

## Evaluate The Correlation Serum Afamin Level With Some Biochemical Parameters In The Sera Of Patients With Type 2 Diabetes Mellitus

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### ARTICLE INFO

### ABSTRACT

#### Keywords

Afamin,  
Oxidative Stress,  
Diabetes Mellitus,  
Uric Acid, Lipid  
Profile.

The purpose of the study was to investigate the relationship between afamin and lipid profiles, renal function, liver enzymes, oxidative stress, and patients with type 2 diabetes (T2DM). The study included 150 adults divided into two groups: 75 patients with type 2 diabetes mellitus and 75 healthy subjects of both sexes; their ages ranged between (35-65 years). Biochemical parameters included (afamin (AFM), Total Cholesterol, HDL, LDL, TG, VLDL, AST, ALT, ALP, urea, creatinine, MDA, GSH, uric acid, and calcium/phosphorous ratio were measured. Results: the results of the study showed that a significant positive correlation was found between the level of AFM with LDL ( $r=0.479$ ;  $p=0.01$ ) TG ( $r=0.256$ ;  $p=0.027$ ), ALT ( $r=0.326$ ;  $p=0.01$ ), AST ( $r=0.325$ ;  $p=0.01$ ), ALP ( $r=0.427$ ;  $p=0.01$ ), creatinine ( $r=0.277$ ;  $p=0.016$ ), urea ( $r=0.436$ ;  $p=0.01$ ), Uric acid ( $r=0.477$ ;  $p=0.01$ ) and MDA ( $r=0.463$ ;  $p=0.01$ ). In contrast, afamin negatively correlates with GSH ( $r=-0.460$ ;  $p=0.01$ ). Conclusion: Afamin is associated positively with LDL and TG, liver enzyme (ALT, AST, and ALP), renal function (creatinine and urea), and oxidative stress (Uric acid and MDA), while it negatively correlates with GSH; thus, increasing the level of afamin may induce type 2 diabetes and its complications, such as fat metabolism, which has consequences related to heart disease and atherosclerosis. In addition, a high level of afamin may lead to disturbances in liver enzymes, kidney function, and oxidative stress.

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## 1. Introduction

Diabetes is one of the most common chronic diseases in all countries. Epidemiological data, according to the International Diabetes Federation, show hazardous values, which indicate the death of more than 4 million people and the injury of more than 450 million adults with diabetes in 2019, and this number is expected to more than double after than 10 years, in addition to the large financial losses that are spent on treatment and medicines for diabetics [1]. There are two types of diabetes: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Type 2 diabetes mellitus is characterized by an increase in glucose levels in the blood due to the cells' resistance to the action of insulin or the insufficient production of insulin by the beta cells in the pancreas to meet the need for excess glucose. Thus, glucose accumulates in the blood [2]. Type 2 diabetes is the most prevalent type of diabetes; it's also considered the most dangerous because its symptoms do not appear at the onset of the disease, which could lead to severe and long-term complications if not controlled, such as cardiovascular disease, diabetic neuropathy, nephropathy, eye disease, skin and mouth diseases, damaged nerves in the legs and feet and other complications [3]. One of the most critical factors that helps control diabetes is changing the lifestyle by following a healthy diet, practicing physical activity, getting rid of excess weight, and taking some medications to prevent the disease [4]. Afamin is a glycoprotein discovered in 1994, mainly excreted in the liver [5]. It is considered the fourth member of the albumin gene family that includes (Albumin,  $\alpha$ -Fetoprotein, and vitamin D-binding protein) [6]. In addition to the abundance of afamin in the blood, the brain, kidneys, testes, and ovaries are other organs from which afamin is secreted [7]. Studies have shown a strong correlation between afamin and some parameters that lead to diabetes, such as glucose, insulin resistance, and dyslipidemia [8, 9]. Afamin plays an essential role in glucose and lipid metabolism. Human plasma afamin is a specific binding protein for vitamin E, Vitamin E is a powerful antioxidant with anti-inflammatory effects [8]. A study showed that afamin carries vitamin E across the blood-brain barrier in a suitable cell culture model. It protects neurons from oxidative stress with its antioxidant function [10]. A study on obese and non-diabetic patients showed a significant negative correlation between afamin with low-density lipoprotein (LDL) and high-density lipoprotein (HDL), indicating that afamin may play a critical role in developing dyslipidemia and heart disease [11]. In addition, insulin resistance in patients with type 2 diabetes leads to increased lipolysis and formation of fatty acids, as increased accumulation of fatty acids in the liver leads to liver poisoning, resulting in increased



secretion of liver enzymes [12]. Urine afamin can also be used as an indicator of kidney injury, where the researchers found a significant increase in the level of urinary afamin and a significant increase in the ratio of afamin to creatinine [7]. Our study aimed to study the correlation between afamin level and some biochemical parameters in patients with type 2 diabetes mellitus.

