

# AMINOGRAM IN EGYPTIAN PATIENTS EXPERIENCING MYOCARDIAL INFARCTION

## Abstract

**Background:** Myocardial infarction is one of the leading causes of death worldwide. Altered amino acids metabolism is linked to cardiovascular disorders. A cross-sectional case control study was set to profile amino acids explore their potential as diagnostic and prognostic tool.

**Methods:** Total of 60 subjects was recruited, comprising 40 myocardial infarction patients and 20 controls. High pressure liquid chromatography was applied to quantify the plasma amino acids.

**Results:** Fourteen amino acids exhibited discriminative values between cases and controls. Leucine, isoleucine, methionine, glycine, threonine, serine, ornithine, arginine, histidine, and tyrosine exhibited low levels compared to healthy controls ( $p \leq 0.001$ ). Meanwhile, cases displayed higher abundance of cysteine, taurine ( $p \leq 0.05$ ), total aromatic amino acids ( $p \leq 0.01$ ), and asparagine ( $p \leq 0.001$ ). Only leucine, isoleucine, and asparagine were correlated to GRACE risk score. Methionine and glycine scored highly (AUC > 0.95), sensitivity (>97%), and specificity (>95%) followed by histidine (AUC > 0.92), sensitivity (>82%), and specificity (>85%).

**Conclusion:** Our study designated a panel of 14 plasma circulating amino acids. Methionine, glycine and histidine were found to be potential markers to differentiate cases from healthy Individuals. Plasma amino acids profiling could help in the diagnosis but is less powerful as a prognostic tool of Myocardial infarction.

**Key words:** Amino acids, myocardial infarction, cardiovascular disorders, liquid chromatography.

## Introduction

Acute myocardial infarction (AMI) is one of major causes of mortality and morbidity worldwide [1]. Every year, about 10% of patients who are present with chest pain are diagnosed with heart attack [2]. Mortality due to AMI was significantly reduced through corrections of risk factors and intensive medical management [3].

AMI is a syndrome which is attributed to ischemic heart disease that occurs when the coronary artery is totally and partially occluded when an atherosclerotic plaque ruptures and develops a thrombus, which decreases blood flow to the heart [4, 5]. The near associations between cardiovascular disorders (CVD), and atherosclerosis has been known for decades. Underestimating the contribution made by other underlying inflammatory condition and/or nutrients, such as amino acids(AAs) are under research.

Amino acids are important metabolites of nutrients and primary precursors to the synthesis of several endogenous products of significant biological interest, necessary for cell survival and growth [6, 7]. AAs have recently been linked to cardiovascular diseases due to their essential role in CVD pathogenesis [8]. Macrophages play a vital function in the development of atherosclerosis. The macrophage atherogenicity is substantially impaired by glycine, cysteine, leucine, and glutamine primarily by the regulation of triglyceride metabolism [9]. Branched chain amino acids (BCAAs) including leucine, isoleucine, and valine, serve as substrates for protein synthesis or energy production and perform several metabolic and signaling functions, particularly via activation of the mammalian target of rapamycin (mTOR) signaling pathway [10]. BCAAs have shown positive associations' with cardiovascular risk, hypertension, metabolic dysregulation, and insulin resistance, platelet functions [11, 12]. Moreover, there are positive and negative atherogenic roles of BCAAs.

Certain BCAAs, in particular Leu, are well-known for their extenuative effects on macrophage lipid accumulation and subsequent formation of foam cells in blood vessel walls while simultaneously enhancing mitochondrial respiration, this role is related to the decrease in cholesterol and triglycerides macrophage content.

The general protective properties of glycine, arginine, histidine, methionine and taurine allow them to play a role in protecting the heart from ischemic events [13-15]. Considerable literature has been published in studies of humans and animals suggesting that Arg may lower blood pressure reduce blood clots and strokes, decrease cholesterol and triglycerides and enhance diabetes and sexual functions as a precursor of NO extracted from endothelium [16]. Glycine also alters the bioavailability of NO as well as synthesis of pro-inflammatory cytokines and has anti-atherogenic effects [17, 18]. Methionine bioavailability influences the synthesis of lipids. Met deficiency contributes to a decrease of GNMT which leads to attenuated liver lipid absorption and thereby raises blood lipid levels, lipid aggregation in macrophages and arterial walls contributing to inflammation of the blood vessels, LDL oxidation which is a strong risk factor for atherosclerosis and venous thrombosis [13, 19, 20]. Taurine, by virtue of its antioxidant activity, has been shown to play a crucial role as a cytoprotectant and in the attenuation of apoptosis. There is a growing consensus that oxidative stress is linked to mitochondrial dysfunction and that the beneficial effects of Tau are a result of its antioxidant properties as well as its ability to improve mitochondrial function by stabilizing the electron transport chain and inhibiting the generation of reactive oxygen species (ROS) [21, 11].

