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Tryptophan metabolite as predictive biomarkers for prognosis of ischemic heart Disease

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Abstract

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Ischemic heart diseases (IHD) a condition that happens when the heart doesn't get enough blood. Inflammation is the underlying common mechanism involved in IHD. Kynurenine pathway, as a major route of tryptophan degradation, its metabolites have been revealed to be crucial in CVD and involved in several biological processes such as immune-regulation, inflammation, and metabolism. Different enzymes have been shown to participate in the kynurenine pathway such as KMO, 3-HHAO. The study aimed to identify whether the metabolites of tryptophan degradation have an association with development of inflammatory event of IHD and its progress A case-control study was conducted at cardiac center in Thi-Qar Governorate, with participants:90 patients with IHD divided into 3 groups, myocardial infraction (M, n=30), angina (AN, n=30) and heart failure (HF, n=30. A 60 sample (n=60) were collected from healthy people as control group. KMO, and 3HHAO were identified by ELISA, while tryptophan concentration was measured by HPLC technique. Statistical tests were applied by using one-way ANOVA and ROC curve analysis. Tryptophan concentration was decreased significantly (P value $\leq$ 0.001) in patient groups compared to control group. The levels of KMO and 3-HHAO were significantly elevated (P value $\leq$ 0.001) in patients than control. These findings suggest inflammatory events in IHD induced tryptophan catabolism through the kynurenine pathway which plays a significant role in modulating immune response and influencing the development of CVD.

INTRODUCTION

Ischemic heart disease refers to a category of heart disease wherein the coronary arteries do not get an adequate amount of blood supply and is very closely associated with a pathological process such as atherosclerosis, thrombosis, and myocardial ischemia(1). The main clinical manifestations of IHD are angina, myocardial infarction, and heart failure. In line with aggravation of the disease, there is a growth of risks for patients as far as adverse cardiovascular events, such as myocardial infarction, development of congestive heart failure, and sudden death(2).

Amino acid metabolism has been reported to be an important participant in the development of CVD(3, 4). For example, branched chain AAs promote endothelial cell dysfunction through increased reactive oxygen species generation and inflammation(5). Aromatic amino acids also have important effects on the natural progression of cardiovascular disease(6). Similarly, tryptophan (Trp) metabolites have been shown to be closely related to inflammation and are thus suggested to be involved in CVD(7, 8).

Tryptophan (Trp), an essential amino acid, constitutes a central component in human and animal protein synthesis, and it serves as the sole source of substrates that facilitate the generation of a range of crucial molecules. Trp precedes and indicates the synthesis of proteins,

nicotinamide adenine dinucleotide (NAD), nicotinic acid, and serotonin (namely, the neurotransmitter)(9). For mammalian species, the kynurenine (Kyn) pathway is Trp's central catabolic route, featured in 95% of peripheral Trp metabolism in mammals; furthermore, it results in NAD's biosynthesis, as NAD functions as a crucial cofactor (10).The highest rates of global morbidity are associated with cardiovascular disease (CVD), and atherosclerosis is the primary etiological factor leading to various manifestations of CVD, including coronary heart disease and stroke(11). One of the critical factors in CVD pathogenesis is the immune response, and a clinical solution remains to be identified(12). Atherosclerosis occurs due to the manner in which low-density lipoprotein (LDL) accumulates and is retained in the arterial wall, and this leads to maladaptive responses from T cells and macrophages(13). Scholars in recent years have directed significant energy towards the examination of the Kyn pathway and the role it plays in CVD pathogenesis, and because several hypotheses have suggested that various factors, including oxidative stress, immune activation, and inflammation, are central to the pathogenesis of atherosclerosis and CVD, a critical area of future investigation is to examine to potential part played by the Kyn pathway in CVD regarding these factors(14).

The study aimed to investigate whether the inflammatory processes involved in IHD pathogenesis is linked with increased tryptophan degradation.

