ISSN: 0067-2904

Changes in Arginase activity, some inflammatory and oxidative stress parameters in Iraqi diabetic patients with valvular heart diseases.

Shaema S. Fadhil *, Jwan A. Zainulabdeen
Department of Chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq

Received: 26/3/2025 Accepted: 21/12/2025 Published: 30/10/2026

Abstract:

Type 2 diabetes mellitus (T2DM) is a significant independent risk factor for the development of valvular heart disease (VHD) in the aging population. The most prevalent valve diseases are aortic valve stenosis (AVD) and mitral valve regurgitation (MVD). Oxidative stress and inflammation undoubtedly play a role in both diseases. Arginase and nitric oxide synthase (NOS) have important roles in inflammatory responses and oxidative stress because they share the same substrate. This study included: 90 T2DM patients with and without VHD, subdivided into three equal groups: patients without VHD (T2DM group), AVD group, and MVD group. Arginase, NOS activities, levels of Arg.1, nitric oxide ('NO), peroxinterite, and oxidized protein, in addition to some inflammation parameters were assessed in patient populations. The overall statistical results showed significant increases in the thoughtful parameters of the two VHD groups compared to the T2DM group. Furthermore, the correlation between arginase activity, and selected parameters (nitric oxide level, peroxynitrite) as well as arginase1 level and nitric oxide synthase activity gave remarkable correlation coefficient values. Likewise, the analysis of receiver operator curves for arginase in VHD groups showed a high ability to differentiate VHD patients from T2DM. Meanwhile, increasing arginase activity enhances oxidative stress and inflammation and may lead to further destruction of valves. Thus, arginase levels can be chosen as the best diagnostic marker and may be a good predictor for VHD, giving a special target for the treatment of T2DM complications.

Keywords: Type 2 diabetes mellitus, Valvular heart disease, Arginase, Nitric oxide synthase.

التغيرات في نشاط الأرجينيز وبعض معايير الالتهاب والإجهاد التأكسدي لدى مرضى السكري العراقيين المصابين بأمراض صمامات القلب.

شيماء سعدون فاضل*, جوان عبدالمحسن زين العابدين
قسم الكيمياء ,كلية العلوم , جامعة بغداد, بغداد, العراق

الخلاصة:

مرض السكري من النوع 2 (T2DM) وأمراض صمامات القلب (VHD) شائعة بين كبار السن في جميع أنحاء العالم. الأشخاص المصابون بمرض السكري من النوع 2 أكثر عرضة للإصابة بأمراض صمامات القلب

*Email: shimaa.s@sc.uobaghdad.edu.iq

Open Access Articles. This articles is licensed under

من غير المصابين به. أكثر أمراض الصمامات انتشارًا هي تضيق الصمام الأبهري (AVD) وارتجاع الصمام التاجي .(MVD) لا شك أن الإجهاد التأكسدي والالتهاب يلعبان دورًا في كلا المرضين. يلعب الأرجينيز وأكسيد النيتريك سنثيز(NOS) دورًا مهمًا في الاستجابات الالتهابية والإجهاد التأكسدي لأنهما يشتركان في المادة الاساس. شملت هذه الدراسة: 90 مريضًا مصابًا بمرض السكري من النوع 2 مع أو بدون أمراض صمامات القلب؛ تم تقسيمهم إلى ثلاث مجموعات متساوية: المرضى غير المصابين بأمراض صمامات القلب)مجموعة (T2DM ومجموعة AVD و مجموعة MVD. تم تقييم فعالية الأرجينيز و أوكسيد النيتريك سنثيز ومستويات الأرجينيز 1 وأوكسيد النيتريك(NO•) والبيروكسي نيتريت والبروتين المؤكسد، بالإضافة إلى بعض معايير الالتهاب في مجموعات المرضى. أظهرت النتائج الإحصائية الإجمالية زيادة كبيرة في المعايير المدروسة لمجموعتي VHD مقارنة بمجموعة .T2DM علاوة على ذلك، أظهرت العلاقة بين فعالية الأرجينيز والمتغيرات المختارة (مستوى أوكسيد النيتريك، مستوى البيروكسي نيتريت) وكذلك مستوى الأرجينيز .1 ونشاط أكسيد النيتريك سنثيز قيم معامل ارتباط ملحوظة. وبالمثل، أظهر تحليل منحنيات المشغل المستقبل للأرجينيز في مجموعات VHD قدرة عالية على التمييز بين مرضى VHDو T2DMفي الوقت نفسه، يؤدي زيادة فعالية الأرجينيز إلى زيادة الإجهاد التأكسدي والالتهاب، وقد يؤدي إلى مزيد من تلف الصمامات. وبالتالي، يمكن اعتبار مستويات الأرجينيز أفضل أداة تشخيصية وقد تكون مؤشراً جيداً للتنبؤ بمرض VHD، مما يوفر هدفاً خاصاً لعلاج مضاعفات T2DM.

Introduction:

The pathophysiological triad of Type 2 diabetes mellitus (T2DM)-hyperglycemia, insulin resistance, and dyslipidemia-acts in concert to induce endothelial dysfunction [1]. This widespread disease is marked by oxidative stress (OS), inflammation, hyperglycemia, and various vascular complications, like valvular heart diseases (VHD) [1]. A major source of cardiovascular morbidity and mortality worldwide VHD in which one or more of the four heart valves (aortic, mitral, pulmonary, or tricuspid) doesn't work properly because the valves lose their elasticity and may not close or open adequately, and regurgitation occurs, impairing the heart's ability to pump blood [1]; aortic and mitral valves are the most common type of VHD worldwide. Knowledge of the cellular mechanisms of VHD is very important because its pathophysiology is complex, and at the same time increasing evidence indicates the important role of redox imbalance OS in this disease in the initiation and metastasis stages [2]. Meanwhile, T2DM and VHD are becoming more prevalent in an ageing population as well and diabetes patients have a risk of hardening of the aortic valve (aortic stenosis; AS) 1.62 times, and a risk of hardening of the mitral valve (mitral stenosis; MS) 2.28 times, higher than non -diabetics [3]. The increased rate of worsening from mild to severe AS and adults with diabetes is approximately twice as likely to develop heart diseases as adults without diabetes [4-6].