Histidine has a strong antioxidant effect and anti-inflammatory. The antioxidant activity of His is mediated by metal ion chelation, the scavenging of ROS and nitrogen species and sequestering advanced lipoxidation end products. Anti-inflammatory action prevents first steps of atherogenesis which can help in preventing development of foam cells [22, 23].

Metabolic profiling using one or group of AAs has been identified as novel biomarkers and metabolic signatures which are associated with incident cardiovascular disorders [9]. eventually this study was set to evaluate amino acids profile in MI patients compared to controls, investigate its

correlation with lipid profile as well as cardiac markers and cardiac outcome, and finally to explicate the diagnostic usage of AAs profile AMI patients.

### **Methods:**

The present study is a cross sectional study with a total of 60 subjects their ages ranged between 30-80 years. Forty diagnosed as myocardial infarcted patients (35 males and 5 females), 14 out of them were candidate for off pump CABG, and 20 healthy individuals acted in the study as controls (15 males and 5 females). Patients were selected randomly from Al-Orman Cardiology Hospital, Assiut University. The study was approved by ethical committee of the faculty of Medicine, Assiut University, IRB no (17101131) and informed consent was obtained from each participant. Full medical history and routine examination were employed to the participants via trained medical expertise. For each patient ECG, Echocardiogram (Echo) and cardiac catheterization were done to confirm the diagnosis in addition to the cardiac markers. In addition, GRACE score was calculated, using age, heart rate, systolic blood pressure and creatinine <https://www.mdcalc.com/grace-acs-risk-mortality-calculator> and KILIP scores were collected from patients' records. Individuals with cancer, autoimmune, neurological endocrine disorders were excluded from the study in addition who were pregnant or lactating.

### **Sample collection:**

After 12 hours overnight fasting, 5 ml of venous blood samples were collected from patients and controls. A total of 3 mL of blood was collected in a heparinized tube, mixed, and centrifuged at 3000 rpm for 15 min, and plasma was separated and stored at  $-80^{\circ}\text{C}$  for analyses of AAs profile. two milliliters was collected in a tube containing potassium ethylene diamine tetra acetic acid (EDTA) centrifuged at a high-speed run (3000 rpm) for 10 min plasma were separated and stored at  $-20^{\circ}\text{C}$  for lipid profile assessment.

### **Laboratory measures**

Profile of plasma AAs were performed by acid protein precipitation using a Sykam Automatic Amino Acid Analyzer S433 provided by Sykam GmbH (Germany, Gilching, catalog number:

1120001). Free AA were prepared from plasma, where the 200- $\mu$ L sulfosalicylic acid solution (10%) was added to 800  $\mu$ L plasma in a micro-centrifuge tube and vortexed and kept  $-4^{\circ}\text{C}$  for 30 min, followed by 10 min centrifugation at 14000 rpm. A supernatant was collected and diluted with the same volume of ready-to-use sample dilution buffer (catalog number: S000015). Both the samples prepared (100  $\mu$ L) and ready-to-use AA functional standard (catalog number: 6006005) were either directly injected or kept at  $-20^{\circ}\text{C}$  for a few days until the time of analysis. Sykam Automatic Amino Acid Analyzer S433 is equipped by Cation separation column: LCA K07/LI, Size: 150 mm x 4.6 mm, Catalogue No. 5112008. Amino acid separation depends on pre-column ninhydrin (catalog number: S000025) derivatization followed by passage in 3 different buffers with pH range from 2.9-8 and final regeneration solution contained lithium hydroxide and EDTA, normality 0.5. The detection method used was UV detection with an absorption wavelength of 570 nm. The reaction was preceded by instrument calibration and standard curve setting using and control amino acid plasma. The specimen chromatogram was matched to the standard assays visually [Figure 1](#). The concentrations of AAs were obtained using the embedded software and multiplied by 2.5 dilution factor.

