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Activation of tryptophan metabolism in cardiometabolic disorders: Prognostic role of IDO and TDO enzymes

Jihad Yaser Kshash[1], Anwar Jasib Almzaiel*[2]

[1]Medical Chemistry Department, College of Medicine, University of AL-Qadisiyah, Al-Diywaniyah, Iraq, [2]Department of Clinical Chemistry, Medical Chemistry Department, College of Medicine, University of AL-Qadisiyah, Al-Diywaniyah, Iraq

***Corresponding author:** Anwar Jasib Thaaban Almzaiel, PhD, Professor, Department of Clinical Chemistry, Medical Chemistry Department, College of Medicine, University of AL-Qadisiyah, Building 77, P.O. Box 8822, Al-Diywaniyah, Iraq

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ABSTRACT

Background: Cardiometabolic disorders, including cardiovascular disease and type 2 diabetes mellitus (T2DM), represent a major global health burden and are closely associated with metabolic abnormalities such as obesity, dyslipidemia, and impaired glucose regulation. Disease progression is strongly influenced by chronic low-grade inflammation and dysregulated immune responses. Tryptophan, an essential amino acid, plays an important role in immune regulation and maintenance of inflammatory homeostasis. Overactivation of the kynurenine pathway leads to the accumulation of toxic metabolites, which promote inflammation and increase the risk of atherosclerosis, pulmonary arterial hypertension (PAH), and non-alcoholic fatty liver disease (NAFLD).

Methods: This study was conducted between October 2024 and February 2025 and included total 150 participants. Ninety patients with cardiometabolic disorders were enrolled, including 30 patients with coronary artery disease (CAD) combined with T2DM, 30 patients with CAD combined with PAH, and 30 patients with CAD combined with NAFLD.

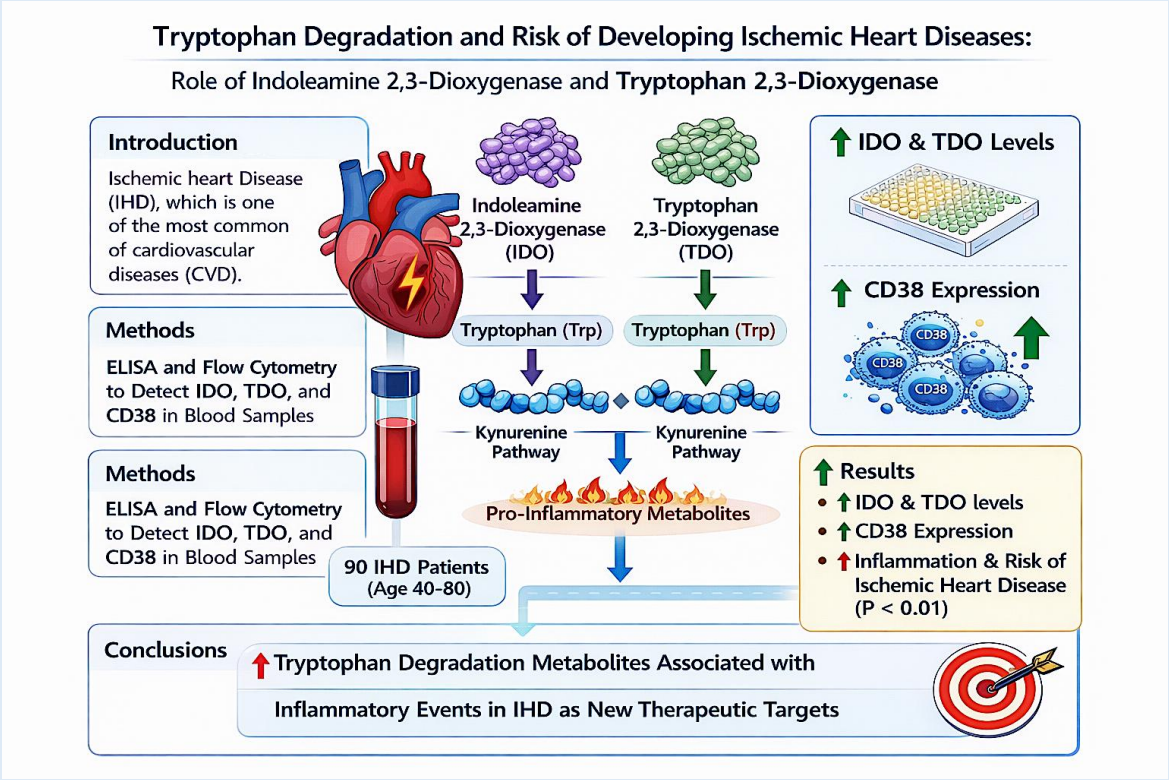
Sixty apparently healthy individuals served as a control group. Venous blood samples were collected from all participants. Hematological parameters were evaluated using an automated complete blood count analyzer. Serum levels of indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) were determined using enzyme-linked immunosorbent assay techniques. CD8+ T cell expression was determined using flow cytometry. All patients had angiographically confirmed coronary artery disease.

Results: Serum IDO levels were significantly elevated in patients with CAD combined with T2DM (4.85 ± 1.21 ng/mL), CAD combined with PAH (4.32 ± 1.08 ng/mL), and CAD combined with NAFLD (5.01 ± 1.34 ng/mL) compared to the control group (2.13 ± 0.74 ng/mL; $P < 0.01$). Similarly, serum TDO levels were significantly higher in CAD + T2DM (3.76 ± 0.98 ng/mL), CAD + PAH (3.54 ± 0.87 ng/mL), and CAD + NAFLD (3.92 ± 1.05 ng/mL) than in controls (1.89 ± 0.63 ng/mL; $P < 0.01$). $CD8^+$ T-cell proportions were also significantly increased in CAD + T2DM (32.4 ± 5.8%), CAD + PAH (30.7 ± 5.1%), and CAD + NAFLD (31.9 ± 6.0%) compared to healthy controls (21.3 ± 4.2%; $P < 0.01$).