METHODOLOGY AND APPROACHES

Subjects

Ninety patients were participated in the study and were divided into three groups: In the first group, 30 patients with myocardial infraction (MI) with mean age (63.7 ±11.4 years), The second group involved 30 patients with angina (AN), with mean age of (59.93 ±11.8 years). The third group 30 with heart failure (HF) patients with mean age (58.23±9.7 yr). After being diagnosed with IHD by expert physician, patients were gathered from the cardiac center in the Thi-Qar Governorate between October 2024 and January 2025.

The healthy control group consisted of 60 samples of healthy individuals with a mean age of 54.18±12.3 years who did not have CAD, diabetes mellitus, hypertension, renal disease, endocrine problems, metabolic abnormalities, infections, or acute or chronic diseases. These healthy subjects visited the hospital for routine check-ups matched for age, sex, and other relevant demographic characteristics. Information like family history of the disease, living situation, treatment type, and sex was collected using structured questionnaires and medical records. Written informed consent was obtained from all subjects, and the study methods were approved by both the Ethical Committee of the College of Medicine, University of Al-Qadisiyah, and the Ministry of Health.

Methods

A 3 milliliters of venous blood were collected and placed in a gel tube and allowed to clot for a few minutes at room temperature. Then serum was obtained after centrifugation at 300 g for 15-20 minutes at room temperature. The resulting serum was transferred into Eppendorf tube and stored at -40°C for further analysis. Serum Tryptophan was detected by using Fluorescence HPLC in accordance with the instructions provided by the Shimadzu/Japan kit, while serum KMO and 3HAAO was identified by the enzyme-linked immunosorbent assay (ELISA) according to procedures of kit (Bioassay/China) respectively.

Statistical Analysis

The statistical analysis was conducted using SPSS, or Version 23 of the Social Sciences Statistical Software. Categorical variables were represented using percentages and frequencies. For continuous variables, Means ± SD was used.

The Shapiro-Wilk test was used to determine if the data distribution was normal. The Student's t-test was used to see if there was a significant difference between the patient and control groups. A one-way analysis of variance (ANOVA) was used to compare significant differences between multiple groups. A *P*-value of less than 0.05 was considered statistically significant for all analyses. Receive operating curve (ROC) was applied to test the sensitivity and specifity for biomarkers

RESULTS

The frequency distribution of patients and control subjects according to sex, was shown in table 1. Patients' group with type MI included 26(86.67%) males and 4 (13.3) females. The second group patients of (Angina)included 10(33.33%) males and 20(66.67%) females. As the third group was patients with HF included 19(63.33%) males and11(36.67%) females, whereas, control group included 47(78.33%) males and 13(21.67%) females.

The mean BMI of patients (MI, Angina, HF) was (25.29±3.8, 26.57±4.06, 25.41± 3.82) respectively and that of control subjects was (25.35±4.89).

The findings of this study demonstrated a significant lower in the serum levels of the Tryptophan in MI, AN, and HF patient groups compared to healthy individuals ($P \leq 0.01$, Figure 1and 2). serum KMO level were significantly increased in IHD compared to control groups ($P \leq 0.01$, Figure 3). Additionally, Serum 3HHAO levels were declared a significant increase in IHD patients as compared to control groups ($P \leq 0.01$, Figure 4).

ROS analysis for diagnosis performance of KMO, and 3HAAO was shown in table (2) in MI, in MI: KMO (>39.05): AUC 0.996, specificity 68.5%, sensitivity 80% while HAAO (>530), AUC (1), specificity 71.5, 77sensitivity. In Angina, with KMO than 39.5 pg/ml, the most accurate result was an are under the curve (AUC) of 0.997, a sensitivity of 80.4% and a specificity of 68.7%. for 3HAAO Sensitivity (78.7%), specificity (70.6%), and AUC was 1. In HF group, KMO cut off mor than 40.55, AUC 0.998. 78.2 Specificity. 71.4 sensitivity, for 3HHAO > 519.9, AUC was 1, specificity 70.5. sensitivity 77.3 which is demonstrated in Figures 5, and table 2).