Arginase (EC:3.5.3.1) is the final enzyme in the urea cycle, responsible for detoxifying ammonia in mammals by breaking down arginine into ornithine and urea [7]. It is constitutively expressed in tissues and cells; it has two isoenzymes, Arginase I and Arginase II, which are similar in mammalian tissue; Arg-1 has a characteristic role to play in the pathogenesis of heart diseases [3,7]. Elevated arginase activity promotes polyamine biosynthesis by dividing the cells of tissues. Cardiovascular disease, kidney disease, and cancer are examples of such situations. These diseases are caused by factors such as oxidized low-density lipoprotein, hypoxia, glucose, and reactive oxygen species [3]. Consequently, arginase negatively affects the reshaping of arteries by directing macrophages to an inflammatory phenotype. Arginase has also been linked to endometrial dysfunction in an increasing number of vascular diseases, including atherosclerosis, hypertension, uremia, ageing, diabetes, and VHD [3,8].

In addition to the arginase pathway, arginine can be metabolized via the nitric oxide synthase (NOS; EC: 1.14.13.39) pathway, generating NO via cell-specific NOS isoforms (iNOS, nNOS, and eNOS) [1]. A ubiquitous molecule (NO) is a small lipophilic second messenger molecule, which at an appropriate level, plays an important role in the heart for basal vascular tone, regulating myocardial contractility, limiting leukocyte adhesion to the endothelium, preventing platelet activation, as well as calcification of the aortic valve [3]. The (•NO) and superoxide can combine to give peroxynitrite (a potent oxidant) [3].

The aim of this study is to measure arginase activity and the level of its isoenzyme (arginase 1), NOS activity, other parameters related to inflammation and OS in the serum of patients with T2DM and VHD. Ultimately, the goal is to determine whether arginase activity can serve as an indicator of T2DM complications and to explorer its relationship with two disease parameters (inflammation and OS). It is hoped that assessing these parameters will help identify potential treatment targets for the diseases under study.

Methods:

The study included 90 patients (54 men and 36 women) classified into three groups: the T2DM, aortic valve stenosis with T2DM (AVD), and mitral valve regurgitation with T2DM (MVD). Each group contains 30 individuals with ages ranging from 50 to 72 years. The duration of T2DM was more than 10 years for the three groups, while in the VHD group, the duration was one year. All patients take metformin in the treatment of T2DM. All samples were collected from patients who attended to Ibn Al- Bitar Center for Cardiac Surgery, from October 2023 until April 2024. In which routine laboratory tests and echocardiography were used to prove the diagnosis of the cases. This protocol of the current study was approved *via* the College of Science's Ethics Committee / University of Baghdad (Ref. CSEC/1123 /0114, November 19, 2023). Patients who have had type 1 DM, hypertension, chronic infections, other types of heart diseases, smokers, and alcohol drinkers were excluded from the study. Collection of venous fasting blood samples occurred from 8:00 to 10:00 a.m. To obtain serum, each sample was centrifuged for 5 mins (at 3000 xg) and aliquoted, then saved at (temperature at -20°C). A complete blood count (CBC) was performed using an Abbott Hematology Autoanalyzer (Cell-DYN Ruby, USA) in a specialized laboratory. Other biochemical tests include fasting serum glucose, albumin, and uric acid were measured using a kit (Linear Chemicals Company, Spain), HbA1c (i-CHROMA /Boditech Med Europe, UK). While total cholesterol and triglyceride were measured using by kit (Spinreact, Spain), and high-density lipoprotein (HDL) was measured using kits (Randox, UK). All assays were performed using colorimetric methods according to the manufacturer's instructions. Arginase activity was determined by an enzymatic reaction that produces the final product ornithine, and nitric oxide synthase (NOS) activity was determined by an enzymatic reaction using Griess reagent. The resulting nitrite was used to calculate NOS activity [9]. The nitric oxide method is based on the reduction of nitrate to nitrite by cadmium, and the resulting nitrite is determined by Griess reagent [10]. Peroxynitrite [11] and oxidized protein [12] levels were evaluated via selected colorimetric methods, while arginase I was evaluated via ELISA techniques (Elabscience, USA).

Anthropometric measurements body mass index (BMI) (weight in kilograms to the length in square meters), WHR (waist to hip ratio), as well as the WHtR (waist to height ratio), The Triglyceride-glucose index (TyG) is calculated by the following: TyG = Ln [fasting triglyceride (mg/dL) × fasting glucose (mg/dL))/2], and AIP (Atherogenic Index of Plasma; AIP= Log (fasting triglyceride / fasting high density lipoprotein) were calculated for each patient [13]. The Statistical Analysis System (SPSS) program version 22.0 (IM SPSS, Chicago, IL, USA) was used to analyze the effect of different factors on the study parameters. One-way ANOVA was used to detect least significant differences (LSD). The correlation

coefficient, the receiver operating characteristic (ROC) curve, and the area under the curve (AUC) were all estimated with it.

Results and Discussion:

As shown in Figure 1, there were no statistically significant differences in age, BMI, WHR, and WHtR among the T2DM, VAD, and VMD patient groups. While there is a highly significant increase (p<0.001) in Hip Circumference and Waist between the two VHD patient groups, and the T2DM group, as well as no significant differences between the AVD and MVD patient groups.

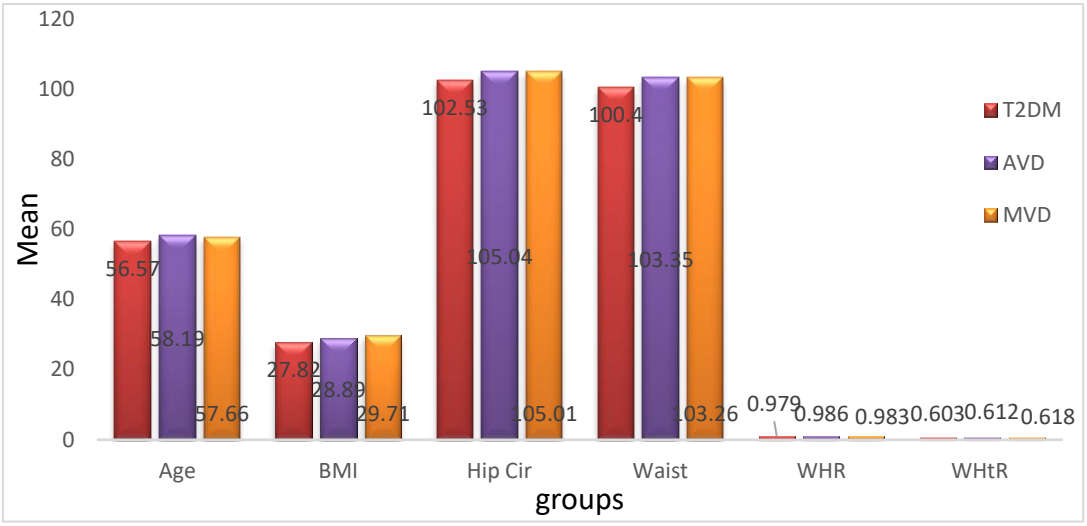


Figure 1: Anthropometric indices for the three study groups (BMI=Body mass index, WHR=waist to hip ratio, and WHtR =waist to height ratio).

