



## Study the Arginase Activity and Its Correlation with Liver and Kidney Functions for Patients with Type-2 Diabetes Mellitus in Nineveh Governorate

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### Abstract

**Background:** Arginase plays an essential role in type 2 diabetes mellitus.. **Aim of the study:** The study aimed to determine the activity of arginase in individuals patients with diabetes mellitus type 2 (DMT2) and study the correlation between arginase activity and the investigated variables Patients and material methods: The study included 201 adults divided into two groups: those with DMT2 (136 people) and control groups (65 people). Blood non-fasting persons had their determination of arginase activity, glucose, glycated haemoglobin (HbA1c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine levels . **Results:** The findings showed that the level of arginase activity in patients with DMT2 was significantly increased ( $p \leq 0.0001$ ) in comparison with control groups, but there was no significant differentiation in the activity of arginase for both gender and age. A positive significant correlation ( $P=0.01$ ) was found between Glucose ( $r=0.584$ ), HbA1c ( $r=0.454$ ), AST(GOT) ( $r=0.433$ ), ALT (GPT) ( $r=0.295$ ), ALP ( $r=0.639$ ), Urea ( $r=0.667$ ), and Creatinine ( $r=0.658$ ). **Conclusion:** In patients with DMT2, increase the activity of arginase plays an active role through it is direct effect on liver and kidney functions.

**Keywords:** Arginase, diabetes mellitus type 2, Glucose , HbA1c %.

### 1.Introduction

Diabetes mellitus is considered one of the common diseases that lead to death in the absence of follow-up of the disease, as it was found that diabetes is on the increase, and in a study that recorded in 2017, approx 462 million people worldwide were influenced by type 2 diabetes, accounting for 6.28 % of the global population (4.4 % of those aged 15-49 years, 15 % of those aged 50-69 years, and 22 % of those over the age of 70 years) [1].

Besides, diabetes mellitus has different types, the most important of which is type 1, called insulin-dependent Juvenile Diabetes. The second type, which is called adult diabetes, is not dependent on insulin [2]. Arginase (EC 3.5.3.1) catalyze L-arginine hydrolysis into L-ornithine as well as urea and needs for its reaction cofactor, especially  $Mn^{+2}$ . Arginase is a part of the urea cycle that regulates L-arginine level [3]. Arginase is one of the important enzymes involved in the metabolism and formation of urea [4,5]. Arginase has two isozymes, the first is found in the cytosol in the liver, which plays a major role in the urea cycle, and

the second is located in the mitochondria of the kidneys and prostate [4,6].

Besides, a study has found that patients with DMT2 have an increase in the activity of arginase [7], and in other studies conducted on rats exposed to diabetes, they have an increase in the activity of the arginase. It was also found that patients with DMT2 have a failure in liver function and a rise in activity of each AST,ALT and ALP [8,9]. Furthermore, It was also found that diabetic patients get a rise in glycated haemoglobin , especially untreated patients, compared to treated patients and difference in liver functions. In addition, DMT2 suffer from impaired kidney function through high levels of urea and creatinine [10,11,12].

Our study aimed to measuring the activity of arginase in the sera of patients with DMT2 and it is relationship to liver and kidney function.

### 2. Materials and Methods

#### 2.1.population study:

The study was conducted on 201 patients who were divided into two groups, the first of which was

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136 patients with type 2 diabetes of both sexes (78 females (58) males, and their ages ranged from (35-75). The healthy group included 65 people without Diabetes and other diseases of both sexes (33 females (32) males; their ages ranged from (35-68). Diabetic patients with other diseases such as heart, cancer and kidney disease were excluded . The study was conducted on diabetic patients who visit the clinics of Al-Wafa Center and the outpatient medical clinics licensed by the Nineveh Health Department. The patients were divided into two groups according to the age group, the first group was (35-45) year, and the second group was ( $\geq 46$ ).

Furthermore, the Ethics Committee of the University of Mosul (College of Science) and the Nineveh Health Department followed the study protocol. All participants were provided with an informed consent questionnaire form to participate in the research.

## 2.2. Samples Collection:

2.2.1. Serum samples were obtained by drawn 5 ml of venous blood, then placing it in a gel tube and leaving it for 15 minutes at a temperature of 37°C and centrifuging 3000xg, then separating the serum and keeping it at a temperature of -20 °C until the necessary tests are performed.