## **2. Material and Method:**

### **2.1. Population Study:**

The current study was conducted from the (1st of September 2022 to the 25<sup>th</sup> of December 2022), at Azadi Teaching Hospital in Duhok Governorate. The study was performed with (150 adults), including (75 patients) suffering from type 2 diabetes mellitus (T2DM) (50 females and 25 males, ages ranging from 35 to 65 years). They attended Azadi Teaching Hospital, licensed by the Ministry of Health in Duhok-Iraq. Seventy-five (75) uninfected adults were considered a control group of both sexes (38 females 37 males aged 35 to 65). The ethics commission at Mosul University and the Duhok Department of Health reviewed the study protocol, and all subjects were informed in a written form of informed consent.

### **2.2. Sample Collection**

After informed consent was obtained from all participants. Serum samples were obtained by drawing (7ml) of venous blood; this blood was placed in a gel tube, Left for (20 minutes) at room temperature, then centrifuged at (3000 xg) for (10) minutes then separate the serum and kept it at (-20° C) until the examination performed [13].

### **2.3. Parameters Assay**

#### **2.3.1. Estimation of Afamin (AFM) Level**

The level of afamin is measured by using a commercial human afamin (AFM) ELISA kit (enzyme-linked immunosorbent assay) [9], donated by Wuhan fine biotech company catalog no. (EH2493).

#### **2.3.2. Estimation of Lipid Profile Level**

Lipid profile levels, including total cholesterol (TC), high-density lipoprotein (HDL), and Triglyceride (TG), were measured by using a commercial Biolabo kit manufactured by Biolabo SAS, Les Hautes Rives, 02160, Maizy, France [14]. Low-density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) were estimated by the Friedewald formula [16].



$$\text{LDL-C (mg/dl)} = \text{TC} - \text{HDL-C} - \frac{T.G}{5}$$

$$\text{VLDL-C (mg/dl)} = \frac{T.G}{5}$$

### 2.3.3 Estimation of liver Enzyme (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP)), Calcium, Phosphorous, and Calcium/phosphorous ratio:

ALT, AST, ALP, Calcium, and phosphorous were estimated using the ready-made analysis kit from Biolabo SAS, Les Hautes Rives, 02160, Maizy, France. Kinza device was used to conduct these tests. The method includes the special reagents for driving tests placed in the place designated for them in the machine. Serum samples are placed in special containers and inside the designated locations inside the device. The sample information, such as the name, age, sample number, and the number of required tests, is entered through the entry screen, then press the start button to turn on the device; after that, the results of the tests appear automatically on the screen. The calcium/phosphorous ratio was measured by dividing the calcium value by the phosphorous value.

### 2.3.4. Estimation of Renal Function (Urea and Creatinine)

Urea and creatinine were measured using enzymatic colorimetric and kinetic methods using the Biolabo kit manufactured by Biolabo SAS, Les Hautes Rives, 02160, Maizy, France [16].

### 2.3.5. Estimation of (Uric Acid (UA), Malondialdehyde (MDA) and Glutathione (GSH))

Uric acid (UA) was measured according to the uricase method. Using the Biolabo kit manufactured by Biolabo SAS, Les Hautes Rives, 02160, Maizy, France [16]. Malonaldehyde (MDA) was estimated in serum. The principle of this method depends on the reaction in the acidic medium between malonaldehyde and thiobarbituric acid (TBA); a colored product and its optical density were measured at a wavelength of 532 nm [17]. Glutathione was estimated in blood serum using the modified method used by the researchers[18], where the method depends on the reduction of the thiol group of glutathione using an Ellman's reagent containing a substance [5,5-Dithio-bis(2-nitrobenzoic acid)] (DTNB) As a result of the reduction a colored substance is produced and its absorption intensity is measured at a wavelength (412nm) and the concentration of the product formed depends on the amount of glutathione present in the blood serum.