Novelty of the Study: This study is among the first to simultaneously evaluate $CD8^+$ T cell expression and IDO and TDO enzyme activity in several cardiometabolic disorders, highlighting their combined prognostic significance in inflammation-driven metabolic pathology.

Conclusions: These findings suggest that inflammation-related activation of tryptophan metabolism through the kynurenine pathway plays an important role in immune disturbances and disease progression in cardiometabolic disorders. IDO and TDO enzymes may serve as valuable diagnostic biomarkers and potential therapeutic targets in inflammation-induced metabolic diseases.

Keywords: Chronic inflammation; Tryptophan metabolism; IDO and TDO enzymes; Kynurenine pathway; Cardiometabolic disorders; Functional food biomarkers



Graphical Abstract: Tryptophan degradation and risk of developing ischemic heart diseases.

INTRODUCTION

Cardiometabolic disorders (CMD), including cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), pulmonary arterial hypertension (PAH), and non-alcoholic fatty liver disease (NAFLD), are a major global cause of morbidity and mortality. [1]. Metabolic abnormalities such as dyslipidemia, central obesity, and impaired glycemic regulation are well-known risk factors that contribute to the development and progression of cardiometabolic diseases [2]. Increasing evidence indicates that dysregulated innate immune responses and persistent low-grade inflammation play a central role in the pathophysiology of insulin resistance, T2DM, and CVD [3-4]. As a result, CMDs represent a major public health challenge and are responsible for a large proportion of healthcare-related morbidity and mortality worldwide [3]. Heart disease and diabetes alone account for approximately 15% of total healthcare expenditure, placing significant pressure on the healthcare system. [4].

Atherosclerosis and ischemic heart disease arise primarily from reduced coronary blood flow and are strongly associated with chronic vascular inflammation [5]. T2DM is a metabolic disorder characterized by persistent inflammasome activation mediated by several proteins, including RNA-binding proteins such as S100A8 and S100A9 [6]. These proteins promote inflammation by interacting with immune cell receptors, particularly Toll-like receptor 4 (TLR4), which leads to increased secretion of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). This inflammatory cascade contributes to microvascular damage affecting tissues such as the retina, kidney and peripheral nerves, which represents an important pathological feature of diabetes-related complications [7].

Tryptophan (TRP) is an essential amino acid obtained exclusively through dietary intake and plays an important role in immune regulation, neurophysiological processes, and maintenance of inflammatory homeostasis [8]. A large part of tryptophan metabolism occurs via the kynurenine pathway, which acts as a critical interface between metabolism and immune signaling [7,8]. Indoleamine 2,3-dioxygenase 1 (IDO1) is widely expressed in the cytoplasm of various cells, with particularly high expression in immune cells such as monocytes and macrophages [8,9]. Together with IDO, tryptophan 2,3-dioxygenase (TDO) plays an important role in regulating tryptophan catabolism through the kynurenine pathway [10].

IDO and TDO catalyze the initial and rate-limiting step of tryptophan degradation by converting tryptophan to N-formylkynurenine [9-10], which is then metabolized into several biologically active compounds. These metabolites are involved in immunomodulation and link chronic inflammation to the pathogenesis of several metabolic and cardiovascular disorders [11-13]. IDO activity is strongly induced by pro-inflammatory cytokines, including IL-6 and TNF-α, resulting in increased tryptophan-to-kynurenine conversion and subsequent changes in immune responses in various pathological conditions [9].

Cytotoxic $CD8^+$ T-lymphocytes play a fundamental role in the immune system but can also contribute to the development of chronic inflammatory conditions such as cardiometabolic diseases. Interactions between tryptophan-metabolizing enzymes and immune cells can increase metabolic inflammation, thereby increasing the risk of atherosclerosis and cardiovascular disease. Chronic inflammation of the vascular endothelium promotes atherosclerotic plaque formation, immune cell infiltration, tissue damage, and thrombosis, ultimately increasing the risk of myocardial infarction and stroke [10]. Diabetes mellitus further increases atherosclerotic risk through vascular dysfunction, mitochondrial impairment, and sustained inflammatory responses [11].

Chronic inflammation associated with insulin resistance and excessive adipose tissue accumulation is a common pathological feature linking T2DM and PAH.

These conditions increase IDO and TDO activity, resulting in increased production of kynurenine metabolites that exacerbate metabolic dysregulation and metabolic disturbances, and increase the risk of coronary heart disease [12]. Non-alcoholic fatty liver disease is one of the most prevalent chronic liver diseases worldwide and is increasing rapidly in both developed and developing countries [13]. NAFLD is characterized by hepatic lipid accumulation, oxidative stress, and inflammation, which increase the risk of progression to cirrhosis and hepatocellular carcinoma [14].

Given the central role of inflammation-driven tryptophan metabolism in cardiometabolic disease pathogenesis, this study aimed to investigate whether inflammation activation in CMD is associated with increased tryptophan degradation via the kynurenine pathway.