The serum levels of triglyceride, total cholesterol, low-density lipoprotein cholesterol, and high density lipoprotein cholesterol were measured using spectrophotometric assay kits (catalog number: TR 20 30, CH 12 20, CH 12 31, CH 12 30) supplied by Biodiagnostic, Zokki, Giza, Egypt, following the manufacturer's instructions.

Plasma creatine kinase-MB (CK-MB), plasma total creatine kinase (CK), plasma lactate dehydrogenase (LDH) and plasma cardiac troponin (cTn) were performed in Al-Orman Cardiology Hospital laboratory using TOSOH AIA-360 and Dimension RxL Max integrated chemistry systems.

### **Statistical analyses**

The statistical analyses were performed using SPSS (version 20, SPSS Inc., Chicago, IL, USA) for windows software. The variables were evaluated for normal distribution using the Shapiro–Wilk W-test. Data were expressed as mean  $\pm$  standard deviation or median (interquartile) for numerical data and number and percentage for categorical data. Student's t-test and Mann–Whitney U test were applied for parametric and non-parametric data respectively. Chi

square test was done to test categorical variables. One-way analysis of variance (ANOVA) with Tukey post hoc was performed for data sets of 3 groups or more. Pearson's correlations were performed to examine the associations between the different parameters. Sensitivity, specificity, the cut-off value, and area under the ROC curve were provided only for AAs that show statistically significant difference between case and controls at  $p$  value  $< 0.05$ . An AUC  $> 0.7$  suggested a discriminating statistical significance capacity, and an AUC  $> 0.8$  indicated the test's excellent discriminating power. A  $p$ -value of  $< 0.05$  was considered statistically significant.

## Results

The mean age of control group was  $42.1 \pm 12.19$  years and mean age of patients' group was  $58.25 \pm 11.97$  years, mean weight, height and BMI of controls and patients were  $82.8 \pm 10.72$ ,  $1.69 \pm 0.04$ ,  $29.02 \pm 3.7$  and  $92.53 \pm 8.71$ ,  $1.68 \pm 0.05$ ,  $32.9 \pm 3.53$  respectively. 30% of controls and 82.5% of cases were obese, 25% of controls and 47.5% of patients were smokers, and 82.5% of patients were dyslipidemic [Table 1](#).

Data showed significant increase in lipid profile of patients compared to controls; LDL and total cholesterol ( $P < 0.001$ ) and significant decrease in HDL of patients ( $P = 0.001$ ), while triglycerides showed no significant difference ( $p = 0.532$ ). Moreover, data showed also mean of plasma cardiac troponin, creatine kinase isozyme, lactate dehydrogenase and total creatine kinase were  $27.02 \pm 27.51$ ,  $234.55 \pm 213.31$ ,  $518.65 \pm 189.93$  and  $1442.68 \pm 991.00$  respectively. GRACE score there were 47.5% of patients had low risk in hospital mortality, 32.5% had intermediate in hospital mortality risk and 20% had high in hospital mortality risk.

Amino acids concentration in controls and patients were shown in [Table \(2\)](#). Branched chain amino acids (BCAAs); valine (Val), isoleucine (Ile) and leucine (Leu) showed variation between all patients and controls, Val showed no significant difference ( $p = 0.247$ ) while Ile, leu and BCAAs\* (sum of Ile and Leu) showed a significant decrease in all patients compared to controls ( $p < 0.001$ ), ( $p = 0.001$ ) and ( $p < 0.001$ ) respectively. In all patients compared to controls ( $p < 0.001$ ), ( $p = 0.001$ ) and ( $p < 0.001$ ) respectively. Sulphur containing AAs including methionine (Met), cysteine (Cys) and taurine (Tau) showed variation between all patients and controls, Met showed a significant decrease

in all patients compared to controls ( $p < 0.001$ ), meanwhile, MI cases displayed higher abundance of cysteine, taurine ( $p \leq 0.05$ ).

