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RESEARCH

Free amino acid profile changes in coronary angiography patients: potential biomarkers for coronary artery disease

Koroner anjiyografi hastalarında serbest amino asit profili değişiklikleri: koroner arter hastalığı için potansiyel biyobelirteçler

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Abstract

Purpose: This study aims to delineate the plasma amino acid profile in patients undergoing coronary angiography (CA) and identify potential biomarkers associated with Coronary artery disease (CAD).

Materials and Methods: A targeted metabolomics approach was employed to analyze plasma levels of 41 amino acids in 25 CAD patients (pre- and post-CA) and 25 healthy controls. Plasma samples from patients pre- and post-CA, as well as from healthy controls, were analyzed using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine free amino acid profiles. Amino acid concentrations were quantified.

Results: In pre-CA patients, levels of arginine (35.124±14.476 µmol/L), asparagine (34.386±6.41 µmol/L), aspartic acid (11.266±4.788 µmol/L), glutamic acid (136.502±54.193µmol/L), and branched-chain amino acids such as leucine (168.451±85.247µmol/L) and isoleucine (66.067±14.605 µmol/L) were markedly lower than in controls. Conversely, hydroxyproline (27.16±21.173 µmol/L), hydroxylysine (0.21±0.116µmol/L), cystine (27.039±11.978 µmol/L), and ethanolamine (13.136±5.812 µmol/L) were elevated. Post-CA, further reductions were observed in asparagine (30.408±7.121µmol/L), leucine (106.362±25.446 µmol/L), isoleucine (57.637±11.83 µmol/L), threonine (81.422±16.043 µmol/L), and tryptophan (36.548±12.014 µmol/L).

Conclusion: These findings highlight the potential of amino acid profiling as a diagnostic and therapeutic tool for CAD, providing insights into disease pathogenesis and opportunities for targeted interventions.

Keywords:. Coronary artery disease, coronary angiography, amino acid, LC-MS/MS, metabolomics.

Öz

Amaç: Bu çalışma, koroner anjiyografi (KA) uygulanan hastalarda plazma amino asit profilini belirlemeyi ve Koroner arter hastalığı (KAH) ile ilişkili potansiyel biyobelirteçleri tanımlamayı amaçlamaktadır.

Gereç ve Yöntem: Hedeflenen metabolomik yaklaşım kullanılarak, 25 KAH hastasının (KA öncesi ve sonrası) ve 25 sağlıklı kontrolün plazmasındaki 41 amino asit düzeyi analiz edildi. KA öncesi ve sonrası hastalardan ve sağlıklı kontrollerden alınan plazma örnekleri, serbest amino asit profillerini belirlemek için yüksek performanslı sıvı kromatografisi tandem kütle spektrometrisi (LC-MS/MS) kullanılarak analiz edildi. Amino asit konsantrasyonları ölçüldü.

Bulgular: KA öncesi hastalarda, arjinin (35.124±14.476 µmol/L), asparajin (34.386±6.41 µmol/L), aspartik asit (11.266±4.788 µmol/L), glutamik asit (136.502±54.193 µmol/L) ve dallı zincirli amino asitlerden lösin (168.451±85.247 µmol/L) ile izolösin (66.067±14.605 µmol/L) düzeyleri kontrol grubuna kıyasla belirgin şekilde daha düşüktü. Buna karşılık, hidroksiprolin (27.16±21.173 µmol/L), hidroksilizin (0.21±0.116 µmol/L), sistin (27.039±11.978 µmol/L) ve etanolamin (13.136±5.812 µmol/L) düzeyleri artmıştı. KA sonrası dönemde ise asparajin (30.408±7.121 µmol/L), lösin (106.362 ± 25.446 µmol/L), izolösin (57.637±11.83 µmol/L), treonin (81.422±16.043 µmol/L) ve triptofan (36.548±12.014 µmol/L) düzeylerinde ek azalmalar gözlendi.

Sonuç: Bu bulgular, amino asit profillemesinin KAH için tanısal ve terapötik bir araç olarak potansiyelini vurgulamakta olup, hastalık patogenezine ilişkin içgörüler ve hedefli müdahaleler için fırsatlar sunmaktadır.

Anahtar kelimeler: Koroner arter hastalığı, koroner anjiyografi, amino asit, LC-MS/MS, metabolomik.