## 2.4. Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 25. The results are expressed as mean  $\pm$  standard deviation (SD); an independent T-test was used to compare the two groups. Also, Pearson's correlation coefficient was used to explore the relationships between the afamin level and the variables studied. P-values of 0.05 were considered statistically significant [19].

## 3. Results and discussion

The current study aimed to investigate the correlation of afamin with lipid profiles, liver enzyme, kidney function, oxidative stress, and the calcium/phosphorus ratio in patients with type 2 diabetes.

### 3.1. The Level of Afamin in Patients' Serum with Type 2 Diabetes Mellitus

According to the results we obtained, there was a significant increase ( $p \leq 0.0001$ ) in the level of afamin ( $63.5 \pm 12$  ng/ml) for patients with type 2 diabetes mellitus (T2DM) compared to the control group ( $30.3 \pm 14.4$  ng/ml), as shown in Table (1), and this is consistent with the results obtained by Kurdiova et al., 2021 [20]. A large population study including nearly 20,000 subjects confirmed that afamin is strongly associated with diabetes-related phenotypes, Adipose tissue dysfunction, inflammation, and insulin resistance. Clinical data indicated that the concentration of afamin was significantly higher in patients with Type 2 diabetes mellitus (T2DM) than in healthy subjects. Therefore, afamin is likely a promising biomarker for predicting type 2 diabetes [9].

Table: The level of Afamin in Patients with Type 2 Diabetes Mellitus

Afamin level	Mean $\pm$ SD	
	Patient n=75	Control n=75
Afamin (ng/ml)	***63.5 $\pm$ 12	30.3 $\pm$ 14.4
***significant at ( $p \leq 0.0001$ ); n= number; ml= Milliliter; ng= nanogram; SD=standard deviation		



### 3.2. The Level of Biochemical Parameters in Patients' Serum with Type 2 Diabetes Mellitus.

#### 3.2.1. Serum Lipid Profile Level in Patients with Type 2 Diabetes Mellitus

Table 2 shows that patients with type 2 diabetes (T2DM) have a significantly increased ( $p \leq 0.0001$ ) cholesterol level ( $197.7 \pm 35$  mg/dl) for patients with type 2 diabetes mellitus compared to control group ( $168 \pm 30.5$  mg/dl), LDL level ( $118.2 \pm 37$  mg / dL) for patients compared to the control group ( $100.6 \pm 32$  mg / dL), and TG level ( $210.4 \pm 123.1$  mg / dL) for patients with type 2 diabetes compared to ( $126.4 \pm 70$  mg / dL) for the control group. As well as a significant increase of VLDL ( $43.3 \pm 27$  mg / dL) for patients with type 2 diabetes compared to the control group ( $25.2 \pm 14$  mg / dL;  $p \leq 0.0001$ ). These results agreed with those obtained by (Sparks, Sparks, & Adeli, 2012) [21]. While the HDL level shows a significant decrease ( $p \leq 0.0001$ ) ( $37.5 \pm 7.4$  mg/dL) for patients with type 2 diabetes compared to the control group ( $46.2 \pm 8.4$  mg/dL). Dyslipidemia is a feature of diabetes, as it increases the incidence of atherosclerosis and vascular and heart diseases. The disorder of carbohydrate metabolism in diabetic patients leads to a disorder of fat metabolism, and insulin resistance in patients with type 2 diabetes works to reduce the response of cells to insulin, in addition to increasing the decomposition of fats in adipose tissues and increasing the release of free fatty acids into the blood, increasing fatty acids in the blood leads to an increase in the build-up of fats in the liver cells, and thus causes an imbalance in the metabolism of blood fats and liver fats [22]. Therefore, to control this dysfunction, a healthy lifestyle can be followed by practicing as little physical activity as possible, trying to lose weight, and eating a healthy diet of saturated fats. Where medical treatment can be initiated if lifestyle changes are insufficient [23].