Alanine (Ala) showed no significant difference between the two groups ( $p = 0.335$ ) while, glycine (Gly), threonine (Thr), serine (Ser) and Ornithine (Orn) showed significant decrease in all patients compared with controls ( $p < 0.001$ ). Lysine (Lys) showed no significant difference between controls and patients ( $p = 0.094$ ) while, arginine (Arg) and histidine (His) showed significant decrease in all patients compared to controls ( $p = 0.002$ ) and ( $p < 0.001$ ) respectively.

Aromatic amino acids (AAA); tyrosine (Tyr) showed a significant decrease in all patients compared with controls ( $p < 0.001$ ) while phenylalanine (Phe) showed no significant difference between the two groups ( $p = 0.291$ ). Meanwhile, MI cases displayed higher abundance of asparagine ( $p \leq 0.001$ ).

The current correlation results of patients were presented in [Table \(3\)](#). There were significant negative correlations between ; LDL-cholesterol and each of Ile, Leu, Met, Cys, Gly, Orn, His and Tau amino acids; CK MB and each of Ile, Leu, Met, Tau, Cys, Arg and His amino acids; Troponin and each of Ile, Leu, Met, Tau, Cys, Arg, Tyr and Gly amino acids, while, there were significant positive correlations between HDL and each of Ile, Leu, Met, Orn, His, Asn, Arg and Cys amino acids.

According the current study, GRACE scores were presented in [Table \(4\)](#) and there weren't significant differences between low, intermediate and high GRACE score groups in AAs levels except for Val and Asn AAs.

[Figure \(2A\) and Table \(5\)](#) showed the ROC for Ile, Leu and BCAAs\*. The AUC for Leu, Ile, and BCAAs\* were 0.843, 0.756 and 0.794, respectively. The sensitivity, specificity, and cutoff point were at 72.5%, 85%, and 80.25  $\mu\text{mol/L}$ ; 72.5%, 70%, and 144.13  $\mu\text{mol/L}$ ; and 75%, 80%, and 228.13  $\mu\text{mol/L}$ , respectively. [Figure 2B and Table \(5\)](#) showed the ROC for Gly, Met, His and Arg. The AUC for Gly, Met, His and Arg were 0.950, 1.00 and 0.925 respectively. The sensitivity, specificity, and cutoff point were at 97.5%, 95%, and 202.04  $\mu\text{mol/L}$ ; 97.5%, 100.0%, and 25.6  $\mu\text{mol/L}$ ; 82.5%, 85.0%, and 75.76  $\mu\text{mol/L}$ ; 70.0%, 80.0%, and 96.44  $\mu\text{mol/L}$  respectively.

## Discussion

Myocardial infarction is the leading cause of morbidity and mortality worldwide. Developing new insights into the pathogenesis, risk stratification and therapeutics is now beyond the available biomarkers. Ischemic heart diseases are characterized by extensive metabolic modifications. Profiling plasma AAs metabolites may improve cardiovascular risk prediction over established risk factors. This study investigated an array of 26 AAs and identified a panel of them that discriminate MI from healthy individuals. Of these, BCAAs (leu & Ile), methionine, glycine, threonine, serine, ornithine, arginine, histidine, and tyrosine exhibited low levels in MI cases compared to healthy controls ( $p \leq 0.001$ ). Meanwhile, MI cases displayed higher abundance of cysteine, taurine ( $p \leq 0.05$ ), total aromatic AAs ( $p \leq 0.01$ ), and asparagine ( $p \leq 0.001$ ). BCAAs (leu, Ile), methionine, glycine, arginine and histidine exhibited diagnostic potential.

Branched chain amino acids are the second major source of energy for the myocardium. Their metabolism by branched-chain  $\alpha$ -keto acid dehydrogenase (BCKDH) provides acetyl-CoA and succinyl-CoA for the respiratory tricarboxylic acid (TCA) cycle. The present results showed that BCAAs (Leu & Ile) were significantly lowered in MI patients compared to controls and in anterior MI compared to both inferior MI and posterior MI. Ile and Leu were also significantly lowered in inferior MI compared to posterior MI.