VHD is one of the most common diseases worldwide, with its incidence increasing with age. The majority of VHD cases, particularly mitral valve disease (MVD) and aortic valve disease (AVD), are diagnosed in patients over 65 years old [14]. Aging is a real risk factor, since it increases the disease prevalence mainly as a result of many factors related to aging including the accumulation of oxidants: Oxidative stress (OS) is a serious effector in the progression of both diseases (T2DM and VHD), especially in older people [15]. Anthropometric measurements (BMI, WHR, and WHtR) are quantitative measurements of the human body that help with health assessment and future disease risk of obesity, which is considered a global health issue [15]. In this study, the estimation of obesity in people was not limited to calculating BMI, but other parameters were measured for humans (as shown in Figure 1) because they are more accurate than BMI in assessing body fat and give a better estimate of "visceral fat"; according to most studies that have investigated the issue, there is a direct proportionality between obesity and diabetes as well as heart disease [16]. The risk factor for T2DM and VHD is not only obesity, but also lifestyle (a high-fat diet, smoking, alcohol intake) and hepatic and inflammatory factors [17]. However, the data showed that the study participants had abdominal obesity, so the first expectation was an association between BMI and the risk of T2DM and its complication VHD.

The fasting serum glucose (FSG), glycated hemoglobin (HbA1c), and lipid profile were all determined, as presented in Table 1, which can be used as important diagnostic or prognostic markers for both diseases. The results indicated a highly significant increase for FSG, HbA1c, TG, VLDL, TyG, and AIP in both groups of VHD patients (AVD and MVD) compared to the T2DM group. However, there was no significant difference between the

subgroups. Whereas there were non-significant differences in the value of TC, HDL, and LDL between the three groups.

Table 1: Comparison of blood glucose and lipid profile parameters between the three study groups

Parameters	Groups			*p*-value		
	T2DM (n=30)	**AVD** (n=30)	**MVD** (n=30)	***Pa***	***Pb***	***Pc***
FSG (mg/dL)	162.76±7.92	211.66±51.44	219.63±49.86	0.0001**	0.0001**	0.312
HbA1c %	7.28±1.30	8.23±1.60	8.42±1.54	0.001**	0.0001**	0.519
TC (mg/dL)	179.47±34.79	189.10±38.14	190.43±31.29	0.256	0.196	0.875
TG (mg/dL)	130.93±32.48	178.43±48.90	180.70±48.79	0.0001**	0.0001**	0.827
HDL (mg/dL)	46.77±12.57	42.80±11.15	42.63±10.58	0.236	0.217	0.960
LDL (mg/dL)	97.27±27.52	106.13±32.73	102.57±26.39	0.191	0.434	0.598
VLDL(mg/dL)	26.18± 6.53	35.68±9.78	36.14± 9.75	0.0001**	0.0001**	0.827
TyG	9.24±0.21	9.77±0.43	9.84±0.36	0.0001**	0.0001**	0.428
AIP	0.44 ±0.16	0.61 ±0.17	0.62 ±0.15	0.0001**	0.0001**	0.895

AIP=Atherogenic Index of Plasma, **FSG=** Fasting serum glucose**,** **HbA1c** = glycated hemoglobin**,** **HDL** = High density lipoprotein cholesterol**,** **LDL** = Low density lipoprotein cholesterol, **TC=** Cholesterol**,** **TG** = Triglyceride**,** **TyG** = Triglyceride-glucose index**,** and **VLDL** = Very low density lipoprotein cholesterol**.** ***Pa*** = the AVD group compared with the T2DM group, ***Pb*** = the MVD group compared with the T2DM group, ***Pc***= the AVD group compared with the MVD group, $p > 0.05$= statistically non-significant differences, * Statistically significant differences at $p < 0.05$, ** Statistically highly significant differences at $p \leq 0.01$ by using LSD post-Hoc test.

The metabolic disease (T2DM) is associated with a sharply increased risk of VHD and death; the risk of developing VHD and its progression from mild to severe is significantly increased by it [18]. Therefore, ascertaining the cross-talk between them is imperative in formulating an efficacious therapeutic approach to forestall or at least decelerate the development and/or progression of AS in patients with T2DM. The deposition of cholesterol in the inner center of the blood vessels that feed the heart leads to an inflammatory disorder in the arterial wall driven by lipids and causes AVD. When the level of TG is lower than elevated, Coagulation factors that promote thrombosis or retard thrombolysis are associated with it. Consequently, platelet aggregation and thrombosis are expedited in patients with elevated triglyceride levels. Furthermore, in diabetics, higher lipid values are related to the prevalence of VHD and might be applied to evaluate metabolic state in clinical settings of the diseases. Diabetic patients accompanied by dyslipidemia are targets of VHD compared with people without diabetes [19]. From known and confirmed information, elevated (TG, VLDL, TyG, AIP) levels are risk factors for heart diseases including VHD [13]. The T2DM is also associated with more lipid dysfunction in the blood, where LDL-C molecules are more thyroid-generating, and endothelial dysfunction is also present, which promotes the adhesion of leukocytes and platelets, thrombosis, inflammation, and blistering coronary plaque [20]. The process of making advanced glycation end products creates cross bond linkages in

collagen molecules, resulting in increased fibrosis with increased stiffness of the heart muscle and poor relaxation of the heart, valvular dysfunction and fibrosis and exacerbating diastolic dysfunction. It has also been seen that very high levels of fatty acid oxidation can lead to problems with energy metabolism and cardiac dysfunction in people with diabetes [21]. So dyslipidemia also plays a role in endothelial dysfunction, which is central to the pathogenesis of multiple sclerosis (MS), IR, thrombosis, and hypertension [22]. Increased accumulation of advanced glycoxidation end products (AGEs); resulting from the glycation of proteins or lipids exposed to sugars, was associated with enhanced valvular OS, inflammation, expresed of coagulation factors and markers of calcification, leading to leaflet thickening, restriction and endothelial damage resulting in lipid infiltration, and fibrosis. Furthermore, AGEs valvular expression was associated with VHD severity. The presence of a correlation between valvular inflammation and long-term glycemic control parameters was observed in diabetic VHD patients [23]. It may affect the level of HDL and LDL, which can increase the risk of coronary arteries and heart valves by converting into oxidized low density lipoprotein (oxLDLs) [19]. These results are similar to a previous study on atherosclerosis in Iraqi patients [7]. A link between lipid and heart valve disease was also found by Hana, Saba, and others [24].