2.2.2. Whole blood 2 ml of venous blood was withdrawn from only patients with diabetes mellitus type 2 to conduct a glycated haemoglobin test and placed in tubes containing an anticoagulant substance EDTA.

## 2.3. Variables Assay

### 2.3.1 Estimation of the arginase activity

Arginase activity was measured by following a modified method [13]. The activity of arginase represents the amount of enzyme needed to convert micromol from arginine to ornithine per minute.

### 2.3.2 Glycated haemoglobin measurement

The glycated haemoglobin was measured using the German-made On-Call device, using the company's supplied kit G136-111.

### 2.3.3 Measurement of glucose concentration

The serum glucose concentration was measured using a colorimetric enzymatic method, using a kit manufactured by the Randox GL364 [13].

### 2.3.4 Measuring the activities of ALT, AST and ALP.

Using the Japanese-origin Fuji automated analyzer device and follow the manufacturer's instructions

### 2.3.5 Measuring the concentration of urea and creatinine

The concentrations of urea was measured using the kit supplied by biosystems company [15] and creatinine was measured using the kit supplied by Biolabo Company [16].

## 2.3. Statistical Analysis

The analysis was done using SPSS version 25. The data were analyzed. Mean  $\pm$  standard deviation (SD) was obtained; two groups were comparable with an independent T-test. The Pearson correlation coefficient is used to explore the correlation of serum arginase activity with the studies variables. The 0.0001 P-values were taken into account statistically significant[17].

## 3. Results and Discussion

Diabetes mellitus Type 2 (DMT2) is a common disease, and failure to monitor it and take the necessary treatment leads to death [18,19]. DMT2 leads to an imbalance in the body's functions, such as a destabilization in the functions of the liver and kidneys [20]. And in turn, it affects the rest of the body's functions as it affects the disorder of enzymes such as arginase [21,22,23]. Therefore, in this study, we decided to study the efficacy of the arginase activity and its correlation with the studied variables, included glucose, glycated haemoglobin (HbA1c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine. Table 1 shows the characteristics of the population study; where the mean  $\pm$  SD age of the controls group and patients with DMT2 is 48.3  $\pm$  10.6 years and 50.7  $\pm$  10.3 years, respectively and that the percentage of females (%57.4) is more affected by the disease than males (%42.6)

The results also demonstrated that level arginase activity in patients with DMT2 (39  $\pm$  6.6 U/ml) rise significantly ( $p \leq 0.0001$ ) when compared to control group, as shown in Table (2) and Figure (1). [24,25]. It was discovered that diabetes patients have a significant 1.7-fold increase in enzyme activity. This discovery is consistent with Kashyap et al. findings. These were based on a small group of 12 diabetes patients. They claim that arginase

activity is high and that insulin infusion can lower it [24]. Reduced substrate concentration is another important mechanism that could explain decreased nitric oxide (NO) generation. Arginase's accelerated

enzymatic conversion of arginine to ornithine. Arginase is consistent with this nitrogen oxide species NOS vasodilatory properties are reduced. [24,26].

Table 1: Shows the characterize of population study

Population study	Control group; n =65	Patient with DM T2; n =136
Age ( years); range	48.3±10.6; (35-68)	50.7±10.3; (35-75)
F(% )/ M(%)	33 (50.8%)/ 32(49.2%)	78 (57.4%)/ 58 (42.6%)
HbA1c %		9.1± 2.4

DM T2 = Diabetes mellitus type 2; n=Number; F =Female; M=Male

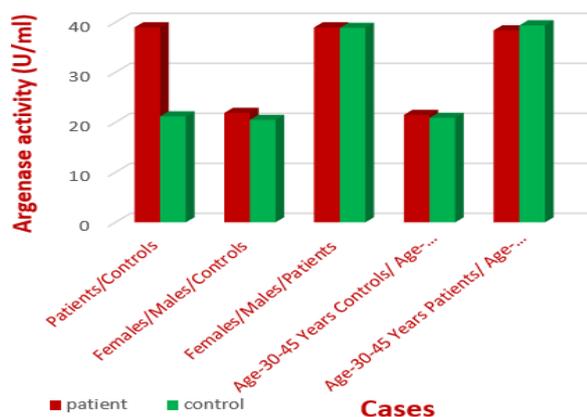
Some factors affecting arginase activity were also studied, including gender and age in patients and control group. Table (2) and Figure 1 show that the sex factor does not affect arginase enzyme activity in patients and control group. The arginase activity is higher in males than females in each of the

patients and Control group, but there is no significant difference

Also, there was no effect of the age factor on the activity of the arginase in patients and control group. However, the activity of arginase increases with age but without a significant difference.