Table 2: The Lipid Profile Level in Patients with Type 2 Diabetes Mellitus

Parameters	Mean $\pm$ SD	
	Patient, n=75	Control, n=75
Cholesterol(mg/dl)	***197.7 $\pm$ 35	168 $\pm$ 30.5
HDL (mg/dl)	***37.5 $\pm$ 7.4	46.2 $\pm$ 8.4
LDL (mg/dl)	***118.2 $\pm$ 37	100.6 $\pm$ 32
VLDL (mg/dl)	***43.3 $\pm$ 27	25.2 $\pm$ 14
TG (mg/dl)	***210.4 $\pm$ 123.1	126.4 $\pm$ 70

mg (Milligram); dl (Deciliter); n (number); SD (standard deviation); TG (Triglyceride); HDL (High-density lipoprotein); LDL (Low-density lipoprotein); VLDL (Very low-density lipoprotein); \*\*\* Significant when the p-value at the level of ( $p \leq 0.0001$ )

### 3.2.2. Serum Liver Enzymes Activity (ALT, AST, and ALP) in Patients with Type 2 Diabetes Mellitus

Table 3 shows that patients with type 2 diabetes mellitus have significantly increased ( $p \leq 0.0001$ ) for ALT ( $48.2 \pm 10.1$  U/L patients, vs. controls  $21 \pm 4.7$  U/L), AST ( $49.2 \pm 7.6$  U/L patients, vs. controls  $19.2 \pm 4$  U/L), and ALP ( $133.1 \pm 44$  U/L patients, vs. controls  $89 \pm 21$  U/L); these results came consistent with Alramadhany et al., 2021 [12]. Elevation of liver enzymes is an important indicator of liver dysfunction due to increased inflammatory stimuli and fatty acids in the liver due to increased insulin resistance in type 2 diabetes mellitus [12]. Alkaline phosphatase is mainly found in the liver and bones, so when these tissues are damaged due to some pathological conditions such as type 2 diabetes, this enzyme is released into the bloodstream [12].

Table 3: The Liver Enzymes Activity (ALT, AST, and ALP) in Patients with Type 2 Diabetes Mellitus

Parameters	Mean $\pm$ SD	
	Patient, n=75	Control, n=75
ALT (U/L)	***48.2 $\pm$ 10.1	21 $\pm$ 4.7
AST (U/L)	***49.2 $\pm$ 7.6	19.2 $\pm$ 4
ALP (U/L)	***133.1 $\pm$ 44	89 $\pm$ 21

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: alkaline phosphatase; U (unit); L (Liter); n (number); SD (standard deviation); \*\*\* Significant when the p-value is at the level of ( $p \leq 0.0001$ ).

### 3.2.3. Serum Renal Function (Creatinine and Urea) in Patients with Type 2 Diabetes Mellitus

The results in Table (4) show a significantly increased ( $p \leq 0.0001$ ) for creatinine level ( $1.4 \pm 0.7$  mg/dl patients, vs.  $0.8 \pm 0.1$  mg/dl controls) and urea level ( $42 \pm 7.4$  mg/dl patients, vs.  $25.3 \pm 4$  mg/dl controls), these results agrees with (Alramadhany et al., 2021; Chen et al., 2017) [12]. Diabetes may damage the kidney's filtration system and reduce the ability to clean the blood of waste products, thus raising urea and creatinine levels [24].





Table 4: The Level of Renal Functions (Creatinine and Urea) in Patients with Type 2 Diabetes Mellitus

Parameters	Mean± SD	
	Patient, n=75	Control, n=75
Creatinine(mg/dl)	***1.4 ±0.7	0.8 ±0.1
Urea (mg/dl)	***42 ±7.4	25.3 ±4
mg (Milligram); dl (Deciliter); U (unit); n (number); SD (standard deviation); *** Significant when the p-value at the level of ( $p \leq 0.0001$ )		