Table (1): Comparison of demographic characteristics between study sample groups

	Patients(n=90)			Control group *n* = 60	*P-values*
	MI *n* = 30	AN *n* = 30	HF		
Age(yr) Mean ±SD	63.7± 11.4	59.93±11.8	58.23±9.7	54.18±12.3	0.56 NS
Male, , N (%)	26(86.67%)	10(33.33%) *	19(63.33 %)	47(78.33%)	0.021 S
Females, N (%)	4 (13.3)*	20(66.67%)	11(36.67 %)	13(21.67%)	
BMI ($kg/m^{2)}$	25.29±3.8	26.57±4.06	25.41± 3.82	25.35±4.89	0.26 NS

n: number of cases; **SD**: standard deviation; **S**: significant at $P \leq 0.05$: NS: not significant at $P \geq 0.05$.

Table (2): ROC for KMO and 3HAAO, in patient group

Parameters	Sensitivity%	Specificity%	Cut off	AUC
MI				
KMO	80	68.5	39.05	0.996
3HAAO	77	71.5	530	178.2
AN				
KMO	80.4	68.7	39.55	0.997
3HAAO	78.7	70.6	519	1.0
HF				
KMO	78.2	71.4	40.55	0.998
3HAAO	77.3	70.5	519.9	1

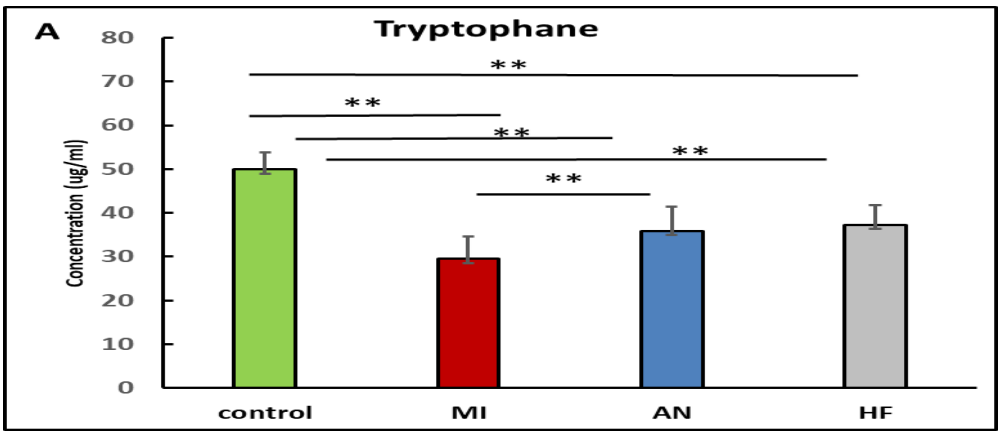

Fig. 1: Serum Trp levels in patients with MI, AN and HF and the control group.