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INTRODUCTION

Amino acids are not only essential for protein synthesis but also play critical roles in metabolic pathways, including those involved in endothelial function, inflammation, and oxidative stress, all of which are implicated in cardiovascular diseases (CVDs)[1]. For instance, arginine is a precursor for nitric oxide (NO), a key molecule in vascular homeostasis, while branched-chain amino acids (BCAAs) have been linked to insulin resistance and increased cardiovascular risk[2].

Metabolomics, a rapidly expanding field in systems biology, provides a powerful approach for the comprehensive analysis of metabolites in biofluids, facilitating biomarker discovery[3]. Among metabolomic techniques, liquid chromatography-mass spectrometry (LC-MS) is highly reliable for the sensitive detection of small-molecule metabolites, offering high accuracy and reproducibility[4]. Plasma, being rich in metabolites, serves as an ideal medium for metabolic profiling[5].

Cardiovascular diseases, particularly CAD, are associated with significant metabolic perturbations[6]. Although numerous circulating metabolites have been implicated in cardiovascular events, studies specifically examining serum amino acid variations in CAD remain limited. The mechanisms underlying these variations are complex and not yet fully understood. Amino acid metabolism plays a key role in immune regulation, glucose, and lipid metabolism in CAD, but the precise relationship between abnormal amino acid metabolism and CAD pathogenesis requires further elucidation[7].

Recent studies have demonstrated that alterations in amino acid levels contribute to both the development and progression of CAD. Elevated concentrations of BCAAs and phenylalanine have been linked to insulin resistance, inflammation, and endothelial dysfunction, serving as independent predictors of CAD risk[8]. Conversely, reduced levels of amino acids such as arginine, ornithine, and citrulline may impair vascular function and promote atherogenesis[9]. Overall, plasma amino acid profiling holds promise for early diagnosis, risk stratification, and disease monitoring in CAD patients[10,11].

Despite advances in cardiovascular metabolomics, studies focusing on amino acid profiles in CAD patients remain inconsistent due to heterogeneity in analytical techniques, sample collection timing, and patient characteristics. Moreover, invasive procedures such as coronary angiography (CA) may influence plasma metabolite levels by inducing acute vascular stress and metabolic shifts.

Given these considerations, the present study aims to characterize plasma amino acid profiles in patients with CAD undergoing CA and to identify candidate metabolites that may serve as potential diagnostic or prognostic biomarkers. The use of a targeted metabolomics approach via LC-MS/MS enables a robust and detailed analysis of amino acid fluctuations before and after angiography, offering insight into both disease-related and procedure-induced metabolic changes.

Although previous studies have explored circulating metabolite profiles in CAD, few have simultaneously evaluated dynamic changes in free amino acid concentrations both before and after coronary angiography using high-sensitivity LC-MS/MS methods. This study is among the first to comprehensively assess temporal shifts in amino acid profiles in CAD patients undergoing invasive evaluation. By identifying amino acids with diagnostic potential through ROC analysis and correlating them with disease pathophysiology, this research offers novel insights that may guide future biomarker-based stratification strategies.

We hypothesize that specific amino acids involved in endothelial function, oxidative stress, and inflammation are significantly altered in CAD patients and undergo further changes following coronary angiography, reflecting both disease-specific and procedure-related metabolic disturbances.

MATERIALS AND METHODS

Study design and participants

This study was conducted as a single-center study at Harran University Medical Faculty Hospital, Şanlıurfa, Turkey. Data collection took place between January 2020 and January 2021. All participants, including both patients and healthy controls, were recruited from the cardiology department of the hospital. This observational case-control study included 25 patients diagnosed with CAD who were scheduled for CA and 25 healthy volunteers. The study included two groups: healthy controls (Group

1) and CAD patients. The CAD patients were evaluated at two time points: pre-CA patients (Group 2), and post-CA patients (Group 3).