The current results were not in line with studies of **Wang et al.**, [24]; **McCormack et al.** [25]; **Wang et al.** [26]; **Wang et al.** [27] and **Li et al.** [28] which found that BCAAs levels increased in MI patients. These studies indicated that high plasma BCAAs concentrations in individuals with ischemic heart disease was related to defective BCAAs catabolism in the myocardium which resulted in their accumulation and subsequent release into the circulation. This was probably due to down regulation of BCKDH; the key limiting enzyme of the BCAAs catabolic process at both protein and RNA levels in ischemic and stressed myocardium [29].

Abundant BCAAs directly inhibited mitochondrial respiratory function, led to superoxide accumulating in the mitochondria of cardiomyocytes. Furthermore, accumulation of BCAAs has been shown to suppress glucose metabolism by inhibiting mitochondrial pyruvate utilization through inhibition of the PDH activity and sensitization the myocardium to ischemic injury [30, 28]. Oral

BCAAs administration activated mammalian target of rapamycin signaling and exacerbated cardiac dysfunction and remodeling in mice with surgically induced MI [29- 31].

Most of the defects of BCAAs metabolism were more evident in diabetic subjects and might be related to insulin resistance and the associated atherosclerosis **Magnusson et al., [32]** and **Tobias et al. [33]**. Interestingly, **Wang et al. [27]** stated that pharmacological enhancement of BCAAs catabolism helped to improve post MI pathologies indicating BCAAs as modifiable risk factor for MI.

Unexpectedly, there were significant negative correlations between Ile, Leu and each of LDL-cholesterol, CK-MB, cardiac troponin, while, significant positive correlations were shown between both and HDL. In that regard, **Grajeda-Iglesias et al. [9]** showed that supplementation of Leu has ability to lower triglycerides and cholesterol contents of macrophage model system through inhibiting the cholesterol biosynthesis rate and increasing cholesterol efflux from macrophages which resembled anti-atherogenic properties of HDL. Also, several investigators found that different animals supplemented with leucine showed significant decrease in hepatic cholesterol and triglycerides [34- 36].

Leucine was also found to increase mitochondrial basal, maximal respiration and ATP production both in vitro and in vivo models which indicated a new potential athero protective feature of leucine [9]. Moreover, this study did not replicate the potential that plasma BCAAs could predict the disease outcome as no variation in BCAAs levels in different GRACE score categories were noticed. This was opposing to **Du et al. [37]** who stated that addition of plasma BCAAs concentrations improved the predictive ability of GRACE risk scores.

Glycine is a non-essential amino acid that can be obtained either via the diet, or synthesized endogenously from serine, threonine, choline and / or glyoxylate in the liver and kidney. It regulates multiple metabolic pathways and contributes vast number of biological molecules that regulates lipid and glucose metabolism as well as providing anti-inflammatory and anti-oxidative effects [38].

The current study showed lower plasma levels of Gly in MI patients compared to controls as well as significant negative correlations between Gly and each of LDL-cholesterol and cardiac troponin. This was in line with the study of 28 who reported negative relationship between

circulating plasma Gly and risk of myocardial infarction. Gly had previously been negatively associated with risk traits of coronary heart disease including diabetes, hypertension and obesity. However, the major possible role of Gly in preventing MI was regenerating GNMT; an enzyme which is responsible for catabolizing excess S-adenosyl methionine by remethylating of glycine into sarcosine [39]. Reduced availability of GNMT was linked to accumulation of S-adenosyl methionine in the liver and macrophages [40]. GNMT also regulated Niemann-Pick type C2 protein which is a small, soluble, lysosomal protein important for cholesterol and sphingolipid transport in the lysosome [41].

According to **McCormack et al.** [25] Gly also had ability to attenuate the uptake of the triglyceride rich VLDL and lower the triglyceride biosynthesis rate in macrophages which was another strong evidence that Gly had anti-atherogenic effects. Moreover, Gly administration to animal models and humans was associated with lower concentration of plasma free fatty acids, triglycerides and cholesterol [42-44].

Oppositely to **Chao de la Barca et al.** [45], Ser was found to be lowered in MI groups. Serine was responsible for replenishing the intracellular Gly level through hydroxymethyltransferase, and both AAs converge at certain point in the pathway of one carbon atom carrier's pathways involving the reversible methylenetetrahydrofolate dehydrogenase 1 flux, that was associated with the production or consumption of NADPH, a crucial reductant in fatty acid and cholesterol synthesis [46].