Table 2 evaluates the efficacy of inflammation indexes derived from CBC and their correlation with both T2DM and cardio metabolic risk in patients with VHD. Upon comparing the two VHD patients with the T2DM group, the results of Table 2 showed significant increases (p<0.01) in all parameters except for monocyte and there were no significant differences between the AVD and MVD groups; the significant increase ($p<0.05$) between the two groups of patients and the T2DM group in PLT may cause non-significant differences in monocyte as well as PLT /Hb ratio.

Table 2: The results of the CBC test for the three diseases included in the study.

Parameters	Groups			*p*-value		
	T2DM (n=30)	**AVD** (n=30)	**MVD** (n=30)	***Pa***	***P b***	***Pc***
WBC (10³/µL)	7.99 ± 1.07	9.80 ±0.96	10.04±1.34	0.0001**	0.0001**	0.423
Neutrophil (10³/ µL)	4.73±0.98	6.07± 1.30	6.11± 1.22	0.001**	0.001**	0.889
Lymphocyte (10³/ µL)	2.29±0.53	1.69±0.48	1.74±0.46	0.0001**	0.0001**	0.729
Monocyte (10³/ µL)	0.55 ±0.10	0.59 ±0.13	0.59 ±0.10	0.171	0.185	0.963
Eosinophil (10³/ µL)	0.22 ±0.06	0.25 ±0.059	0.26 ±0.051	0.015*	0.004**	0.608
Basophile (10³/ µL)	0.08 ±0.01	0.10 ±0.02	0.11 ±0.02	0.001**	0.0001**	0.054
Hb (g/dL)	13.42±1.58	14.43±1.50	14.49±1.46	0.006**	0.004**	0.877
PLT (10³/µL)	±47.26 254.83	288.83±49.75	±53.74 284.73	0.012*	0.027*	0.760
PLT /HB Ratio	19.41±5.05	20.18±4.06	19.74±3.70	0.494	0.773	0.962
NLR Ratio	2.16±0.65	3.71±0.73	3.60±0.61	0.0001**	0.0001**	0.577

Hb= hemoglobin, **NLR ratio**= Neutrophil to Lymphocyte Ratio, **PLT**=Platelet, **PLT /HB ratio**= Platelet to hemoglobin ratio, ***P*a** = the AVD group compared with the T2DM group, ***P*b** = the MVD group compared with the T2DM group, ***P*c**= the AVD group compared with the MVD group, p> 0.05= statistically non-significant differences, * Statistically significant differences at $p < 0.05$, ** Statistically highly significant differences at $p \leq 0.01$ by using LSD post-Hoc test.

It has been demonstrated that circulating WBCs closely mirror the metabolic and transcriptional status within the heart to an unexpectedly high degree, as the number of WBCs

strongly correlates with components of the metabolic syndrome such as lipids, blood pressure, and BMI, independently of risk factors and inflammation (such as interleukin and tumor necrosis factor). Meanwhile, inflammation plays an important role in the pathogenesis of various cardiovascular diseases, including coronary heart disease. Abnormalities in inflammatory parameters may indicate endothelial dysfunction [25,26]. The NLR is an important and influential factor in various inflammatory diseases, including T2DM and VHD; it can be used as a disease monitoring tool in diabetic patients (during follow-up) [27]. Elevated NLR in these patients may indicate systemic inflammation and an increased risk of valve damage, cardiac complications, and worse clinical outcomes. The fundamental process underlying the genesis of VHD is intimately associated with the instigation of persistent inflammation, predominantly engendered by the incessant secretion of cytokines, and adipokines from adipose tissue. This process can mobilize leukocytes and contribute to the progression of VHD [28]. In the course of an inflammatory response, the body initiates a series of events involving circulating WBCs such as neutrophils [26], This may lead to an increase in the secretion of cytokines and proteolytic enzymes. These molecules have the potential to induce tissue damage by means of activating the coagulation cascade. They also mess up the endothelial cells. Inflammation and the immune response play a central role in the development and progression of diabetes. Organ dysfunction and tissue damage can result from chronic inflammation, which can lead to insulin resistance and impaired insulin secretion [29]. These findings are similar to those of a previous study conducted on patients with hypertension [28].

Table 3 presents the selected biochemical and oxidative markers in the current work. The results indicated that uric acid levels were elevated but did not differ significantly across the groups. In contrast, there was a highly significant increases in Albumin, and UA/[A] ratio between AVD and MVD compared to the T2DM group. However, there were no significant differences when comparing AVD and MVD.

Table 3: The level of uric acid and Albumin, Arginase, Arg.1, NOS, NO, Peroxynitrite, and Oxidized protein for the three included diseases.

Parameters	Groups			*p*-value		
	T2DM (n=30)	AVD (n=30)	MVD (n=30)	*Pa*	*Pb*	*Pc*
UA (mg/dL)	5.37±0.73	5.68±0.68	5.51±0.87	0.135	0.489	0.420
Albumin (g/dL)	4.60±0.65	4.11±0.45	4.23±0.47	0.0001**	0.002**	0.295
UA/[A] ratio	1.18±0.22	1.39±0.21	1.31±0.23	0.0001**	0.023*	0.139
Arginase activity (U/L)	12.87 ±1.97	17.54±1.84	17.81±1.71	0.0001**	0.0001**	0.495
Arg.1 Conc. (ng/mL)	69.89±11.85	134.96 ±28.02	±26.97 139.43	0.0001**	0.0001**	0.354
NOS activity (U/L)	55.77±9.50	91.35±14.71	86.57±16.43	0.0001**	0.0001**	0.099
NO (µg/mL)	65.44±5.72	91.70±8.79	89.13±10.90	0.0001**	0.0001**	0.123
Peroxynitrite (µM/L)	6.90±1.37	11.93±1.76	11.06±1.72	0.0001**	0.0001**	0.003**
Oxidized protein (µM/L)	9.12±0.83	12.23±1.66	11.81±0.96	0.0001**	0.0001**	0.081

Arg.1 Conc.= Arginase1 concentration**, [A]=** Albumin**, UA=** Uric acid, **UA/[A] ratio=** Uric acid to Albumin ratio**, NO** = Nitric oxide concentration, and **NOS activity** = Nitric oxide

synthase activity, ***P*a** = the AVD group compared with the T2DM group, ***P*b** = the MVD group compared with the T2DM group, ***P*c**= the AVD group compared with the MVD group, $p > 0.05$= statistically non-significant differences, * Statistically significant differences at $p < 0.05$, ** Statistically highly significant differences at $p \leq 0.01$ by using LSD post-Hoc test.