All coronary angiography procedures were performed at the hospital's dedicated Coronary Angiography Unit by experienced interventional cardiologists under standardized institutional protocols to ensure procedural consistency and patient safety. Blood samples from CAD patients and healthy controls were collected and processed according to established clinical guidelines. Plasma amino acid analyses were carried out at the Metabolism Laboratory of Harran University, which operates under validated clinical protocols and regularly participates in external quality assurance programs. The laboratory is equipped with high-precision LC-MS/MS instruments (Shimadzu 8045, Japan). Sample collection, preparation, and analysis were performed by trained clinical laboratory specialists with expertise in metabolomics, ensuring high reliability and reproducibility of results.

The sample size was determined based on a priori power analysis using G*Power software (version 3.1.9.7, Düsseldorf, Germany). Assuming an effect size of 0.80 for detecting significant differences in plasma amino acid levels between CAD patients and healthy controls, with a statistical power of 80% (β=0.20) and a two-sided significance level of α=0.05, a minimum of 22 participants per group was required. To account for potential dropouts and to strengthen the robustness of the findings, 25 patients and 25 healthy controls were included in the study. This sample size provides sufficient power to detect clinically meaningful differences in amino acid concentrations and supports the validity of the ROC analyses performed.

A total of 33 patients scheduled for coronary angiography at Harran University Medical Faculty Hospital (January 2020–January 2021) were approached. Of these, 25 met the inclusion criteria and were enrolled. Eight patients were excluded due to recent myocardial infarction or acute coronary syndrome (n=3), chronic kidney or liver disease (n=3), and active infection or inflammatory disease (n=2). Inclusion criteria for CAD patients were: age 40–75 years, ≥50% luminal narrowing in at least one major coronary artery, and no prior coronary interventions. Healthy controls (n=25) were recruited from the same hospital if they had no clinical or angiographic evidence of CAD, no chronic diseases, and were not using medications affecting amino acid metabolism. The final study population consisted of 25 CAD patients and 25 healthy controls.

Procedure

This study was conducted in accordance with the Declaration of Helsinki and received approval from the Ethics Committee of Harran University Medical Faculty (Approval No. 19/04/07, Document No. E.49752). Written informed consent was obtained from all participants before inclusion in the study.

Coronary angiography (CA) procedure

CA was performed using standard femoral approach techniques with a 6-7F guiding catheter. Angiographic projections of the left and right coronary arteries were acquired to assess stenosis severity. CAD was defined as ≥50% luminal narrowing in at least one major coronary artery.

Plasma sample collection and amino acid analysis

Venous blood samples were obtained from patients before and after CA. Samples were collected in EDTA tubes, centrifuged at 4°C at 5000 rpm for 10 minutes, and stored at -80°C until analysis. Plasma free amino acid concentrations were measured using a JASEM amino acid kit on an LC-MS/MS system (Shimadzu 8045, Japan). Quality control measures were applied to ensure reproducibility and accuracy.

Statistical analysis

All statistical analyses were performed using SPSS 25.0 (IBM, USA). Continuous variables, including plasma amino acid concentrations and clinical parameters such as age and BMI, were tested for normality using the Shapiro–Wilk test. Differences in amino acid levels between CAD patients and healthy controls were analyzed using the independent samples t-test for normally distributed variables or the Mann–Whitney U test for non-normally distributed variables. Comparisons among control, pre-CA, and post-CA groups were performed using one-way ANOVA with Bonferroni correction for normally distributed data or the Kruskal–Wallis test followed by Dunn's post-hoc test for non-normal data. For within-patient comparisons of pre- and post-CA samples, paired t-tests or Wilcoxon signed-rank tests were used, depending on data distribution. Correlations between amino acid concentrations and clinical variables, including age, BMI, and

angiographic stenosis severity, were assessed using Spearman's rank correlation coefficient. Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of significantly altered amino acids, specifically hydroxyproline, arginine, asparagine, leucine, and cystine, for CAD diagnosis. All tests were two-tailed, and a p-value <0.05 was considered statistically significant.

RESULTS

The mean age of male patients was 59 years, and female patients had a mean age of 59.92 years. In the control group, the mean age was 58 years for men and 61 years for women. A total of 41 amino acids were identified and quantified in plasma samples from the control group, pre-CA patients (Group 2), and post-CA patients (Group 3). Significant alterations in amino acid levels were observed between the groups, as summarized in Table 1.