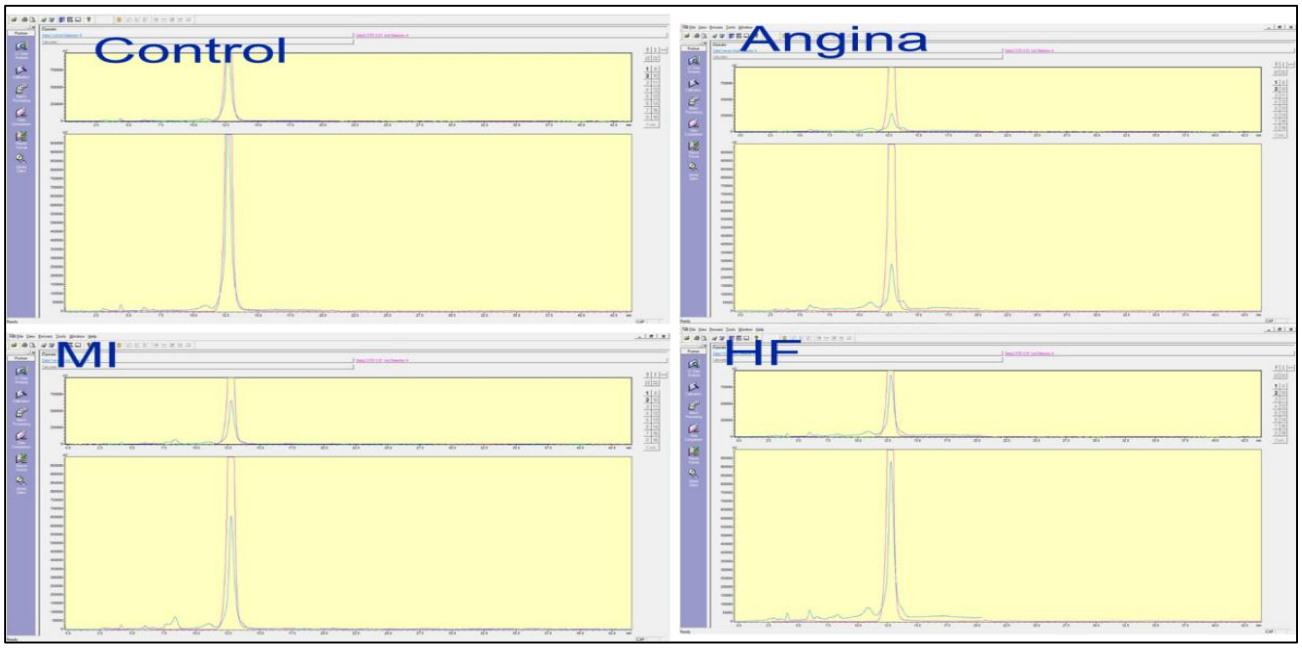

Fig. 2: HPLC peaks for serum Tryptophan in patients with MI, AN and HF and the control groups**.**

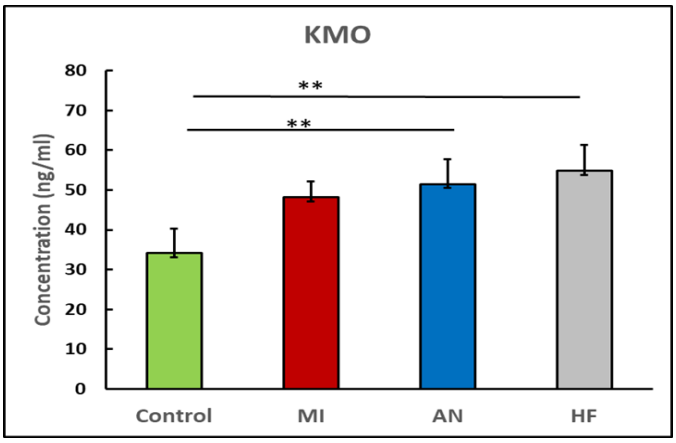

Fig. 3: Serum KMO levels in patients with MI, AN, HF, and the control group.

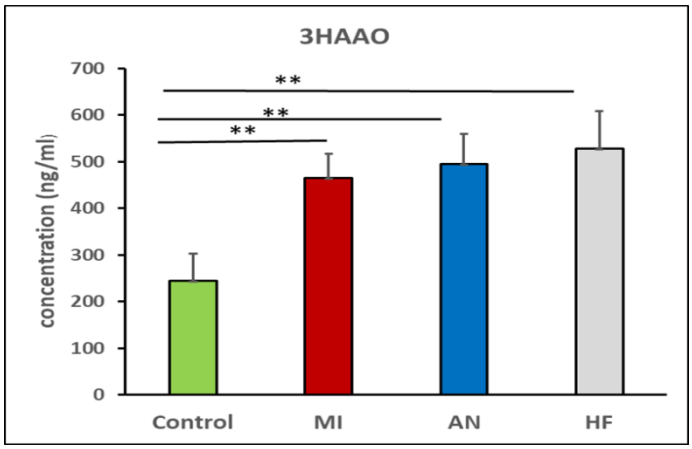

Fig. 4: Serum 3HAAO levels in patients with MI, AN, HF and the control group.