Reduced Met level was obvious in patients` group compared to controls, along with negative correlations between Met and each of LDL-cholesterol, CK-MB and cardiac troponin while significant positive correlation was shown between Met and HDL. The current study came in line with **Dhar et al.** [47] and **Calderón-Larrañaga et al.** [48] who found that there was an association between the plasma level of Met and MI incidence and atherogenic lipid profile. Met deficiency led to reduce GNMT flux that attenuated lipid uptake by the liver and so caused hyperlipidemia as aforementioned. Low Met led to the formation of venous thrombosis which was and added risk factor for arteries atherosclerosis [43]. Also, low Met led to hypomethylation of and overexpression of proprotein convertase subtilisin/kexin type 9 (PCSK9). Increased PCSK9 led to the degradation of

LDL receptors in the liver which in turn accumulated circulating LDL with augmentation of atherogenic profile [49].

Moreover, low Met levels led to deficiency of GSH, a major intracellular antioxidant, which has been linked to metabolic and CV diseases [50]. Additionally, Met was important for mitochondrial fatty acids oxidation as it was important for carnitine synthesis that carries fatty acid into the mitochondria [51].

Cysteine is a by-product of homocysteine metabolism and is an important precursor to intracellular GSH, which is an essential cellular antioxidant. Cys was found to be higher in MI cases. Data on the association of cysteine and CVD risk was conflicting. In line with our study, **van den Brandhof et al. [52]** and **Page et al. [53]** indicated that elevated Cys was positively associated with MI risk. However, **Özkan et al. [54]** did not find any association. Elevated plasma Cys may thus be a marker of the body's attempt to increase intracellular GSH in response to increased oxidative stress [53].

The protective effect of Tau on cardiac muscles was reported by several studies as it has anti-apoptotic, anti-inflammatory, and free radical scavenging properties as well as regulating the calcium ion channels functions [55, 56].

**Sabeena et al., [57]** had previously shown that Tau administration increased GSH a naturally occurring endogenous antioxidant and protected against isoprenaline induced MI in animal model. In the current study, the higher levels of Tau in MI patients compared to controls were suggested as compensatory mechanisms in myocardial ischemia during the course of chronic ischemia and the associated atherosclerosis.

In fact, we noticed negative correlations between Tau and each of LDL- cholesterol, CK-MB and cardiac troponin. The current study was in line with **Bhatnagar et al. [58]**; **Singh et al. [59]** and **Holeček, [60]** who reported higher Tau levels in post MI patients. Considering that the myocardium was known as a good reservoir for Tau amino acid, its release from the ischemic tissues up on arterial occlusion and the acute ischemic attack could also explain its higher level in MI patients. Indeed, posterior MI showed lowest levels of Tau as it was expected to have small infarction volume.

The current results presented low levels of Arg in MI group compared to controls. This was in accordance with previous studies which indicated an inverse association between circulating Arg concentrations and MI events [61, 62].

Arg is the source for NO synthesis by converting Arg into citrulline. NO has direct effects on arteries endothelial cells as it promotes vasodilator action [63, 64]. In addition, **Luo et al.**, [65] found the administration of Arg in patients with ischemia improved epithelium nitric oxide synthase (eNOS) expression, which produced NO and had potential cardio-protective action, reduced infarction area, promoted angiogenesis and ultimately enhanced myocardial tissue perfusion. Also, Arg had indirect antioxidant actions which enhancing its role as a cardioprotective amino acid. Antioxidant effect could be explained through scavenging of oxygen free radicals by NO [14]. Inhibition of oxidation activity in inner aortic lumen had direct effect for lowering LDL oxidation and led to prevention of atherosclerosis which was directly affecting MI development [66--68]. Moreover, In vitro adhesion of endothelial cells to monocytes [69], endogenously oxidative stress and lesion formation in animal models was shown to increase by asymmetric dimethylarginine [70].

Study of **Walker et al.** [71] suggested cardio-protective effects of Arg were due to reversing the competitive inhibitory effect of asymmetric dimethylarginine on eNOS to and eventually preserving NO. Our study stated significant negative correlations between Arg and each of cardiac troponin and CK-MB and significant positive correlation was shown between Arg and HDL. Similarly, to **Tripathi et al.** [72] found that high plasma concentration of L-Arg associated with lipid lowering affects mainly serum cholesterol and LDL.