MATERIALS AND METHODS:

Study Population: A total of 150 participants were included in this study. Ninety participants were diagnosed with cardiometabolic disorders and classified into three groups. The first group included 30 patients with coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM), with a mean age of 57.95 ± 10.34 years. The second group included 30 patients with coronary artery disease and pulmonary arterial hypertension (PAH), with a mean age of 56.38 ± 13.14 years. The third group included 30 patients with coronary artery disease and non-alcoholic fatty liver disease (NAFLD), with a mean age of 55.38 ± 10.70 years. Participants were recruited from Al-Karama Teaching Hospital and Al-Zahra Teaching Hospital in Wasit Province, Iraq, between October 2024 and February 2025. Demographic data including age, sex, and family history were recorded for all participants. In addition, 60 apparently healthy individuals were registered as a control group. Control subjects had a mean age of 53.28 ± 9.46 years and had no history of coronary heart disease, hypertension, renal disease, endocrine disorders, metabolic diseases, infections, or acute or chronic inflammatory conditions. These people went to hospital for routine health checks.

General demographic data, including age, sex, and medical history were collected for each participant. All participants provided written informed consent, and the study protocol was approved by the Ethics Committee of the College of Medicine, Al-Qadisiyah University, and by Iraqi Ministry of Health.

METHODS

Venous blood samples (5 mL) were collected under aseptic conditions from all participants. Two milliliters of whole blood were transferred into EDTA-containing tubes for complete blood count (CBC) analysis. Hematological parameters, including white blood cell count, hemoglobin concentration, neutrophil count, lymphocyte count, and neutrophil-to-lymphocyte ratio (NLR) were measured using an automated hematology analyzer (Sysmex, Japan).

$CD8^+$ T-cell expression was analyzed using a BD FACSCalibur flow cytometer (BD Biosciences, USA). Peripheral blood lymphocytes were identified based on forward scatter (FSC) and side scatter (SSC) characteristics. $CD8^+$ T cells were detected using a fluorochrome-conjugated anti-human CD8 monoclonal antibody. Compensation controls and isotype controls were used to ensure specificity. $CD8^+$ T-cell proportion was calculated as the percentage of CD8-positive cells within the total lymphocyte gate.

The remaining 3 ml of blood was allowed to clot at room temperature, after which the serum was separated by centrifugation at 400 × g for 15–20 min. The obtained serum samples were poured into Eppendorf tubes and stored at -40 °C until further analysis.

Serum levels of indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) were determined using enzyme-linked immunosorbent assay (ELISA) assayed according to the manufacturer's instructions (LabScience & Bioassay Technology Laboratory, China).

Serum IDO and TDO levels were measured using commercially available ELISA kits (LabScience, China; IDO Cat. No: ELS-IDO-001; TDO Cat. No: ELS-TDO-002). The assay detection range for IDO was 0.5–15 ng/mL with a minimum detectable concentration of 0.12 ng/mL, while the detection range for TDO was 0.4–12 ng/mL with a sensitivity of 0.10 ng/mL. The intra-assay and inter-assay coefficients of variation were <8% and <10%, respectively. All serum samples were analyzed in duplicate, and quality control samples were included in each assay run according to the manufacturer's protocol.

Statistical analysis: Statistical analysis was performed using SPSS software version 23. Data were expressed as mean ± standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. The Shapiro-Wilk test was used to assess the normality of the data.

Comparisons between patient and control groups were made using Student's t-test for normally distributed variables. One-way analysis of variance (ANOVA) was used to evaluate differences between multiple groups. Chi-square test was used to analyze categorical variables. A *p*-value of less than 0.05 was considered statistically significant for all analyses.

RESULTS

General characteristics of CMD patients and the control group: A total of 90 patients with cardiometabolic disorders were included in the study, including 30 patients with CAD and T2DM (10 women and 20 men), whose mean age was 57.95 ± 10.34 years, and 30 patients with CAD combined with PAH (12 women and 18 men), whose mean age was 56.38 ± 13.14 years In addition, 30 patients with NAFLD (14 women and 16 men) were included, with a mean age of 55.38 ± 10.70 years. A healthy control group consisting of 60 subjects (33 women and 27 men) with a mean age of 53.28 ± 9.46 years was included. Table 1 provides a summary of the clinical and demographic characteristics of the study population. No significant difference in mean age was observed between the patient groups and the control group (P = 0.56). Similarly, no significant difference in body mass index (BMI) was observed between the patient groups and the control group (P = 0.26; Table 1).

Table 1: Comparison of demographic characteristics between study sample Groups**.**

Characteristic	Patients			Control group	*P-values*
	CAD + T2DM n = 30	CAD + PAH n = 30	CAD + NAFLD n = 30	*n* = 60	
Age (years), Mean ± SD	**57.95 ± 10.34**	**56.38 ± 13.14**	**55.38 ± 10.7**	**53.28 ± 9.46**	**0.56**
Male, N (%)	**20 (66.6%)**	**18(60%)**	**16(53.3%)**	**27 (45%)**	**0.21**
Females, N (%)	**10 (33.3%)**	**12 (40%)**	**14(46.6%)**	**33 (55%)**	
BMI (kg/m²), Mean ± SD	**31.71 ± 7.90**	**27.30 ± 4.5**	**32.78 ± 6.29**	**27.27 ± 3.1**	**0.26**

N: numeral of subjects NS: not significant; The biochemical assessment

Biochemical parameters for patients with cardiometabolic disorders were compared with patients in the control group. The results are presented in Table 2. The average levels of C-reactive protein (CRP) and troponin were significantly higher in the patient groups compared to the control group. These differences were statistically significant (P = 0.035 and P = 0.015, respectively).

Table 2: Comparison of CRP and Troponin between patient and control groups.