Compared to healthy controls, pre-CA patients exhibited significantly lower levels of arginine, asparagine, aspartic acid, glutamic acid, leucine, isoleucine, alloisoleucine, lysine, phenylalanine, proline, serine, threonine, argininosuccinic acid, gamma-aminobutyric acid (GABA), and thioproline ($p<0.05$). Conversely, hydroxylysine, hydroxyproline, cystine, and ethanolamine levels were significantly elevated in pre-CA patients ($p<0.05$). Post-CA, asparagine, leucine, isoleucine, threonine, tryptophan, argininosuccinic acid, beta-alanine, and hydroxylysine levels showed significant reductions ($p<0.05$).

Asparagine, leucine, and isoleucine levels were significantly reduced in pre-CA patients compared to healthy controls, and these levels decreased even further after CA. This suggests that these amino acids may play a role in the progression of CAD. Hydroxylysine and hydroxyproline levels were significantly higher in pre-CA patients compared to controls, indicating their potential involvement in the development of CAD. Cystine and ethanolamine levels were also elevated in pre-CA patients, which may be linked to increased oxidative stress and disruptions in lipid metabolism associated with CAD.

Asparagine, leucine, isoleucine, threonine, and argininosuccinic acid were consistently lower in CAD patients, while hydroxylysine levels were elevated but decreased post-CA.

The discriminative power of some plasma amino acids in the diagnosis of CAD was evaluated using ROC analysis (Table 2).

Table 1. Amino acid levels in control, pre-CA, and post-CA groups.

	Control (Group 1)	**Pre-CA (Group 2)**	**Post-CA (Group 3)**	**Group 1 and Group 2**	**Group 2 and Group 3**
	Mean±SD (µmol/l)	Mean±SD (µmol/l)	Mean±SD (µmol/l)	p	P
Alanine	480.196 ± 258.927	361.137 ± 110.316	303.86 ± 100.143	0.058	0.058
Arginine	60.02 ± 39.055	35.124 ± 14.476	36.326 ± 13.195	**0.035***	0.719
Asparagine	50.88 ± 23.807	34.386 ± 6.41	30.408 ± 7.121	**0.002***	**0.041***
Aspartic Acid	59.444 ± 36.854	11.266 ± 4.788	9.524 ± 4.011	**0.001***	0.308
Citrulline	40.464 ± 21.184	31.361 ± 12.609	28.778 ± 7.375	0.086	0.420
Glutamine	519.396 ± 240.236	500.518 ± 205.392	394.076 ± 128.384	0.749	0.064
Glutamic acid	259.808 ± 134.949	136.502 ± 54.193	139.14 ± 67.665	**0.001***	0.691
Glycine	300.88 ± 174.811	208.112 ± 51.163	190.944 ± 69.108	0.051	0.079
Histidine	60.34 ± 29.757	53.096 ± 31.497	35.268 ± 12.3	0.256	0.050
Leucine	218.456 ± 108.407	168.451 ± 85.247	106.362 ± 25.446	**0.012***	**0.006***
Isoleucine	103.168 ± 55.287	66.067 ± 14.605	57.637 ± 11.83	**0.014***	**0.024***
Alloisoleucine	0.592 ± 0.248	0.236 ± 0.057	0.206 ± 0.04	**0.001***	0.060
Lysine	216.124 ± 107.953	161.888 ± 51.587	137.914 ± 41.206	**0.020***	0.089
Methionine	27.816 ± 13.661	22.125 ± 6.071	19.651 ± 4.528	0.093	0.159
Ornithine	168.412 ± 78.155	153.473 ± 56.3	123.284 ± 53.094	0.607	0.079
Phenylalanine	86.464 ± 42.212	57.462 ± 12.653	52.005 ± 12.984	**0.002***	0.089
Proline	242.508 ± 110.163	180.482 ± 52.819	156.614 ± 39.231	**0.008***	0.064
Serine	161.872 ± 86.659	114.009 ± 27.74	102.135 ± 32.194	**0.016***	0.123