Ornithine is a byproduct of Arg catabolism by arginase enzyme after release of urea. The current study reported low level of Orn in MI patients. This low level inversely affected bioavailability of Arg. Then again Orn concentration decrease could be a result of both an inhibition of arginase activity combined with an increased activity of ornithine decarboxylase in the polyamine pathway. Orn is decarboxylated by ornithine decarboxylase to form putrescine, spermidine and spermine. There was evidence that polyamines, particularly spermine, played beneficial roles in calcium homeostasis in ischemia/reperfusion (I/R) injury settings, scavenging free radicals and

reducing lipid peroxidation. It has been shown that MI and I/R injury elicited the polyamine stress response characterized by increased ornithine decarboxylase. Our results were in agreement with [45].

Contrary to our study, two recent studies by **Yu et al. [61]** and **Molek et al. [73]** who indicated lack of association of Orn and the incidence of MI although both indicated same pattern of Arg as our study. This could be explained by variation in metabolic status and medication pattern in these studies as well as the timing of sampling and the sample size.

Although no significant change in plasma Phe levels in MI patients compared to control low tyrosine level was evident in MI patients in our study. This depicted higher Phe / Tyr ratio in patients compared to controls and potential failure to convert Phe into Tyr. The inverse relation between Phe/Tyr ratio in relation to CAD was reported previously [74]. It was suggested that phenylalanine hydroxylase activity was reduced as a result of reduced tetrahydropyridine secondary to sustained inflammation, immune stimulation and oxidative stress in CAD patients [75]. Reduced conversion of Phe into Tyr by phenylalanine hydroxylase and tyrosine hydroxylase might increase metabolites of phenylalanine (Trans-cinnamate, phenylacetate, 3-phenylpropionate) and tyrosine (4-hydroxyphenylpruvate and p-hydroxyphenyllactate). These metabolites increased mitochondrial dysfunction by interfering with NAD-dependent oxidation, increased production of ROS, induced mitochondrial pore opening, fostered platelet responsiveness and thrombosis potential [76].

Histidine is semi-essential amino acid which is precursor of many biomolecules in the body including histamine, carnosine and the histidyl dipeptide. Consequently, His has vast biological impact on cardiovascular health. Low His levels were reported in MI patients compared to controls of the current study. Our results showed significant negative correlations between His and each of LDL-cholesterol and CK-MB, while, showed significant positive correlation between it and HDL. This was in line with **Aa et al. [62]**. While cross sectional studies to describe His levels in MI were limited its biological role had been largely studied. Cardioprotective effects of His on isoproterenol-induced myocardial infarction were reported in study by **Moradi-Arzelloo et al. [22]**. Also, it has been shown that His reduced myocardial mitochondrial damage and prevented post ischemic reperfusion injury in isolated hearts by inhibiting ROS generation and preserving high-energy phosphate.

Histidine has a good antioxidant and anti-inflammatory action (77, 78 and 38). Anti-inflammatory action prevents first step of atherogenesis, and antioxidant action can help to scavenge ROS and free radical which prevent formation of foam cells. The net result of dual action of His is to prevent atherosclerosis and development of MI. Also, low histidine may contribute to low histamine levels. Histamine confers a protective effect during MI, its deficiency exacerbates AMI-induced cardiomyocyte apoptosis [79]. Moreover, carnosine is a naturally occurring dipeptide ( $\beta$ -alanine-L-histidine) and other histidyl dipeptides are believed to play a generally protective role, neutralizing reactive biomolecules that contribute to tissue injury in stress by attenuating changes in intracellular pH and preventing the accumulation of lipid peroxidation derived aldehydes [80, 81]

Circulating asparagine was associated with decreased BMI, abdominal obesity, and insulin resistance. Recently, **Ottosson et al.** [82] showed that circulating levels of Asn provided protection against the incidence CAD. Asn was converted into aspartate via asparaginase enzyme. This enzyme was used in treatment of leukemia. Interestingly, patients who received this medication reported high incidence of hyperglycemia and cardiovascular events. The same was also reported for those who carried polymorphism that increased the enzyme activity. So cardioprotective effects of Asn were evident. Indeed, Asn supplementation restored intracellular deoxynucleoside triphosphate (dNTP), mTORC1 signaling, protein synthesis, endoplasmic reticulum stress response and redox imbalances and protected endothelial cells from damage induced by glutamine-deprived [83]. However, our results indicated high Asn in MI cases and not in the control. This could be attributed to the fact that our samples were collected after overnight fasting.