Parameter	CAD + T2DM (n=30) Mean ± SD	CAD + PAH (n=30) Mean ± SD	CAD + NAFLD (n=30) Mean ± SD	Control (n=60) Mean ± SD	P-value
CRP (mg/L)	8.42 ± 2.31	7.95 ± 2.18	8.76 ± 2.54	3.12 ± 1.07	0.035
Troponin (ng/mL)	0.041 ± 0.012	0.038 ± 0.010	0.044 ± 0.013	0.016 ± 0.006	0.015

Neutrophils/lymphocytes in CMDs patient and control groups: Table 3 summarizes the haematological characteristics of patients with cardiometabolic disorders and healthy controls. In this study, the neutrophil-to-lymphocyte ratio (NLR) was calculated as an indicator of the inflammatory response in patients with coronary artery disease combined with T2DM, PAH, or NAFLD. No significant difference was observed in hemoglobin levels between the CMD group and the control group (P = 0.55; Table 3). The number of neutrophils was significantly higher in the patient groups compared to the control group (P = 0.041; Table 3). No significant difference in the lymphocyte count was observed between the patient groups and the control group (P = 0.235). In contrast, the neutrophil-to-lymphocyte ratio was significantly higher in all patient groups compared to the control group (P = 0.033).

Table 3: Comparison of hematological parameters between CAD-combined cardiometabolic groups and healthy controls.

Characteristic	Patients			Control *(n=60)*	*P-value*
	CAD + T2DM (n=30)	CAD + PAH (n=30)	CAD + NAFLD (n=30)		
Hemoglobin (g/dL)	13.6 ± 1.39	13.26 ± 1.37	14.42 ± 1.66	13.48 ± 1.3	0.55
Neutrophils X10⁹/L	6.02 ± 0.77*	6.14 ± 0.83*	6.41 ± 0.92*	4.6 ± 0.73	0.041
Lymphocytes X 10 ⁹/L	37.33 ± 5. 3	38.52 ± 5.17	38.04 ± 5.85	40.55 ± 4.76	0.235
NLR	1.65 ± 0.36*	1.62 ± 0.36*	1.72 ± 0.35*	1.15 ± 0.25	0.033

NS: Non-significant * $P < 0.05$

Serum IDO levels in individuals with CMD and the control groups

As shown in Figure 1, serum IDO levels were significantly elevated in patients with T2DM (4.85 ± 1.21 ng/mL), PAH (4.32 ± 1.08 ng/mL), and NAFLD (5.01 ± 1.34 ng/mL) compared to the control group (2.13 ± 0.74 ng/mL; $P < 0.01$). The highest IDO levels were observed in patients with NAFLD, followed by T2DM and PAH.

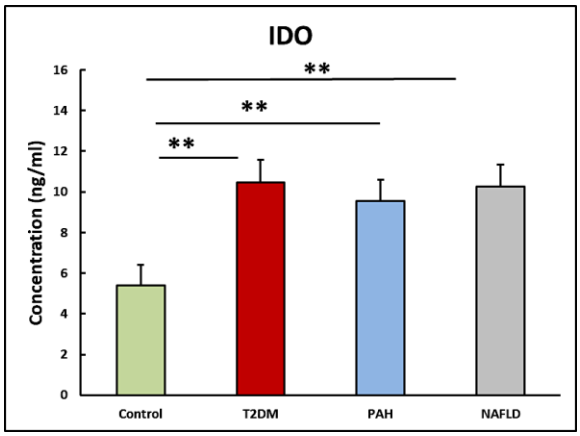


Figure 1: Serum IDO levels in patients with CAD + T2DM, CAD + PAH, CAD + NAFLD, and the control group. The data were shown as mean ± SD. ** indicated a statistically significant difference between the patient and control groups ($P \leq 0.01$).

Serum TDO levels in individuals with CMD and the control groups: Serum TDO levels were significantly higher in patients with T2DM (3.76 ± 0.98 ng/mL), PAH (3.54 ± 0.87 ng/mL), and NAFLD (3.92 ± 1.05 ng/mL) compared to the control group (1.89 ± 0.63 ng/mL; $P < 0.01$). TDO levels were significantly higher in patients with NAFLD and T2DM compared to the control group ($P < 0.01$; Figure 2).

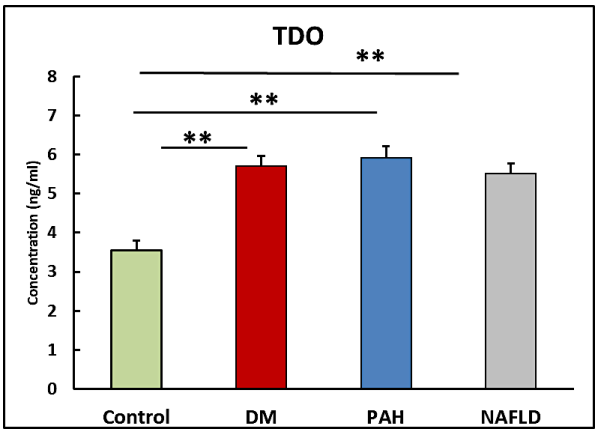


Figure 2: Serum TDO levels in patients with T2DM, PAH, and NAFLD as well as the control group. The data is shown as means ± SD, indicating a significant difference between the patient and control groups ($P \leq 0.01$).