The UA has a dual effect (oxidant or antioxidant) through experimental studies, and is also known to induce inflammation in vascular endothelial and intracellular OS, and smooth muscle cells, leading to endothelial dysfunction. It refers to a condition in which there is an imbalance due to an imbalance between endothelium-derived vasodilator and vasoconstrictor factors. Therefore, the UA level is often useful as a marker to predict subsequent clinical events in patients with established T2DM, and VHD [8,24]. The results suggested that UA acts as an oxidant rather than an antioxidant. Moreover, albumin is an important factor and a useful indicator of malnutrition and prognostic relevance in diabetic patients with VHD [1]. These results are consistent with those of a study conducted by Lu et al. on Chinese patients with heart valves [1]. Albumin is an antioxidant that works by binding copper and iron to dispose of free radicals, so its deficiency decreases the elimination of free radicals, therefore, increases the lipid peroxidation. Due to the physiological properties of albumin, which include anti-inflammatory, antioxidant, anticoagulant and platelet aggregation activity as well as colloidal osmosis, low levels of serum A can be associated with T2DM, and VHD [30,1,31]. Therefore, both UA and A represent the antioxidant condition in the body, taking into account the UA/A ratio as an accessible oxidizing biomarker conventional and inexpensive, used as a new composite biomarker that provides a superior predictive value for inflammation and OS compared to UA and [A] alone [32].

The results in Table 4 showed a highly significant increase ($p \leq 0.01$) of Arginase and NOS activities, in addition to Arg.1, NO, peroxynitrite, and oxidized protein levels in both AVD, and MVD patient groups when compared with the T2DM, while when comparing the AVD, and MVD groups of patients, there was no significant difference between them except in peroxynitrite level.

Cells are protected from free radical damage by antioxidants including enzymes, which are molecules present in low concentration that neutralize oxidants through certain mechanisms However several pathways associated with increased OS of cells have been identified; thereafter, OS eventually increases the production of reactive oxygen species (ROS), such as hydroxyl radicals, peroxide oxides, and superoxide anions. If ROS levels are high, they may overcome or exceed antioxidant defence mechanisms by antioxidants [32].
Diabetes alters glucose metabolism and the resulting use of secondary metabolism, including the shift in the use of glucose in heart muscle towards less efficient oxidation of fatty acids contributes to structural and functional abnormalities of the heart, and negatively affects heart contraction and relaxation. A relationship is found between high glucose levels and endothelial dysfunction in smooth vascular muscles. Due to the malignant effects of hyperglycemia on lining injury and the activation of macrophages and leukocytes, inflammatory cytokines, and growth factors, leading to accelerated progression in microvascular complications and large blood vessels, which is a major consequence of diabetes [24]. Furthermore, hyperglycemia and dyslipidemia in T2DM patients enhance oxidative stress (OS), and high oxidant levels are risk factors. These factors might be used in combination with antioxidants to assess OS in T2DM and vascular heart disease (VHD) patients, indicating a significant presence of OS in those patients. Meanwhile, most of the studied parameters serve as a useful indicator of VHD. In this study, increased levels of oxidized proteins in the VHD groups indicated significant oxidative stress (OS) in these patients, which aligns with a study on Iraqi patients with VHD that evaluated OS status using

malondialdehyde and OS index [24]. These results are consistent with a previous study by Suhad [7]. In diabetes, food consumption increases the ratio of blood glucagon to insulin, and amino acid metabolism increases, leading to an increase in the activity of the urea cycle and arginase activity.

The results show significant increases in arginase and NOS activities in diabetic patients with VHD (AVD and MVD). As far as we know, this is the first study to address diabetic patients with VHD in Iraq. In fact the regulation of Arginine metabolism is complex and it should not be showed as a simple view of two enzymes competing on a substrate and arginase does not outperforms NOS for the same substrate or *vice versa* [33], this compatible with the findings study in coronary arteries (CA) of diabetes , which are not affected by NO synthesis (NOS) inhibition, but by restriction an arginase inhibitor or L-arginine, the development of endothelial dysfunction is primarily influenced by an increase in arginase activity, and may show increased gene expression in arginase 1 using eNOS in endothelial cells [34]. Arginase-1 is abundantly expressed in endothelial cells. It is used with eNOS in the coronary vessels of diabetics [34]. A possible explanation for the higher arg1 level is that it may contribute to the development of T2DM and its implications, which is due to its regulatory role in vascular dysfunction, as well as its influence on β-cell functioning by affecting L-arginine metabolism, OS, and inflammatory responses [26]. The NO is very important for many biological functions, including processes related to the pathophysiology and cardiovascular systems, such as inflammation, neurotransmission, and immune function; also, endothelial cells is capable of NO production. Various isoforms of NOS control the endogenous production of this molecule [35]; in this work, total NOS was evaluated. The increasing iNOS expression in various cardiovascular disorders, such as heart failure, cardiomyopathy, specifically, iNOS, but NO produced by eNOS can be a useful tool, while NO produced by iNOS can have adverse effects [36]. Hypoxia has been reported to be a synergistic inducer of iNOS expression that favors the reaction between NO and oxygen, resulting in nitrosative modifications. Therefore, the measured elevation in NO in the VHD patients may be a result of cytokines released by inflammatory mediators due to the presence of hypoxia, which was reported to result in overexpression of iNOS synthesis [36]. As well, the reason for the increase in the level of NO may also be the metformin medication taken by T2DM and VHD patients where it can increase the expression of NOS mRNA by activating AMP-activated protein kinase (AMPK), increasing the production of NO in aortic endothelial cells [37].
Peroxynitrite ($ONOO^-$) produced upon the reaction of superoxide and nitric oxide can modulate the effects of the other, and the ratio of their concentrations is important in systems in which both are produced, is believed to be at least partially responsible for the toxic and detrimental effects of NO in biological system, this molecule can oxidize lipids such as those found in LDL, or arachidonic acid, it can also act as peroxide substrates for peroxidases such as those found in cyclooxygenase and nitrate DNA[36]. These results are consistent with a previous study conducted by Momma [33]. The lower low-density lipoprotein (LDL) leads to a lower risk of CVD [17]; in the current study significant increases in the [peroxynitrite] were reported between T2DM, and VHD groups and also between AVD and MVD patients proving the OS status.