Table 2: Shows study the activity of arginase in the sera of DM T2 patient compared to control group

Arginase activity (U/ml); Mean ±SD					
Control group		Patient T2DM		p-value	
21.2±3.5		***39±6.6		0.0001	
Arginase activity according (U/ml) sex factor					
M	F	p-value	M	F	P-value
21.9±3.7	20.5±3.3	0.119	39±6.8	38.8±6.4	0.903
Arginase activity (U/ml) according to age factor					
20.9±3.3		0.504	21.5±3.7		0.382
*** Significant at the level of ( $p \leq 0.0001$ )					



**Figure 1:** Shows study the activity of arginase in the sera of DM T2 patient

Table (3) compares the studied variables, including glucose, glycated haemoglobin, liver and kidney functions in DMT2 patients and their comparison with controls. A significant increase comparison with control group. A significant increase ( $p \leq 0.0001$ ) in the glucose concentration for patients compared to control group agrees with (kashyab et al. 2008) [24]. Also, increased HbA1c % agrees with (shatanawi et al. 2017)[24,26,27].

Studies have shown that there is a significant increase ( $p \leq 0.0001$ ) in AST, ALT and ALP in patients with DMT2 compared to control group, and this is consistent with [28,29]. This study is comparable to a study conducted by Salman et al [29] and the two additional studies conducted by Idris et [30], who reported an obvious rise in ALT and AST. Ni et al. Htet, also conducted a study on patients with diabetes in Malaysia, and the study showed that these patients had liver function tests that were abnormal [31]. To supplement, Wang et al. had conducted a study in which they examined DMT2 patients and control. Their research discovered that ALT was significantly associated with a heightened risk of type 2 diabetes in the Chinese population [32]. This study discovered that ALT was significantly associated with an increased risk of DMT2 in Chinese individuals. The occurrence of these enzymes could be attributable to the significant hepatotoxic effect intense insulin resistance (measured as insulin resistance or HbA1c) may lead to higher levels of fatty acids (i.e., free fatty acids) in the liver [28,33]. This accumulation may be due to disruption of the cell membrane, malfunction of the mitochondria, and increased production of substances like transaminases, fatty acids, and toxic metabolites. These disruptions may also cause mitochondrial dysfunction, oxidative stress, and a rise in pro-inflammatory cytokines [34,29].

The results also showed in Table 3 the effect of the of arginase activity on kidney function, which included

urea and creatinine, where the results showed a significant increase ( $p \leq 0.0001$ ) for each of urea, creatinine in patients with DMT2 compared to control group, this is consistent with other studies [35,36,37,38].

According to this study, high concentrations of urea and creatinine in the blood of diabetes patients indicate a pre-renal disease [36]. In 2017, Abdul Rahman Al-Dakhil conducted a study in Saudi Arabia that revealed a comparison [39]. Iraq had the highest prevalence of diabetic nephropathy among arab countries, whereas the United Arab Emirates had the lowest majority in Bahrain [39].

**Table 3:** Shows the level of the variable study in the patients with D2M

Variables	Control group	Patient DMT2	p-value
	Mean $\pm$ SD		
Glu. mg/dl	88.1 $\pm$ 8	***217.1 $\pm$ 75.4	0.0001
ASTU/L	15.5 $\pm$ 3.5	***22 $\pm$ 6.6	0.0001
ALT U/L	16.4 $\pm$ 3.2	***21.4 $\pm$ 4.1	0.0001
ALP U/L	90.4 $\pm$ 55	***159.5 $\pm$ 55	0.0001
Urea mg/dl	22.3 $\pm$ 55	***40 $\pm$ 55	0.0001
Creatinine mg/dl	0.61 $\pm$ 0.2	***1.2 $\pm$ 0.4	0.0001

\*\*\* Significant when the p-value at the level of ( $p \leq 0.0001$ )