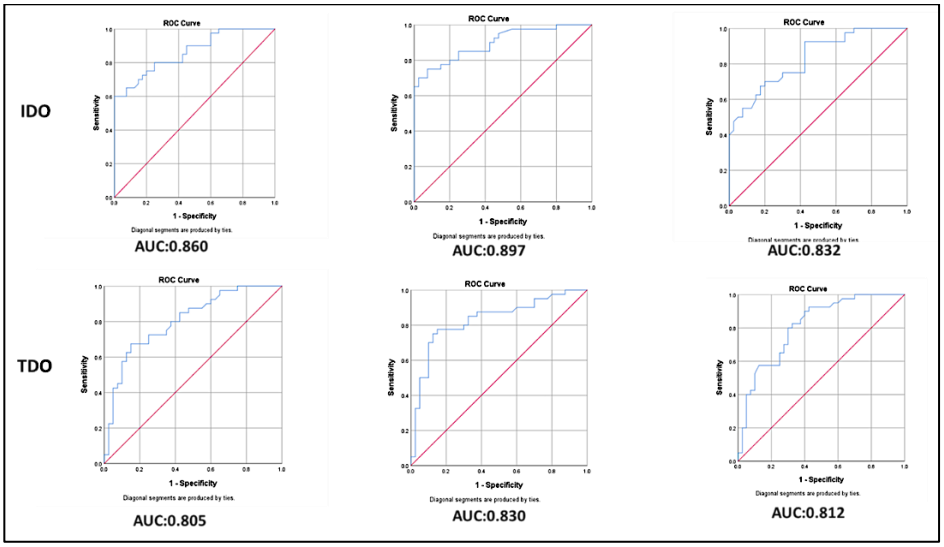


Figure 3: ROC arch examination to find the best cutoff assessment of IDO and TDO that can forecast a positive diagnosis of CMD in terms of sensitivity and specificity. ROC curve analysis was performed to evaluate the diagnostic performance of IDO and TDO in distinguishing CMD patients from healthy controls. IDO demonstrated an area under the curve (AUC) of 0.84 (95% CI: 0.76–0.91; P < 0.001). A cut-off value of 3.20 ng/mL yielded a sensitivity of 80% and a specificity of 78%, determined using the Youden index. Similarly, TDO showed an AUC of 0.81 (95% CI: 0.73–0.89; P < 0.001), with an optimal cut-off value of 2.75 ng/mL providing 76% sensitivity and 74% specificity.

Quantitative of CD8+ population by flow cytometry in CMD patients and the control groups: Cytotoxic $CD8^+$ T cells play an important role in controlling cardiovascular inflammatory responses; therefore, flow cytometry was used to assess variation in CD8+ T cell populations. $CD8^+$ T-cell proportions were significantly increased in patients with T2DM (32.4 ± 5.8%), PAH (30.7 ± 5.1%), and NAFLD (31.9 ± 6.0%) compared to the control group (21.3 ± 4.2%; $P < 0.01$). Representative flow cytometry plots and incidence distributions of CD8+ T cells are shown in Figure 5.

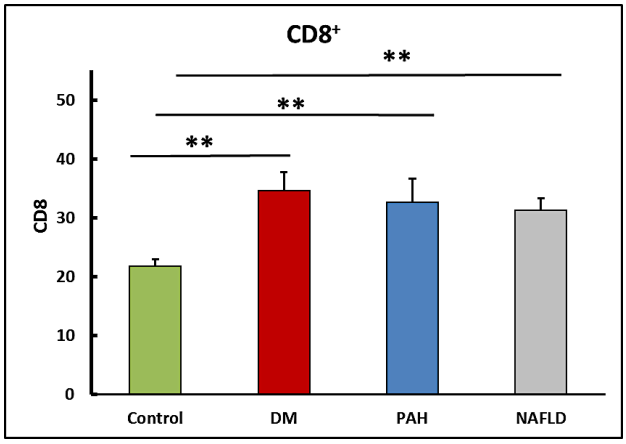


Figure 4: CD8+ T cell proportion in CMD patients with T2DM, PAH, NAFLD, and the control. Data are expressed as mean ± SD. ** Demonstrated a substantial disparity between the patient and control groups ($P < 0.01$).