### 3.2.4. The Level of (Uric acid, MDA, and GSH) in the Serum of Patients with Type 2 Diabetes Mellitus

Table 5 shows that uric acid has a significantly increased ( $p \leq 0.0001$ ) in the patients' group ( $9 \pm 1.4$  mg/dl) compared to the control group ( $4.2 \pm 1$  mg/dl); this is consistent with (K. Singh et al., 2019) [25]. Hyperuricemia may indicate nephropathy or may be due to many factors, such as insulin resistance, obesity, and metabolic syndrome. Otherwise, uric acid is an antioxidant, so it increases as a response to the oxidative stress state for patients [25]. Also, the results showed a significant increase ( $p \leq 0.0001$ ) of the Malonaldehyde (MDA) level for patients with type 2 diabetes mellitus ( $6 \pm 0.7$   $\mu\text{mol/L}$ ) compared to the healthy group ( $1.7 \pm 0.4$   $\mu\text{mol/L}$ ). This agrees with (Aouacheri, Saka, Krim, Messaadia, & Maidi, 2015) [26]. The reason for the increase in malonaldehyde is due to the increase in oxidative stress, which is associated with an increase in blood sugar in diabetic patients, which leads to an increase in free radicals and lipid peroxidation, and thus an increase in malonaldehyde [26]. In contrast, a significant decrease ( $p \leq 0.0001$ ) showed in the level of Glutathione (GSH) in the patients' group ( $0.8 \pm 0.1$   $\mu\text{mol/L}$ ) compared to the healthy group ( $2 \pm 0.5$   $\mu\text{mol/L}$ ). The decrease in glutathione, one of the types of antioxidants that contribute to the removal of free radicals, indicates an increase in its consumption rate or an increase in oxidative stress.





Table 5: The Level of Uric Acid, MDA, and GSH in Patients with Type 2 Diabetes Mellitus

Parameters	Mean± SD	
	patients, n=75	Control, n=75
Uric acid (mg/dl)	***9 ±1.4	4.2 ±1
MDA (µmol/L)	***6 ±0.7	1.7 ±0.4
GSH (µmol/L)	***0.8 ±0.1	2 ±0.5

mg :Milligram; dl :Deciliter; µmol :Micromole; L :Liter; n: number; SD: standard deviation; MDA: Malonaldehyde; GSH: Glutathione; \*\*\* Significant when the p-value at the level of (p ≤0.0001).

### 3.2.5. The Level of Calcium, Phosphorous, and Calcium/phosphorous (ratio) in the Serum of a Patient with Type 2 Diabetes Mellitus

Table 6 shows that the level of phosphorous increased significantly (4.4 ±1.5 mg/dl; p≤0.05) compared to the control group (3.9 ±0.7 mg/dl). There is a significant difference between calcium (9.7±0.5 mg/dl patients vs. 9.6 ±0.4 mg/dl controls) and calcium/ phosphorus ratio (2.2±0.4 patients vs. 2.4±0.6 controls). Calcium and phosphorous are essential minerals that must be available in the human body, and they can be obtained in many foods and from some nutritional supplements. Calcium and phosphorous have many benefits for the human body, as they are abundant in the bones and teeth. It is necessary for the growth and maintenance of bones. Calcium and phosphorous also regulate muscle contraction and majorly in normal blood clotting. Phosphorus deficiency is rare because it is available in most foods, but there are some diseases such as diabetes or taking certain medications lead to low levels of phosphorus in the body [27].

Table 6: The Level of Calcium, Phosphorous, and Calcium/Phosphorous (ratio) in the Serum of Patients with Type 2 Diabetes Mellitus

Parameters	Mean± SD	
	patients, n=75	Control, n=75
Calcium (mg/dl)	9.7±0.5	9.6 ±0.4
Phosphorous (mg/dl)	*4.4±1.5	3.9±0.7
Calcium /Phosphorous ratio	2.2±0.4	2.4±0.6

Mg (Milligram); dl (Deciliter); n (number); SD (standard deviation); \* Significant when the p-value at the level of (p≤0.05)



### 3.3. Correlation of Afamin with Lipid Profiles, Liver Enzymes, Kidney Function, Uric Acid, MDA, GSH, and Calcium/phosphorous (ratio) in the Serum of Patients with Type 2 Diabetes Mellitus:

Table 7 shows the correlation of the afamin level studied with the biochemical parameters in type 2 diabetes patients, which included total cholesterol, HDL, LDL, VLDL, TG, AST, ALT, ALP, Creatinine, Urea, Uric acid, MDA, GSH, Ca<sup>+</sup>, Phosphorous, and Calcium /Phosphorous ratio.