Threonine	125.904 ± 60.761	96.731 ± 23.262	81.422 ± 16.043	**0.032***	**0.025***
Tryptophan	54.656 ± 30.731	48.111 ± 15.374	36.548 ± 12.014	0.528	**0.003***
Tyrosine	65.104 ± 32.946	57.733 ± 15.527	49.976 ± 11.496	0.491	0.118
Valine	251.432 ± 129.395	223.842 ± 50.293	195.414 ± 41.247	0.528	0.050
2-Amino-Adipic Acid	0.488 ± 0.234	0.265 ± 0.172	0.345 ± 0.218	0.118	0.264
Amino Pimelic Acid	0.356 ± 0.229	0.277 ± 0.122	0.255 ± 0.198	0.490	0.900
Anserine	1.716 ± 1.285	2.209 ± 1.647	1.6 ± 1.027	0.409	0.165
Argininosuccinic Acid	0.344 ± 0.099	0.07 ± 0.048	0.042 ± 0.037	**0.001***	**0.026***
2-Amino-Buutiric Acid	6.256 ± 2.197	5.956 ± 3.021	6.329 ± 4.605	0.961	0.599
3-Amino-Isobutyric Acid	3.892 ± 2.14	2.735 ± 2.064	2.701 ± 1.455	0.114	0.756
Gamma-aminobutyric acid	6.096 ± 2.899	0.727 ± 0.079	2.203 ± 1.321	**0.001***	0.705
Beta Alanine	3.24 ± 2.075	3.27 ± 1.913	2.316 ± 1.309	0.946	**0.010***
Cystathionine	0.148 ± 0.022	0.143 ± 0.101	0.126 ± 0.022	0.354	0.938
Thioproline	0.668 ± 0.409	0.087 ± 0.015	0.098 ± 0.032	**0.001***	0.356
1-Methyl Histidine	4.068 ± 2.176	3.387 ± 2.055	2.917 ± 1.122	0.225	0.420
3-Methyl Histidine	1.176 ± 0.998	1.497 ± 0.871	1.925 ± 1.045	0.705	0.432
Hydroxylysine	0.132 ± 0.095	0.21 ± 0.116	0.186 ± 0.041	**0.012***	**0.013***
Hydroxyproline	9.216 ± 4.747	27.16 ± 21.173	21.01 ± 15.7	**0.001***	0.116
Cystine	0.916 ± 0.676	27.039 ± 11.978	29.572 ± 10.479	**0.001***	0.204
Serotenin	0.236 ± 0.056	0.160 ± 0.094	0.193 ± 0.064	0.170	0.053
Histamine	0.090 ± 0.050	0.058 ± 0.035	0.069 ± 0.038	0.103	0.119
Ethanolamine	6.044 ± 4.081	13.136 ± 5.812	11.235 ± 4.194	**0.001***	0.070
Taurine	47.632 ± 25.642	49.141 ± 24.087	46.397 ± 23.992	0.884	0.778

Pre-CA: Before coronary angiography Post-CA: After coronary angiography

Table 2. ROC analysis of significant amino acids

Amino Acid	AUC (95% GA)	Optimal Cut-off Value (µmol/L)	Sensitivity (%)	Specificity (%)	p
Hydroxyproline	0.92 (0.85–0.99)	18.5	88	84	<0.001
Arginine	0.88 (0.79–0.97)	45.2	80	76	<0.001
Asparagine	0.85 (0.75–0.95)	38.1	76	80	0.002
Leucine	0.82 (0.71–0.93)	120.3	72	78	0.004
Cystine	0.79 (0.67–0.91)	12.8	68	82	0.008

AUC (Area Under Curve): AUC > 0.7 suggests diagnostic potential

Hydroxyproline demonstrated the highest diagnostic power with an Area Under the Curve (AUC) of 0.92 (95% CI: 0.85–0.99), showing 88% sensitivity and 84% specificity (AUC values >0.7 suggest clinically relevant diagnostic potential). Significant diagnostic potential was also observed for arginine (AUC: 0.88), asparagine (AUC: 0.85), leucine (AUC: 0.82), and cystine (AUC: 0.79) ($p<0.01$). The optimal cut-off values for these amino acids were calculated as 18.5, 45.2, 38.1, 120.3, and 12.8 µmol/L, respectively. ROC curves of the significant amino acids are presented in Figure 1. These results indicate that these compounds may serve as potential biomarkers for CAD diagnosis.