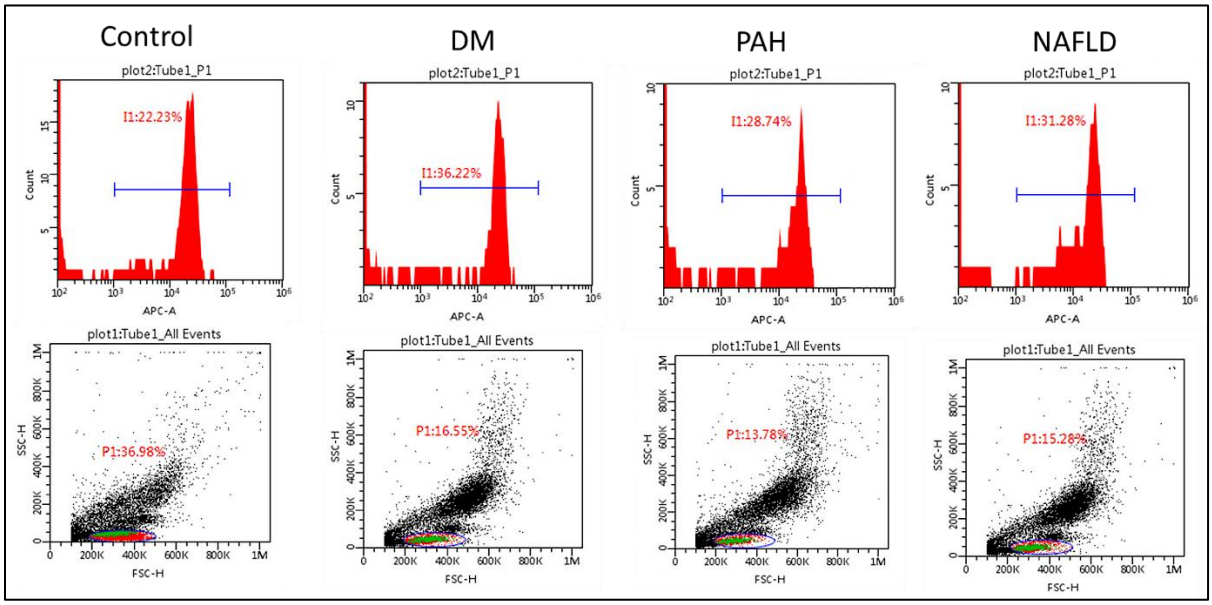


Figure 5: Representative flow cytometry plots showing CD8+ T-cell gating strategy and distribution in CMD patients and controls.

DISCUSSION

Cardiometabolic disorders (CMDs) encompass a spectrum of conditions characterized by shared risk factors and underlying pathophysiological mechanisms, including cardiovascular and metabolic diseases. The present study specifically evaluated patients with established CAD and T2DM, PAH, or NAFLD. CMDs are characterized by common etiological risk factors, including obesity, insulin resistance, chronic inflammation, sedentary lifestyle, and suboptimal dietary habits. The rising global incidence of chronic medical disorders represents a considerable public health challenge due to their association with increased morbidity and mortality rates. Timely recognition and intervention for these interrelated disorders are essential to mitigate their long-term effects on both individual and public health.

Elevated C-reactive protein (CRP) levels were observed in patients with T2DM, PAH, and NAFLD compared with the control group ($P = 0.035$). CRP reflects the presence of chronic low-grade inflammation in these diseases. It serves as an important biomarker of

inflammatory response and an indicator of systemic inflammation associated with disease progression. In the T2DM group, CRP levels were significantly higher than in the control group. Elevated CRP levels are associated with chronic hyperglycemia and insulin resistance, which contribute to the development of atherosclerosis and increased cardiovascular risk in patients with T2DM. This is consistent with the study by Tsalamandris et al. [15]. This finding confirms the association between C-reactive protein levels and cardiovascular risk in patients with diabetes. In the PAH group, a significant increase in CRP levels was observed, reflecting chronic, low-grade inflammation, a hallmark of the disease. A study by Santos-Gomez et al. [16] indicated that CRP is a key indicator of systemic inflammation in PAH and is associated with increased levels of pro-inflammatory cytokines such as IL-6 and MCP-1, which contribute to disease progression. In NAFLD, data have shown a significant increase in CRP levels, which indicates chronic liver inflammation due to fat accumulation, a risk marker for progression to NASH or cirrhosis. The elevated CRP levels are associated with an increased prevalence of NAFLD even in non-obese individuals, reinforcing the importance of measuring CRP in clinical practice to assess the risk of disease progression [17].

The results in Table 2 demonstrated a statistically significant difference in troponin levels between patients with T2DM, PAH, and NAFLD and compared with the control group (P = 0.015). This elevation reflects potential myocardial injury or cardiac stress and is unlikely to be incidental. The study demonstrated a significant increase in neutrophil counts across all patient groups compared with the control group (*P* = 0.041; Table 3), indicating cardiac damage caused by the disease, which is consistent with the results of the study by Arno D. Kazi et al. [18]. PAH patients have elevated troponin levels reflecting right ventricular muscle strain due to pulmonary hypertension, an important indicator for assessing disease severity, as shown by Steven Hsu et al. [19]. In the NAFLD group, elevated troponin levels indicate cardiac stress or muscle injury, with clear statistical significance (*P* = 0.015). These results support the study by Zhao et al., which indicated that high-sensitivity troponin can predict the risk of death and cardiac complications in NAFLD patients [20].

The study showed a significant increase (*P* = 0.041) in neutrophil counts across all patient groups compared to the control group, Table 3. This finding suggests a chronic inflammatory state in these patients, as neutrophils play a pivotal role in the innate immune response and represent key regulators of chronic inflammation resolution [7,19]. These findings support a study by Zhang et al., which indicated that neutrophil dysfunction or imbalance can contribute to the development of many diseases, such as cancer and inflammatory disorders [21]. Comparison of lymphocyte counts did not show a significant difference (*P*=0.235) between patients and controls. This suggests that lymphocyte counts are not significantly affected in these chronic conditions, or that changes are insufficient to demonstrate statistical significance. It is possible that lymphocyte counts remain stable in some chronic diseases, not associated with acute immune disorders. The neutrophil-to-lymphocyte ratio (NLR) is a sensitive indicator of chronic inflammation and immune system activation. In this study, NLR values were significantly higher in all three patient groups compared with the control group (P = 0.033). Patients with T2DM, PAH, and NAFLD had markedly increased NLR compared to healthy controls, corresponding with indicators of disease severity and systemic inflammation. This elevation reflects the presence of a chronic inflammatory state, and NLR is considered a powerful indicator in assessing the severity of chronic inflammation and associated diseases. Chronic low-grade systemic inflammation is a prevalent underlying characteristic in diabetes mellitus, pulmonary arterial hypertension, and non-alcoholic fatty liver disease. The NLR indicates the equilibrium between

innate (neutrophils) and adaptive (lymphocytes) immunity, hence functioning as a sensitive indicator of systemic inflammation. It surpassed conventional inflammatory markers in forecasting unfavorable outcomes and served as a dependable prognostic predictor in these chronic diseases. This is consistent with a study [22].