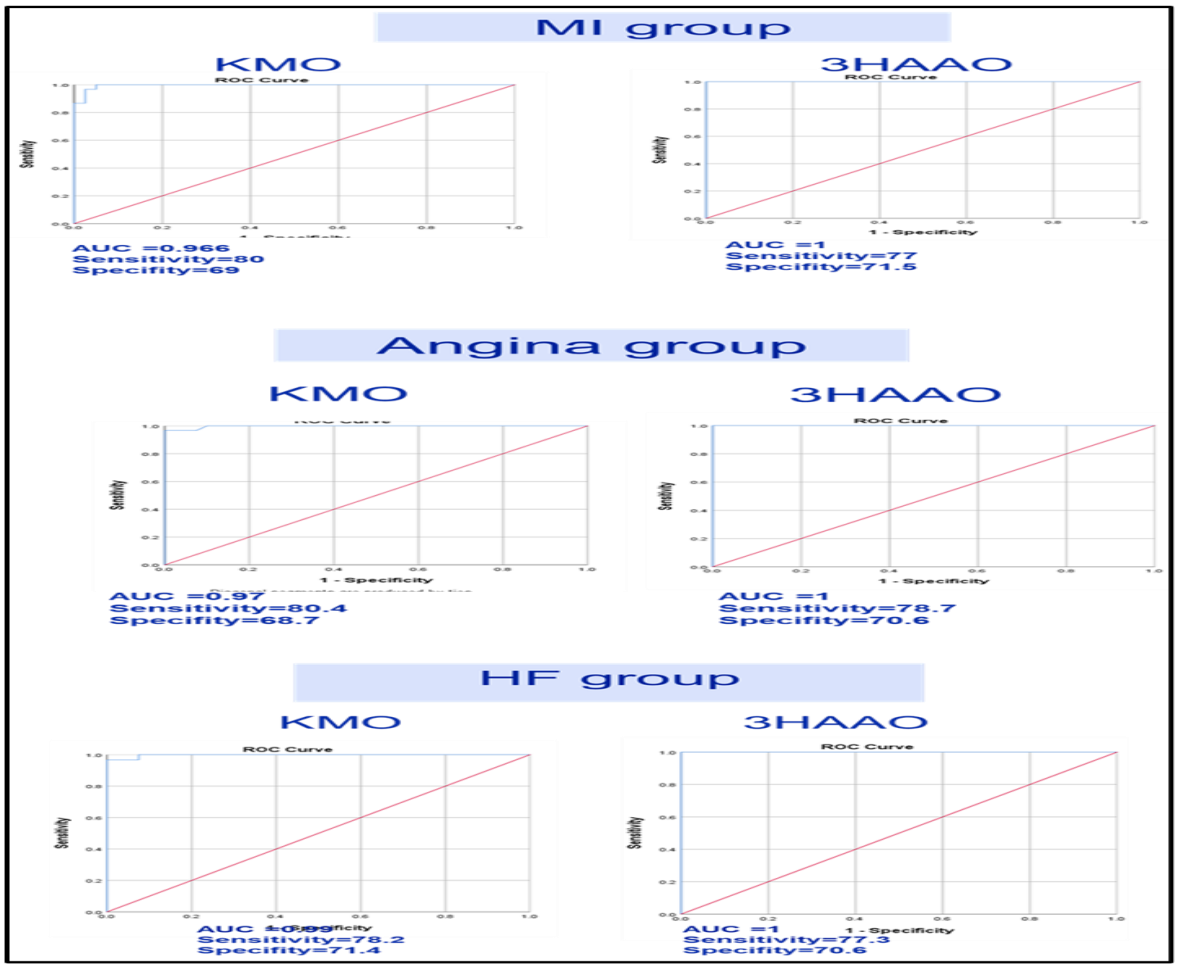

Fig. 5: ROC arch examination to find the best cutoff assessment of KMO and 3HAAO that can forecast a positive diagnosis of IHD in terms of sensitivity and specificity.