The correlations between arginase activity (Arg), and arginase1 (Arg I) were examined with biochemical parameters according to the correlation coefficient value for AVD and MVD patients, as shown in (Figure 2).

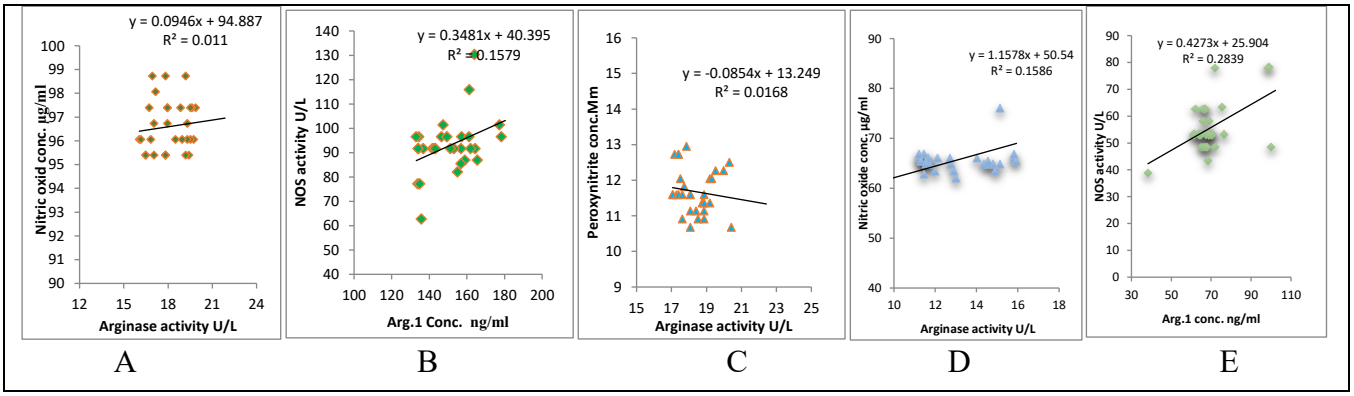


Figure 2: The correlation between **(A&B** for **AVD group**). **A:** arginase activity and nitric oxide level. **B:** arginase.1 level and nitric oxide synthase activity. **(C** for **MVD group**). **C:** arginase activity and peroxynitrite level. **(D&E** for **T2DM group)**. **D:** arginase activity and nitric oxide level. **E:** arginase.1 level and nitric oxide synthase activity.

For the AVD group, the results indicated that the arginase activity was a significant positive moderate correlation with nitric oxide level (r=0.105, p=0.019), while ArgI was positively moderately correlated with NOS activity (r=0.397, p=0.0168). For the MVD group, arginase activity indicated a significant positive moderately correlation (r= 0.13, p=0.0168 with peroxynitrite. On the other hand, T2DM group, arginase activity showed a significant positive moderate correlation with nitric oxide level (r=0.398, p=0.029), Arg I was positively moderately correlated with NOS activity (r=0.533, p=0.002).

In order to estimate the ability of the arginase activity and Arg.1 level to distinguish between the active AVD, MVD patients' group and the T2DM group as a diagnostic biomarker, ROC curve analysis was used. The results showed that the arginase activity in AVD groups had a high ability to differentiate patients from T2DM individuals. The AUC, sensitivity, and specificity were 0.940, 86.7%, and 96.7%, respectively, while in the MVD group were 0.904, 83.3%, and 96.7%, respectively, at the cutoff values of 15.9455 and 16.8233. The value of AVD and MVD prediction was exactly right. On the other hand, Arg.1 level had a good ability to discriminate AVD patients from T2DM individuals. The AUC, sensitivity, and specificity were 0.961, 96.7%, and 90.0%, respectively, while in the MVD group were 0.957, 96.7%, and 90.0%, respectively. At the cutoff value of 76.7844 and 77.2155, it was a good value of AVD and MVD prediction, as shown in Figs. 3 and 4.

Items	AVD (n=30)	MVD (n=30)
AUC	0.940	0.904
SE	0.032	0.047
***p*-value**	0.000	0.000
Cut-off	15.9455	16.8233
Sensitivity	86.7%	83.3%
Specificity	96.7%	96.7%

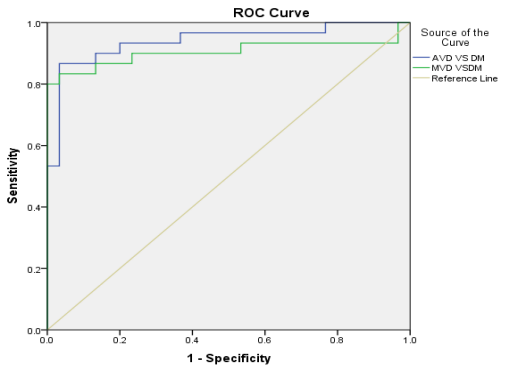


Figure 3: The ROC analysis for the value of predicting the levels of arginase activity (U/L) in patients with AVD and MVD vs T2DM. AUC = area under the curve; SE = standard error.

Items	AVD (n=30)	MVD (n=30)
AUC	0.961	0.957
SE	0.025	0.029
***p*-value**	0.000	0.000
Cut-off	76.7844	77.2155
Sensitivity	96.7%	96.7%
Specificity	90.0%	90.0%

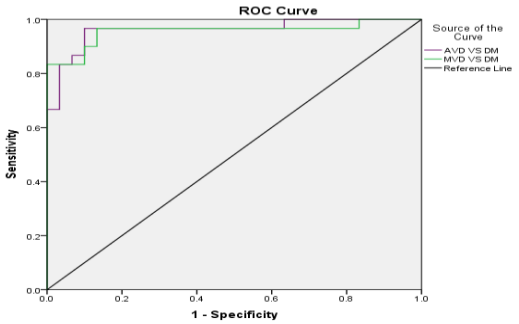


Figure 4: The ROC analysis for the predictive value of circulating levels of Arg.1 Conc. (ng/ml) in AVD and MVD patients vs T2DM.