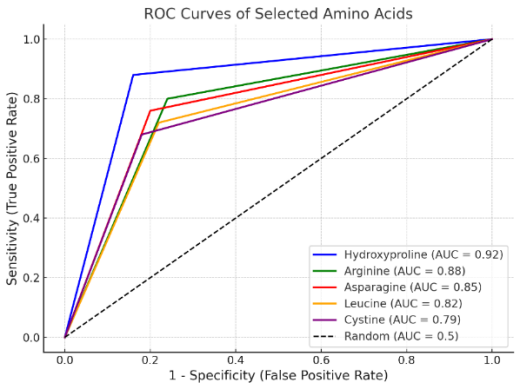

Figure 1. ROC curves of amino acids with significant discriminative performance.

DISCUSSION

CAD is closely associated with alterations in amino acid metabolism. Previous studies have demonstrated the predictive value of branched-chain amino acids (BCAAs) in cardiovascular events among CAD patients[12]. Glutamic acid, essential for vascular function and ATP production, has been linked to CAD risk, though its precise role remains debated[13]. Some studies have reported increased levels of leucine, isoleucine, glutamic acid, and methionine in CAD patients[6]. However, our findings revealed a significant reduction in proline, leucine, isoleucine, and glutamic acid levels before angiography, with further decreases observed post-procedure. These differences highlight the complex relationship between amino acid metabolism and CAD pathogenesis.

This discrepancy may be attributed to various factors, including the clinical characteristics of the patient population, disease stage, metabolic status, timing of sampling (before and after the acute procedure), and the analytical methods employed. Specifically, coronary angiography an acute intervention may induce metabolic stress and trigger an inflammatory response, thereby increasing amino acid consumption. Indeed, several studies have reported reduced plasma amino acid levels in conditions of acute physical stress or inflammation[14–16]. Furthermore, amino acids such as proline, which are essential for tissue regeneration and collagen synthesis[17,18], may decrease due to heightened tissue repair demands following vascular injury. Thus, the reductions observed in our study may reflect metabolic adaptations associated with CAD-related inflammatory processes and acute procedural effects.

Elevated cysteine levels have been identified as potential markers of CAD[19]. Our study found significantly increased cystine levels in pre-CA patients, supporting previous findings that cysteine may be a more reliable CAD indicator than homocysteine[20]. Additionally, BCAAs have been implicated as important markers of CAD, often compared to LDL cholesterol[21,22]. However, in contrast to studies reporting elevated BCAA levels in CAD patients, our results showed significantly lower leucine and isoleucine levels, while valine exhibited a non-significant decrease. These findings suggest a possible link between BCAA metabolism and CAD progression, indicating their potential role as therapeutic targets[23].

L-arginine has been widely studied in CAD, with conflicting results regarding its benefits. Some studies suggest its role in preserving endothelial function, while others find no significant effects[24]. In our study, arginine levels were significantly reduced in pre-CA patients, while citrulline levels were lower but not statistically significant. This aligns with previous reports showing decreased plasma levels of arginine, citrulline, and ornithine in acute coronary syndrome patients compared to those with stable angina pectoris[24]. Similarly, tryptophan has been associated with immune activation and inflammation in CAD patients[22]. Our study found decreased tryptophan levels, supporting previous findings by Zaric et al.[23] but contrasting with Yong Fan et al.[25], who reported elevated tryptophan-arginine-leucine levels in acute myocardial infarction (AMI) patients.

Furthermore, phenylalanine and tyrosine have been linked to immune activation and inflammation in CAD[25]. While some studies reported elevated phenylalanine and tyrosine levels in CAD patients[26], our findings indicated reduced tyrosine levels in pre-CA patients. Since pro-inflammatory factor activity may be more relevant to disease pathogenesis than absolute amino acid levels, future research should explore both protein concentrations and activity levels[7].

Hydroxyproline levels have been proposed as a protective factor against myocardial infarction (MI)[7]. However, our study found significantly elevated hydroxyproline levels in pre-CA patients, in contrast to previous findings that reported lower levels in MI patients.

Zhang et al. reported significantly lower plasma levels of arginine, citrulline, and ornithine in patients with acute coronary syndrome (ACS) compared to those with stable angina pectoris[27]. Similarly, our study found significantly reduced arginine levels ($p<0.05$) in pre-CA patients, along with lower citrulline and ornithine levels compared to controls. Other studies have highlighted differences in amino acid profiles, such as citrate, glycine, histidine, and hydroxyproline, between CAD patients and those with acute aortic dissection[28]. In our study, hydroxyproline levels were significantly elevated in pre-CA patients and increased further post-CA, while glycine, histidine, and serine levels were lower but not statistically significant.