#### Correlation of arginase activity with variables study

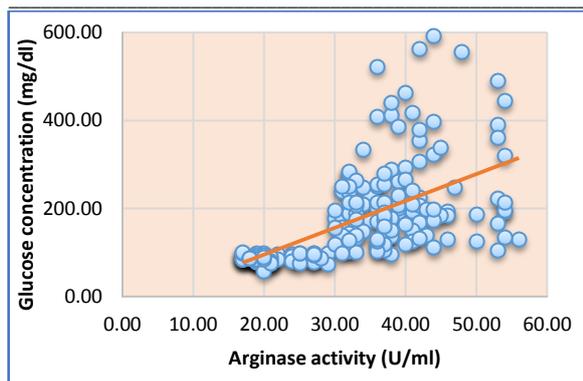
Table 4 and Figure 2 show the correlation of arginase activity was studied with the variables, which included glucose, HbAc%, AST, ALT, ALP, urea and creatinine

#### 1. Correlation of arginase activity with glucose

The results showed in Table (4) and Figure (2,1) that there is a positive relationship between the activity of arginase and the concentration of glucose in the blood. This result agrees with [40]. While our study does not agree with Shatanawi and Momani, 2017. Where they founded that the levels of arginase do not show any significant relationship with the concentration of blood sugar [25].

**Table4:** Shows the correlation of arginase activity with the variables study in patients with DMT2 compare with control group

Biochemical parameters	Arginase r (p-value)
Glucose mg/dl	**0.584( 0.01)
HbA1c %	**0.454( 0.01)
AST U/L	**0.466( 0.01)
ALT U/L	**0.513( 0.01)
ALP U/L	**0.639( 0.01)
Urea mg/dl	**0.667( 0.01)
Creatinine mg/dl	**0.658( 0.01)



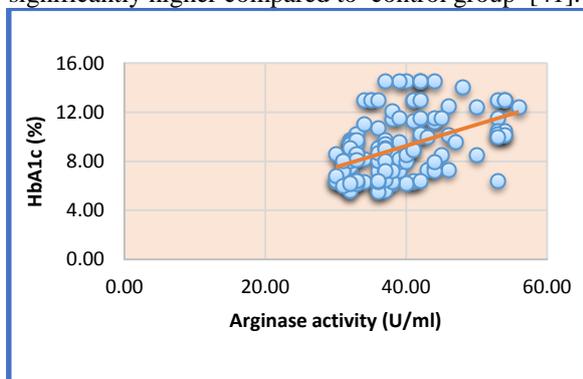
**Figure 2.1:** Shows the correlation of arginase activity with glucose concentration in patients with DMT2

### 2-correlation of arginase and HbA1c

The results showed in Table (4) and Figure (2, 2) that there is a positive correlation between arginase activity and HbA1c. This is consistent with a previous study [25]. That found a positive correlation between the arginase activity and HbA1c because that chronic exposure to high glucose levels is necessary to increase arginase activity .

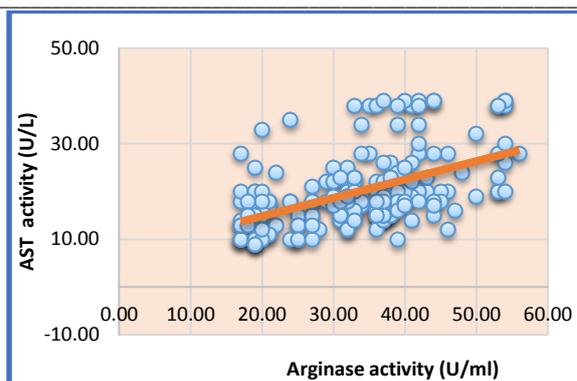
### 3-correlation of arginase and liver function

The results showed in Table (4) and Figure (2.3), (2.4) and (2.5) a significant increase and a positive correlation between the activity of arginase and liver function in patients with DMT2 compared to control group AST, ALT and ALP. This result is consistent with a study that found that the results of liver function tests for patients with DMT2 were significantly higher compared to control group [41].