The data indicated a marked elevation in IDO concentrations among patients with T2DM compared with the control group. Chronic inflammation in diabetes activates pro-inflammatory cytokines, including IFN-γ and IL-6, which stimulate IDO activity and enhance the kynurenine pathway. This stimulates the synthesis of chemicals that affect beta cell function and increase insulin resistance. These findings are consistent with Oxenkrug's study [21], which indicated that dysfunction in the TRP, KYN, and NAD metabolic pathways contributes to IR. The initial phase that regulates the activity of the TRP-KYN pathway is influenced by enzymes activated by pro-inflammatory factors, including TNF factor (IL-6). Pyridoxal 5'-phosphate (P5P), an active form of vitamin B6, is necessary for key enzymes of the KYN-NAD pathway and serves as a cofactor in this system. A deficiency in this form alters KYN-NAD metabolism, redirecting it from NAD synthesis towards the overproduction of xanthric acid (XA) and various KYN derivatives that influence insulin function. These findings indicate that elevated IDO concentrations play a major role in immune–metabolic regulation, which in turn increases insulin resistance, a hallmark of T2DM. The cytosolic IDO catalyzes the oxidative cleavage of the indole ring of tryptophan, producing N-formyl-kynurenine, which then breaks down into format and L-kynurenine. IDO has been connected to the suppression of asthma because of its anti-inflammatory and immunosuppressive properties, which aid in regulating immunological responses [24]. Numerous studies have investigated the role of IDO in pulmonary hypertension. A study by Yingjie Xiao [25], suggests that endothelial IDO protects against the development of PAH. This finding is consistent with the present study, in which an increase in IDO concentration was observed ($P \leq 0.01$) and was considered statistically significant in NAFLD. Referred to as the hepatic manifestation of metabolic syndrome, it is intricately linked to obesity, insulin resistance, heightened systemic inflammation, and the advancement of severe atherosclerosis [26]. The increased concentration of IDO was found in the NAFLD group, which indicates the role of IDO in the exacerbation of this disease, especially since the pathogenesis of this disease is associated with T2DM, obesity, and symptoms of CVD. Cytokines play an important part in the invasion of inflammatory cells into the liver [18], and this is consistent with study results.

TDO is an important enzyme involved in tryptophan metabolism. The results showed a significant increase in TDO levels in patients with T2DM, pulmonary arterial hypertension, and NAFLD compared with the control group ($P < 0.01$). TDO is activated in cases of inflammation or metabolic stress, which stimulates TDO gene expression. Given that TDO functions in concert with IDO, an increase in TDO concentration was observed in patients with T2DM, indicating that its involvement and influence on inflammation plays a major role in the pathogenesis of the disease. TDO levels also increase in patients suffering from PAH ($P \leq 0.01$ Fig. 2). This is due to cytokines that enable the infiltration of inflammatory cells into liver tissue, a mechanism consistent with the findings from Simpson et al. [3], who demonstrated changes in the kynurenine pathway in relation to inflammatory processes. In both human and rodent models of PAH, the TDO2 gene, which encodes TDO, the enzyme that facilitates the transformation of tryptophan into kynurenine demonstrated considerable upregulation and was closely linked to key characteristics of PAH. Enhanced metabolism of the kynurenine pathway has been observed at early stages of PAH, predominantly localized within pulmonary tissues, and

appears to be modulated by TDO. Metabolites of the kynurenine pathway may function as promising biomarkers for pulmonary arterial hypertension, and tryptophan 2,3-dioxygenase represents a potential novel therapeutic target that requires further investigation. The findings indicate that this metabolic shift correlates with heightened disease severity and reduced survival rates. Additionally, it was noted that the expression of TDO2, the enzyme that catalyzes the conversion of tryptophan to kynurenine, increases concurrently with the progression of PAH. The results indicated a notable increase in the levels of TDO in patients with NAFLD compared with the control group. This observation suggests an upregulation of the kynurenine pathway involved in tryptophan metabolism in the context of NAFLD. These findings are in line with recent data highlighting alterations in tryptophan metabolism in NAFLD pathophysiology [14]. The loss of TDO2 in a mouse model NAFLD was shown to attenuate significantly hepatic steatosis and fibrosis. Additional studies have highlighted the involvement of downstream kynurenine-pathway enzymes, including kynurenine-3-monooxygenase, in regulating hepatic inflammation and disease progression [26]. This effect happened because it blocked the NF-κB signaling pathway that causes inflammation and reduced the activation of hepatic stellate cells (HSC). Therefore, higher levels of TDO2 are important in the NAFLD and suggests it could be a possible treatment target for it.

From the perspective of functional food science, the present findings provide mechanistic support for targeting inflammation-driven tryptophan metabolism as a modifiable pathway through diet-based interventions. Functional foods are defined as foods that provide health benefits beyond basic nutrition by modulating specific physiological pathways and biomarkers associated with disease risk. In this context, IDO and TDO enzymes, together with $CD8^+$ T-cell activation, represent clinically relevant biomarkers linking immune dysregulation, chronic inflammation, and cardiometabolic disease progression.

Previous studies within the Functional Foods in Health and Disease ecosystem have demonstrated that dietary bioactive compounds—including polyphenols, prebiotics, probiotics, and gut microbiota–modulating components—can influence immune–metabolic pathways, inflammatory signaling, and metabolic homeostasis [8–10,15]. These functional food interventions have been shown to indirectly regulate tryptophan availability and kynurenine pathway activity, thereby contributing to improved immune balance and reduced cardiometabolic risk.