The biological role of the AAs on pathogenesis of MI either via molecules that alter lipid metabolism, cellular oxidative stress, endothelial function, vascular reactivity help understanding the disease process and lead to new therapeutic targets. The differential metabolites between MI and healthy individuals have also central roles in lipid plaque rupture and warning the occurrence of MI. Six out of the 18 tested AAs showed good potential as a diagnostic or predictive marker for the occurrence of MI. According to the ROC curve Met and Gly showed high sensitivity and specificity that exceeding 95% in discriminating MI cases and controls. BCAAs, Arg and His came next in such regard with sensitivity and specificity ranged from 72 to 80%. These data coordinated with the earlier

studies which reported the value of Met, Arg [62] and BCAAs in predicting high risk of CVD and MI [32, 61]. Unsurprisingly, only marginal variations in the levels of BCAAs and Asn were noticed in different GRACE risk score categories. This was opposing to Du et al. [37] who stated that addition of plasma BCAAs concentrations improved the predictive ability of GRACE risk scores.

These contradictory findings in relation to AAs profile in our cohort and other studies suggested that multiple confounding factors and traits might depict the levels of these AAs in MI patients especially in our small sample size. These confounders included and not limited to; comorbidities like diabetic status, the chronicity of the disease before the acute attack, the presence type of medication, time of sampling, fasting, the nutritional status and the mixed gender of the participants. Numerous factors like dietary consumption, genetic variation, altered metabolic homeostasis and recently gut microbiome might also impact circulating levels of amino acid.

## **Conclusion**

In conclusion, our study designated a panel of 14 plasma circulating AAs that were altered in MI. Met, Gly and His were found to be potential markers to recognize MI cases from healthy individuals. Eventually, plasma AAs profiling could help in confirms diagnosis of MI patients. Indeed, the role of plasma AAs profiling could be more helpful in understanding the disease process and set new lines of treatment.

Myocardial infarction is a serious acute disease, and it leads to complications and too many deaths annually, controlling and preventing myocardial infarction complications is consider the first concern all times.

Based upon our results we recommend the following:

- ✓ Monitoring the plasma free AAs especially Ile, Leu, Gly, Met, Arg and Tau could help in the confirm diagnosis of myocardial infarction.
- ✓ Introduction of Arg, Met, His, Leu and Tau in diet could help in prevention of MI in susceptible patients.

Finally, researches are needed to identify the role of these AAs in atherosclerosis and MI, which may open the way for developing anti-atherogenic nutritive and medicinal approaches.

## **Limitations**

The present study had several limitations. First was with small sample size, but this was due to funding limitations. The small sample size was reflected on studying the subgroups and stratifying the patients according to the known risk factors like diabetes, dyslipidemias and the BMI. Second, the study was also limited by the fact that blood samples were taken from mixed gender groups, the wide range of the age of participants. Third, our findings were based on serum metabolite profiles which were not necessarily representative of myocardial metabolism, along with the limited data regarding the nutritional state of the participants. Lastly, we had limited follow up data regarding the incidence of complications; such data could enhance our findings in relation to prediction of complications.

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## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## **Contributorship**

**Tahia H. Saleem:** Research design, participating in performing the experiments, contributed in writing and reviewing of the manuscript; **Ahmed Farouk & Mohamed Abdel Ghany:** patient recruitment, patient consents and sample collection; **Ayat A. Sayed:** writing and reviewing the manuscript; **Abdel-Raheim M.A. Meki:** Experiment design; **Ahmed H. Hussein:** performed the experiments and data analysis; **Fify Alfy Gabra:** Writing and editing the manuscript.

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**Figure legends:**

**Fig. 1** Aminogram of A) standard amino acids, B) plasma amino acids of controls, and C) patients with myocardial infarction.

**Fig. 2** Receiver operating characteristic curve of A) ROC curve of Ile, Leu and BCAAs\* amino acids, B) Gly, Met, His and Arg amino acids.