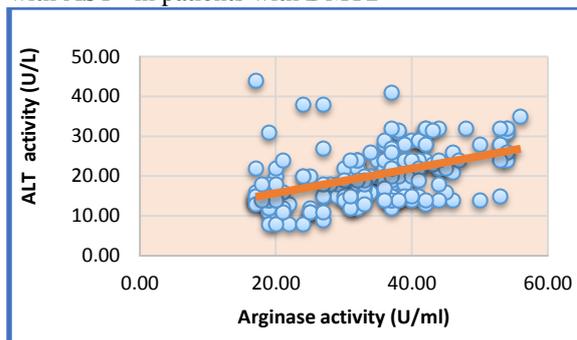


**Figure 2.2:** Shows the correlation of arginase activity with HbA1c in patients with DMT2

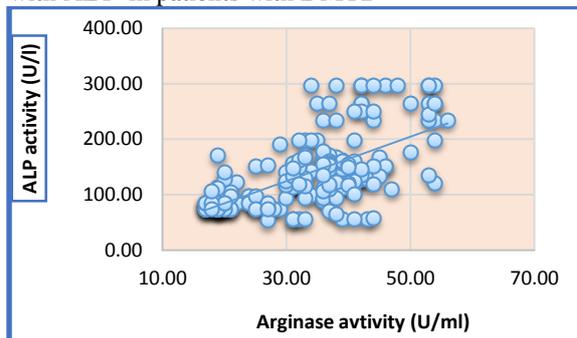
The positive correlation of arginase activity with these enzymes is due to the toxic effect of the liver due to the fatty acids that are formed in excess quantities on the liver cells as a result of insulin resistance [33].



**Figure 2.3:** Shows the correlation of arginase activity with AST in patients with DMT2



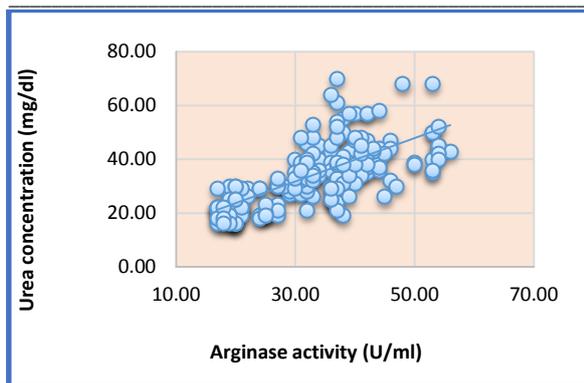
**Figure 2.4:** Shows the correlation of arginase activity with ALT in patients with DMT2



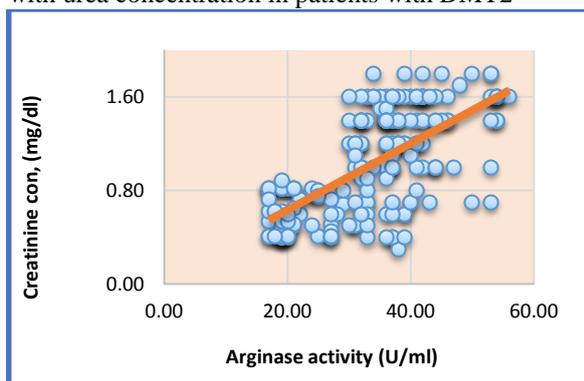
**Figure 2.5:** Shows the correlation of arginase activity with ALP in patients with DMT2

### 3-correlation of arginase and kidney function urea and creatinine

The results showed in Table (4) and Figure (2.6), (2.7) a significant positive correlation between the activity of arginase and kidney function in patients with DMT2. A study found that there was a agree with [42]. The findings of this study also revealed that poorly regulated blood sugar levels could result in high glucose levels and blood urea levels, putting diabetes individuals at a higher risk of developing nephropathy. This is in line with the findings of a prior study, which demonstrated that the most common cause of increasing kidney injury is hyperglycemia [36].



**Figure 2.6:** Shows the correlation of arginase activity with urea concentration in patients with DMT2



**Figure 2.7:** Shows the correlation of arginase activity with creatinine concentration in patients with DMT2

### Conclusions

The increased activity of arginase in patients with DMT2 directly affects liver and kidney functions. Besides, disorders in liver and kidney function generally coincide DMT2 diabetes in Nineveh Governorate. Therefore, screening for liver and kidney dysfunction in diabetic patients is required to prevent further complications associated with it with the liver and kidneys.

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### Conflicts of interest

The author does not report any conflicts of interest.

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