The identification of elevated IDO and TDO levels in cardiometabolic disorders aligns with the Functional Food Center's biomarker-driven model and the 17-Step Functional Food Product Development framework [10,15], particularly Step 6 (biomarker identification and validation). These findings provide a scientific foundation for the development of functional foods targeting inflammation-driven tryptophan metabolism, with the aim of reducing chronic inflammation and improving cardiometabolic health outcomes [8–10].

Therefore, the present findings not only advance the understanding of inflammation-related tryptophan metabolism in cardiometabolic disorders but also support the translational application of these biomarkers within functional food science, reinforcing the relevance of this study to the scope of Functional Foods in Health and Disease [15].

Cytotoxic T lymphocytes, or $CD8^+$ T-cells, are essential for the cellular immune response. The results demonstrated a significant increase in $CD8^+$ T-cell populations in CMD patients with T2DM, PAH, and NAFLD compared with the control group ($P < 0.01$). The T2DM group exhibited the highest $CD8^+$ cells, then PAH, and NAFLD. The high $CD8^+$ levels in T2DM patients due to low-grade chronic inflammation, triggered by factors like fat buildup, oxidative stress, and gut microbiota changes.

These factors increase the secretion of inflammatory cytokines, stimulating the immune response, particularly $CD8^+$ cytotoxic T-cells. These cytotoxic T-cells may contribute to tissue damage, exacerbating insulin resistance, and amplifying inflammatory responses. Recent studies have demonstrated that metabolic and hormonal alterations influence $CD8^+$ T-cell activity and chronic inflammation [20]. The results showed that insulin, adiponectin, and IGF-1 levels affect $CD8^+$ T-cell activity, causing chronic inflammation and associated effects. The research also shows that hormonal changes reduce $CD8^+$ T-cell activity, which increases the inflammatory response and impairs glucose management and immunological function. This matches how fat gain, oxidative stress, and gut microbiome changes increase the immunological response, increasing $CD8^+$ Tcell counts, and insulin resistance and inflammation.

A significant difference was observed in $CD8^+$ cell population between the PAH group and the control group ($P \leq 0.01$). Recent studies suggest $CD8^+$ cells contribute to the exacerbation of inflammation associated with PAH. Multiple studies have demonstrated the activation of $CD8^+$ cells in this condition. Research suggests that these cells play a role in endothelial damage and pulmonary vascular remodeling by secreting toxic molecules such as granzyme B—a serine protease with caspase-like activity released by cytotoxic T lymphocytes, which serve as key effector cells in the immune system—and perforin, which exacerbates fibrosis and pulmonary arterial constriction. Cell-mediated immunity data in PAH patients have indicated increased levels of inflammatory cytokines, which in turn enhances $CD8^+$ T-cell activation and inflammatory signaling [20]. The $CD8^+$ T-cell population was markedly increased in CMD patients with NAFLD, T2DM and PAH compared to the control group (Figure 4), the increased proportion of CD8+ cells in NAFLD patients identify a distinct immune response that contributes to hepatic inflammation. CD8+ T lymphocytes penetrate the liver and become activated, leading to hepatocyte apoptosis by mechanisms including direct cytolysis or the secretion of inflammatory cytokines, such as IFN-γ and TNF-α. This facilitates the advancement of the illness from uncomplicated fatty liver to nonalcoholic steatohepatitis (NASH), which may subsequently progress to non-alcoholic steatohepatitis, cirrhosis, or hepatocellular carcinoma [18].

The novelty of the present study lies in its integrated evaluation of inflammatory biomarkers, immune cell activation, and tryptophan-metabolizing enzymes across distinct cardiometabolic disorders. Unlike previous studies that focused on IDO or TDO independently, this work demonstrates their concurrent upregulation alongside increased $CD8^+$ T-cell activity in T2DM, PAH, and NAFLD. This combined immunometabolic profile provides novel prognostic insight into inflammation-associated cardiometabolic disease progression.

Recent studies within the Functional Foods in Health and Disease ecosystem further support the role of dietary bioactive compounds in modulating inflammation and metabolic pathways, reinforcing the translational relevance of tryptophan metabolism in functional food science.

Scientific Innovation and Practical Implications: According to the Functional Food Center's 17-Step Functional Food Product Development Model, the present study primarily corresponds to Step 6: establishing relevant biomarkers. The identification of IDO and TDO enzymes, together with $CD8^+$ T-cell activation, as inflammation-associated biomarkers in cardiometabolic disorders provides foundational evidence that may support subsequent stages of functional food development, including preclinical efficacy evaluation.

CONCLUSION

In conclusion, this study highlights significantly elevated levels of IDO and TDO in patients with cardiometabolic disorders, including T2DM, PAH, and NAFLD. These findings suggest that inflammation-induced tryptophan catabolism through the kynurenine pathway plays a significant role in modulating immune responses and influencing the development of cardiometabolic disorders. The elevated levels of IDO and TDO not only indicate their potential utility as diagnostic biomarkers but also establish them as promising therapeutic targets. These targets may be relevant for the management and reduction of chronic inflammation-associated metabolic disorders. These findings provide a mechanistic foundation for future functional food interventions aimed at regulating tryptophan metabolism to mitigate inflammation-associated cardiometabolic disorders.

List of Abbreviations: T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; PAH, pulmonary arterial hypertension; IDO, indoleamine 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase; CMD, Cardiometabolic disorders; CVD, cardiovascular disease; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; TRP, Tryptophan; IDO1, Indoleamine 2,3-dioxygenase 1; CAD, coronary artery disease; CBC, complete blood count; NLR, neutrophil-to-lymphocyte ratio; SD, standard deviation.

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