Discussion

Recent studies have suggested a potential link between the metabolism of tryptophan (Trp) and CVD(15). Trp, an essential amino acid, where the amino acid metabolism, as well as the involvement of tryptophan metabolism, plays a crucial role as substrates for the CVD, providing a comprehensive and quantitative value for human cardiac fuel using in IHD. TRP is mainly degraded into N-formylkynurenine leading to the generation of several active metabolites known as Kynurenine (Kyn) metabolites(16).

Moreover, a potential role of downstream Trp metabolites from the kynurenine pathway as biomarkers or as causal risk factors for CVD. Level of serum Tryptophan were lower significantly in IHD patients in comparison with healthy control ($P \leq 0.01$) in Figure1). It agrees with studies that show the relationship between Trp and CVD. This suggests that there is a decrease in the plasma Trp concentration in CVD patients, which indicate that inflammation is also a potential factor contributing to decreased levels of Trp in patients with CVD(17). The Th1-type cytokine IFN-γ in CVD patients, causes increased 2,3-dioxygenase activity, which ultimately decreases the serum levels of Trp(18). Moreover, plasma Trp is negatively associated with cardiovascular events. A reduction in circulating Trp levels may serve as a significant predictor of adverse outcomes in patients with CVD(6).

It was also found in the current study the KMO levels in the IHD patients were significantly greater than those in the control group ($P \leq 0.01$, figure 2).The results of this study are consistent with the results of the recent study that found an elevated plasma levels of KYN indicating an increased risk of adverse cardiovascular events(19). Where elevated production of these toxic metabolites and free radicals in the bloodstream triggers neuronal apoptosis. It also agrees with the results of recent Mendelian randomization studies which have indicated a positive correlation between KYN and IHD(20). IDO1demonstrates a positive correlation not only with IHD (21). Moreover, research evidence suggests that downstream metabolites including KMO for the kynurenine pathway serve as risk factors for various CVD, including atherosclerosis, hypertension, and vascular inflammation(22). Where KMO is primarily present in peripheral tissues, macrophages, and monocytes, while in the nervous system, it is mainly concentrated in microglial cells(23).

In pathological conditions, IDO1 experiences strong induction by inflammatory mediators, resulting in a substantial increase in downstream metabolites of the kynurenine pathway. These downstream metabolites, including KYN, 3-hydroxykynurenine, 3-HAA, and quinolinic acid (QA), have demonstrated the capacity to induce apoptosis in various immune cells like T cells, B cells, NK cells, and neutrophils. Consequently, they aid in tempering inflammatory responses. Moreover, in atherosclerotic aneurysms, kynureninase and KMO exhibited significant elevation within macrophages(24).

Finally, The results shown mean levels of serum 3-HAAO was significantly increased in patients with IHD in comparison with healthy control ($P \leq 0.01$, figure 4) .This is agree with studies have found an increase in the enzyme 3-HAAO which is considered one of the factors contributing to the Kyn pathway(25, 26), where 3HAAO catalyzes the conversion of 3HAA to QA, a neurotoxin found in excess levels in various diseases(26), As many inflammatory-related diseases exasperated by age, including cardiovascular, liver, and kidney disease, as inhibition of 3HAAO hinders QA and other downstream metabolites(27).The Kyn pathway plays an important role in several fundamental biological processes including infections(28). As inflammation, oxidative stress (OS), and immune activation have been postulated to be crucially involved in the pathogenesis of atherosclerosis and CVD(29).

The study's ROC curve analysis of MI, AN, and HF biomarkers, such as KMO and 3HAAO revealed a larger area under the curve with good sensitivity and specificity values of IHD, which explains why these biomarkers are applicable in IHD. Additionally, ROC analysis has demonstrated that these biomarkers are more predictive, with AUC =1 for 3HAAO(Figure 4).

Conclusion

These findings highlight the potential relevance of tryptophan degradation in CVD. The elevated serum levels of KMO and 3HAAO in IHD not only indicates their potential utility as biomarkers for early detection and disease monitoring, but also establishes them as promising therapeutic targets to reduce IHD chronic inflammatory risk.

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