Ottosson et al. linked elevated glutamate and reduced asparagine levels to both CAD and type 2 diabetes,

suggesting their potential as biomarkers for disease progression[29]. Our findings align with this, showing significantly lower asparagine levels in pre-CA patients compared to controls. Additionally, research has demonstrated the anti-atherogenic effects of glycine and leucine, contrasting with the pro-atherogenic role of glutamine[30]. In our study, leucine levels were significantly reduced in pre-CA patients, while glycine and glutamine levels were lower but not statistically significant. These findings suggest that reduced levels of anti-atherogenic amino acids may contribute to coronary stenosis in CAD patients. Notably, leucine's role appears complex, with some studies reporting elevated levels in CAD and AMI patients[6].

Elevated hydroxyproline levels may indicate an adaptive response to vascular damage, highlighting its potential as a biomarker for CAD progression. Additionally, taurine has been recognized for its cardioprotective effects, including anti-inflammatory properties and blood pressure regulation[31]. In our study, taurine levels were similar across groups, supporting its role in maintaining cardiovascular homeostasis.

In ROC analysis, hydroxyproline demonstrated the highest AUC value (88% sensitivity, 84% specificity). Its plasma levels likely reflect collagen turnover and tissue remodeling, suggesting a significant role in CAD pathogenesis. The high AUC value (0.88) and 80% sensitivity of arginine indicate its association with endothelial dysfunction and impaired NO synthesis. Furthermore, asparagine and leucine may contribute to disease progression through their involvement in both metabolic and immune regulatory processes. Elevated cystine levels could be linked to oxidative stress and inflammatory pathways. These findings suggest that selected amino acids not only mirror pathophysiological processes but may also have clinical utility for early diagnosis and risk stratification. The significant reductions in key amino acids post-CA suggest metabolic disturbances potentially linked to oxidative stress and endothelial dysfunction. These findings underscore the importance of amino acid metabolism in CAD and highlight the potential of metabolomic profiling for diagnosis and treatment.

This study has several limitations, including a relatively small sample size and a single-center design, which may affect the generalizability of the findings. The cross-sectional nature of the study limits the ability to establish causal relationships between amino acid levels and CAD, and potential confounding factors such as diet, physical activity, genetics, and medication use were not fully controlled. Additionally, post-coronary angiography changes in amino acid levels may reflect procedural stress rather than CAD-specific metabolic alterations. While the study identifies potential amino acid biomarkers, their clinical applicability requires further validation. This study focused exclusively on amino acid profiles and did not include additional clinical or laboratory data, such as inflammatory markers, lipid profiles, or dietary habits. Future studies incorporating these variables could provide a more comprehensive understanding of the metabolic changes associated with CAD and their relationship with disease progression. Despite these limitations, the findings provide valuable insights into the metabolic changes associated with CAD.

In conclusion, this study provides novel insights into plasma amino acid alterations associated with CAD and demonstrates that coronary angiography may further influence these metabolic profiles. Elevated levels of hydroxylysine, hydroxyproline, cystine, and ethanolamine highlight the significance of these amino acids in CAD among patients undergoing CA. Conversely, asparagine, leucine, isoleucine, threonine, tryptophan, argininosuccinic acid, and beta-alanine, which were already reduced pre-CA, showed further declines post-procedure, underscoring the dynamic fluctuations in amino acid levels associated with CAD.

These findings highlight specific amino acids with potential diagnostic value, supporting their future evaluation as biomarkers for CAD risk stratification and management. By enhancing our understanding of CAD pathogenesis in relation to amino acid metabolism, this research may aid in identifying novel therapeutic targets. Regular monitoring of plasma amino acid levels in CAD patients, combined with metabolomic analysis, could lead to more effective treatments and optimize resource utilization. Further multicenter studies with larger cohorts and comprehensive metabolic assessments are warranted to validate these results and explore their clinical applicability. Future research should also investigate longitudinal changes in amino acid metabolism, integrate inflammatory and genetic markers, and explore therapeutic modulation of these pathways to develop personalized management strategies for CAD.

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