According to the results of ROC curve analysis, it showed that Arginase activity and Arg.1 Conc. in AVD and MVD groups could represent a perfect predictor for VHD diagnosis. These findings suggest that these two biomarkers could be highly accurate and effective diagnostic tools for distinguishing between affected and unaffected individuals. Their potential clinical utility could be further supported through validation in larger-scale studies.

Conclusions

The significant observed in Inflammation and OS parameters led to the key conclusion that these pathways were demonstrated for the first time through the measurement of arginase and its related pathway markers. Elevated arginase activity, particularly arginase-1, has been associated with issues such as vascular dysfunction in both diseases (T2DM and VHD). This can give an idea for use as a marker for predicting complications of T2DM; it could also be used as a diagnostic criterion for monitoring arginase activity for both diseases.

Acknowledgments:

We would like to thank the University of Baghdad for their partial support of this project. The authors were responsible for the project's funding.

References:

[1] Q. Lu, J. Lv, Y. Ye, Z. Li, W. Wang, B. Zhang, et al., "revalence and impact of diabetes in patients with valvular heart disease," *iScience* ,vol. 27, no. 3, pp. 109084, 2024. https://doi.org/10.1016/j.isci.2024.109084.

[2] L. Shu, Z. Yuan, F. Li, and Z. Cai, "Oxidative stress and valvular endothelial cells in aortic valve calcification," *Biomedicine & Pharmacotherapy*, vol. 163, pp. 114775, 2023. https://doi.org/10.1016/j.biopha.2023.114775 .

[3] F. M. Khaleel, N. N. Oda, and B. A. Abed, "Disturbance of Arginase Activity and Nitric Oxide Levels in Iraqi Type 2 Diabetes Mellitus," *Baghdad Science Journal*, vol. 15, no. 2, pp. 189-191, 2018. http://dx.doi.org/10.21123/bsj.2018.15.2.0189

[4] Y. Chen, F. Xiao, avd R. Wang, "Calcified aortic valve disease complicated with and without diabetes mellitus:the underlying pathogenesis," *Reviews in Cardiovascular Medicine*., vol. 23, no.1, pp.7, 2022 . https://doi.org/10.31083/j.rcm2301007

[5] D. Arcy, S. Coffey, M. A. Loudon, A. Kennedy, J. P. Stuttard , J. Birks , et al., "Large-scale community echocardiographic screening reveals a major burden of undiagnosed valvular heart disease in older people : the OxVALVE Population Cohort Study," *European Heart Journal*, vol. 37, no. 47, pp. 515-3522, 2016. https://doi.org/10.1093/eurheartj/ehw229.

[6] S. Coffey, R. R. Thomson, A. Brown, J. Carapetis, M. Chen, M. E. Sarano, et al., "Global epidemiology of valvular heart disease," *Nature Reviews Cardiology*, vol.18, no.12, pp. 853–864, 2021.
https://doi.org/10.1038/s41569-021-00570-z

[7] S. A. Ibrahim, J. A. Zainulabdeen, and H. M. Jasim, "Levels of Arginase Isoenzymes in Sera of Iraqi Patients with Atherosclerosis and Type 2 Diabetes Mellitus," *Research Journal of Biotechnology*, vol. 14, no.1,
pp. 201-207, 2019.

[8] Z. Li, L. Wang, Y. Ren, Y. Huang, W. Liu, Z. Lv, et al., "Arginase: shedding light on the mechanisms and opportunities in cardiovascular diseases," *Cell Death Discovery*, vol. 8, no. 413, pp.1-14, 2022.
https://doi.org/10.1038/s41420-022-01200-4.

[9] J. A. Zainulabdeen, and A. A. Al-kinani, "New Modified Method for Determination of Nitric Oxide Synthase Activity in Plasma of Vitiligo Patients," *Oriental Journal of Chemistry*, vol. 34, no. 5, pp. 2502, 2018.
http://dx.doi.org/10.13005/ojc/340536.

[10] N. K. Cortas, and N. W. Wakid, "Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method," *Clinical chemistry*, vol. 36, no. 8, pp. 1440-1443, 1990.
https://doi.org/10.1093/clinchem/36.8.1440.

[11] B. E. Vanuffelen, J. V. Der Zee, B. M. D. Koster, J. Vansteveninck, and J. G. Elferink, "Intracellular but not extracellular conversion of nitroxyl anion into nitric oxide leads to stimulation of human neutrophil migration
Biochemical Journal, vol.*330*, no.2, pp.719-722, 1998. https://doi.org/10.1042/bj3300719

[12] R. L. Levine, N. Wehr, J. A. Williams, E. R. Stadtman, and E. Shacter, "Determination of carbonyl groups in oxidized proteins," *Methods in Molecular Biology*, vol. 99, pp. 15- 24, 2000.
https://doi.org/10.1385/1-59259-054-3:15

[13] S. Saeed, M. R. Dweck, and J. Chambers, "Sex differences in aortic stenosis: from pathophysiology
to treatment," *Expert Review of Cardiovascular Therapy*, vol. 18, no. 2, pp. 65–76, 2020.
http://dx.doi.org/10.1080/14779072.2020.1732209.

[14] B. Iung, G. Baron, E. G. Butchart, F. Delahaye, C. G. Bärwolf, O.W. Levang, et al., "A prospective survey of patients with valvular heart disease in Europe: the Euro Heart Survey on Valvular Heart Disease,
European Heart Journal, vol. 24, no.13, pp.1231, 2003. https://doi.org/10.1016/s0195-668x(03)00201-x.

[15] A. Vahanian, F. Beyersdorf, F. Praz, M. Milojevic, S. Baldus, J. Bauersachs, et al., "ESC/EACTS Guidelines for the management of valvular heart disease," *European Heart Journal*, vol. 43, pp. 561–632 ,2021.
https://doi.org/10.1093/eurheartj/ehab395

[16] C. L. Ogden, M. D. Carroll, B. K. Kit, and M K., "Flegal Prevalence of childhood and adult obesity in the united states," *Journal of the American Medical Association*, vol. 311, pp. 806–814, 2014.
http://dx.doi.org/10.1001/jama.2014.732.

[17] S. C. Larsson, A. Wolk, N. Håkansson, and M. Bäck," Overall and abdominal obesity and incident aortic valve stenosis: two prospective cohort studies," *European Heart Journal*, vol.38, no. 28, pp. 2192-2197,
2017. https://doi.org/10.1093/eurheartj/ehx140

[18] I. M. Timón, C. S. Collantes, A. S. Galindo, and F. J. Del, "Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength," *World Journal of Diabetes*, vol. 5, no. 4, pp. 444 –470,2014.
https://doi.org/10.4239/wjd.v5.i4.444.

