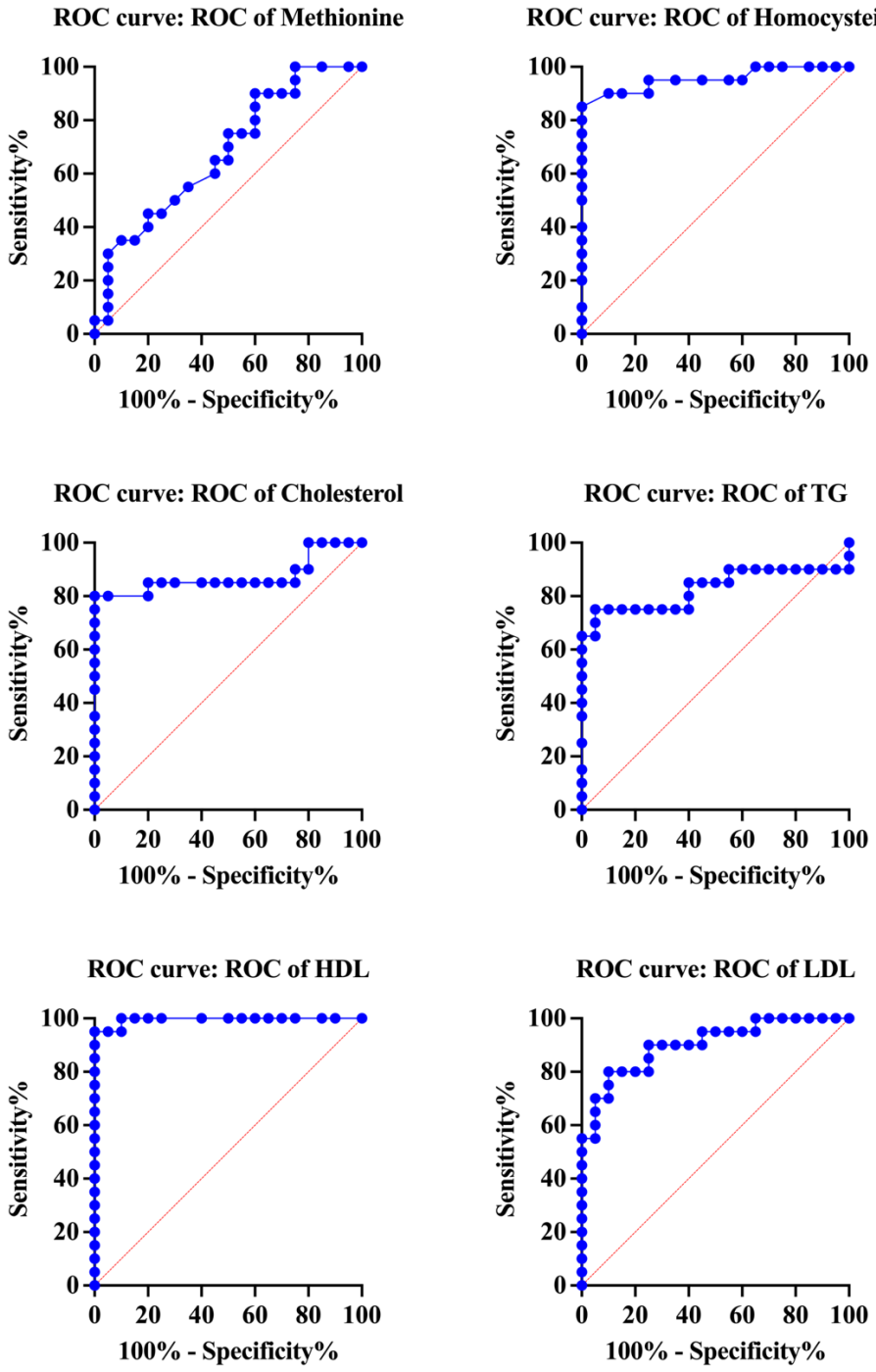

Figure 1: ROC curve of parameters

Conclusion

Serum homocysteine was found to be significantly elevated in patients with myocardial infarction, and that methionine levels were lower in comparison to healthy controls, two major findings in the study. These findings indicate a disturbed methylation and oxidative stress pathway in MI pathology. Dyslipidemia and lower HDL are in line with atherogenic profiles affecting cardiovascular risk. Among them, homocysteine displayed an excellent performance and may be considered a promising biomarker for clinical risk factors. Methionine, though less specific, could still shed light into disturbance in the underlying metabolism. Such knowledge could improve (early) diagnosis and individualized treatment in these MI-prone patients.

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