[19] M. K. Ismael, H. N. Nasser, and M. F. Al. Halbosiy, "Correlation Between Chlamydia pneumoniae Infection and Lipid Profile in Patients with Cardiovascular Diseases," *Iraqi Journal of Science*, vol. 60, no.10,
pp.2129-2135,2019 .https://doi.org/10.24996/ijs.2019.60.10.4.

[20] A. J. S. Coats,“ Common Co-Morbidities in Heart Failure-Diabetes, Functional Mitral Regurgitation and Sleep Apnoea,” *International Journal of Heart Failure*, vol. 1, no.1, pp. 25-41, 2019. https://doi.org/10.36628/ijhf.2019.0004.

[21] N. Fillmore, J. Mori, and G. D. Lopaschuk, “ Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy,” *British Journal of Pharmacology*, vol. 171, no.8, pp. 2080-2090, 2013. https://doi.org/10.1111/bph.12475.

[22] Y. Higashi, “Endothelial Function in Dyslipidemia: Roles of LDL-Cholesterol, HDL-Cholesterol and Triglycerides”., *Cells*, vol. 12, no. 9, pp. 1293, 2023. https://doi.org/10.3390/cells12091293

[23] J. Natorska,“Diabetes mellitus as a risk factor for aortic stenosis: from new mechanisms to clinical implications,” *Kardiologia Polska*, vol. 79, no. 10, pp. 1060-1067, 2021. https://doi.org/10.33963/kp.a2021.0137.

[24] H. K. Sacheat, S. Z. Hussein, and S. S. Al-Mudhaffar,“Estimation of oxidative stress and some related parameters in Iraqi patients with valvular heart diseases,” *Biochemical and Cellular Archives*, vol. 20 , no. 2, pp. 5875-5879, 2020. https://connectjournals.com/03896.2020.20.5875.

[25] M. A. H. Elsanan, I. H. H. H. Tahoon, G. I. Mohamed, S. G. ZeinElabdeen, and I. E. Shehata,“Relationship between inflammatory markers and coronary slow flow in type 2 diabetic patients,” *BMC Cardiovascular Disorders*, vol. 23, no. 244, 2023. https://doi.org/10.1186/s12872-023-03275-y.

[26] A. Marra, A. Bondesan, D. Caroli, and A. Sartorio,“Complete Blood Count (CBC)-Derived Inflammation Indexes Are Useful in Predicting Metabolic Syndrome in Adults with Severe Obesity,” *Journal of clinical Medicine*, vol. 13, no. 5, pp.1353, 2024. https://doi.org/10.3390/jcm13051353.

[27] M. Hussain, M. Z. M. Babar, L. Akhtar, M.S. Hussain, “ Neutrophil lymphocyte ratio (NLR): A well assessment tool of glycemic control in Type-2 diabetic patients,” *Pakistan Journal of Medical Sciences.*, vol. 33, no. 6, pp.1366-1370, 2017. https://doi.org/10.12669/pjms.336.12900.

[28] X. Zhang, R. Wei, X. Wang, W. Zhang, M. Li, T. Ni, et al.,“The neutrophil-to-lymphocyte ratio is associated with all-cause and cardiovascular mortality among individuals with hypertension,’’ *Cardiovascular Diabetology*, vol. 23, no.117, 2024. https://doi.org/10.1186/s12933-024-02191-5 .

[29] G. S. Aljuraiban, F. J. Alharbi, A. O. Aljohi, A. Z. Almeshari, M. N. Alotaibi, S. S. AlShammari, et al., “ Systemic Immune-Inflammation Index in Relation to Diabetes Markers in Saudi Adults: A Retrospective study,’’ *Medicina (Kaunas),* vol. 60, no. 3, pp. 442, 2024. https://doi.org/10.3390/medicina60030442 .

[30] R. K. Ibrahim, K. K. Ghudhaib, and A. A. D. Allawi, “Studying Sex Effect on CTGF, TGF-B1 Levels and Some Relevant Parameters in Iraqi Diabetic Patients with Glomeruli and Renal Tubules Fibrosis,” *Ibn Al-Haitham Journal for Pure and Applied Sciences*, vol. 37, no. 2, pp. 260-269, 2024. https://doi.org/10.30526/37.2.3302

[31] S. Arques, “ Human serum albumin in cardiovascular diseases,” *European Journal of Internal Medicine*, vol. 52, pp. 8-12, 2018. https://doi.org/10.1016/j.ejim.2018.04.014

[32] R. Yin, Z. Ye, H. You, Y.W. MB, M. W. Chen, and T. J. MD, “ Elevated uric acid/albumin ratio as a predictor of poor coronary collateral circulation development in patients with non-ST segment elevation Myocardial infarction,” *Clinical Cardiology*, vol. 47, no.1, pp. e24215, 2024. https://doi.org/10.1002/clc.24215.

[33] T. Y. Momma, and J. I. Ottaviani, “There is no direct competition between arginase and nitric oxide synthase for the common substrate l-arginine,” *Nitric Oxide*, vol.129, pp.16-24, 2022. https://doi.org/10.1016/j.niox.2022.09.002

[34] X. Weiling, F.T. Kaneko, S. Zheng, S. A. A. Comhair, A. J. Janocha, T. Goggans, et al., “Increased arginase II and decreased NO synthesis in endothelial cells of patients with pulmonary arterial hypertension,” *Federation of American Societies for Experimental Biology Journal*, vol.8, pp.1746-1748,

2004. https://doi.org/10.1096%2Ffj.04-2317fje

[35] S. Papi, F. Ahmadizar, and A. Hasanvand, "The role of nitric oxide in inflammation and oxidative stress," *Immunopathologia Persa*, vol.5, no.1, pp. e08, 2019. https://doi.org/10.15171/ipp.2019.08.

[36] R. A. Babel, and M. P. A. Dandekar, "Review on Cellular and Molecular Mechanisms Linked to the Development of Diabetes Complications," *Current Diabetes Reviews*, vol. 17, pp. 457-473, 2021. https://doi.org/10.2174/1573399816666201103143818

[37] A. M. Barciszewska, A. Belter, J. F. Barciszewski, I. G. nska, M. G. Pietraszuk, and M. ZN. Barciszewska, "Mechanistic Insights on Metformin and Arginine Implementation as Repurposed Drugs in Glioblastoma Treatment," *International Journal of Molecular Sciences,* vol. 25, no. 9460, pp. 1-15, 2024. https://doi.org/10.3390/ijms25179460