#### 3.3.1. Correlation of Afamin Level with Lipid Profile (Total Cholesterol, HDL, LDL, VLDL, and TG)

The results show in Table 7 and Figure (1a), (1b) that there is a negative insignificant correlation between afamin and Total Cholesterol ( $r=-0.036$ ,  $p=0.761$ ) and HDL ( $r=-0.072$ ,  $p=0.539$ ) in the serum of type 2 diabetes patients. Figure (1d) showed non-significant correlations between afamin level and VLDL ( $r= 0.167$ ,  $p=0.151$ ). on other hand, afamin shows in Figure (1c) and (1e) a significant positive correlation with LDL ( $r=0.479$ ,  $p=0.01$ ) and TG ( $r =0.256$ ;  $p=0.027$ ) in patients' group. A study on young women suffering from Polycystic Ovary Syndrome diagnosed with insulin resistance revealed a significant positive correlation between afamin with LDL and TG. In contrast, the correlation was negative and insignificant between afamin and HDL [28].

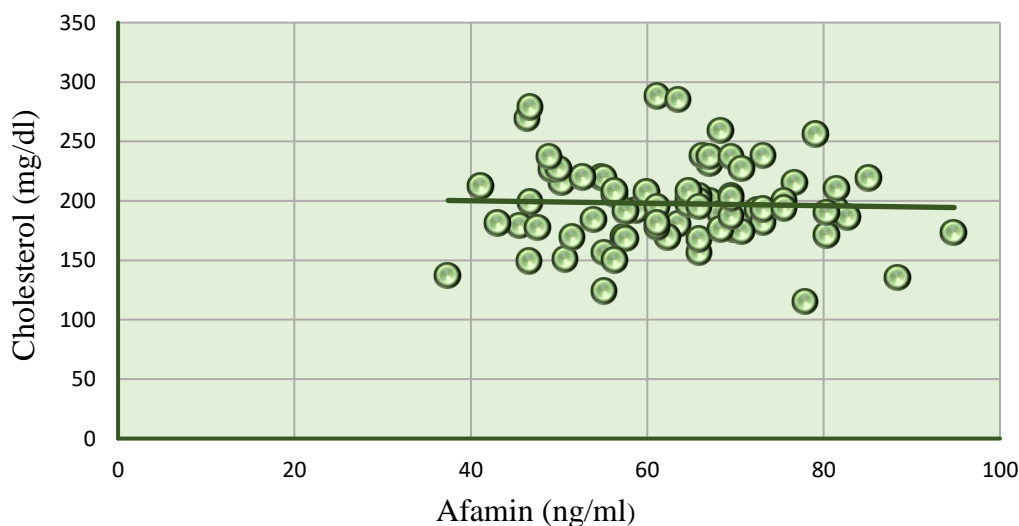


Figure 1a: The Correlation of Afamin with Total Cholesterol in Type 2 Diabetes Mellitus

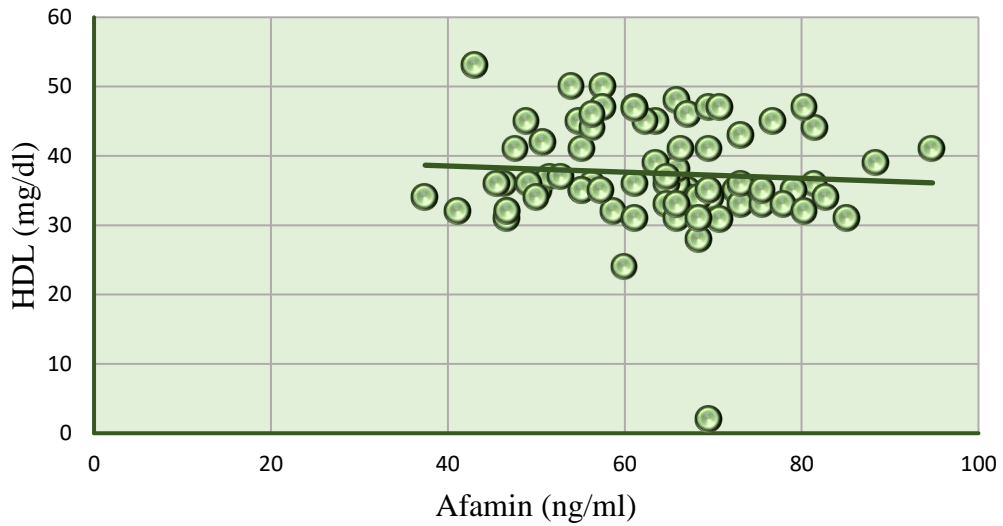


Figure 1b: The correlation of afamin with HDL in type 2 diabetes Mellitus

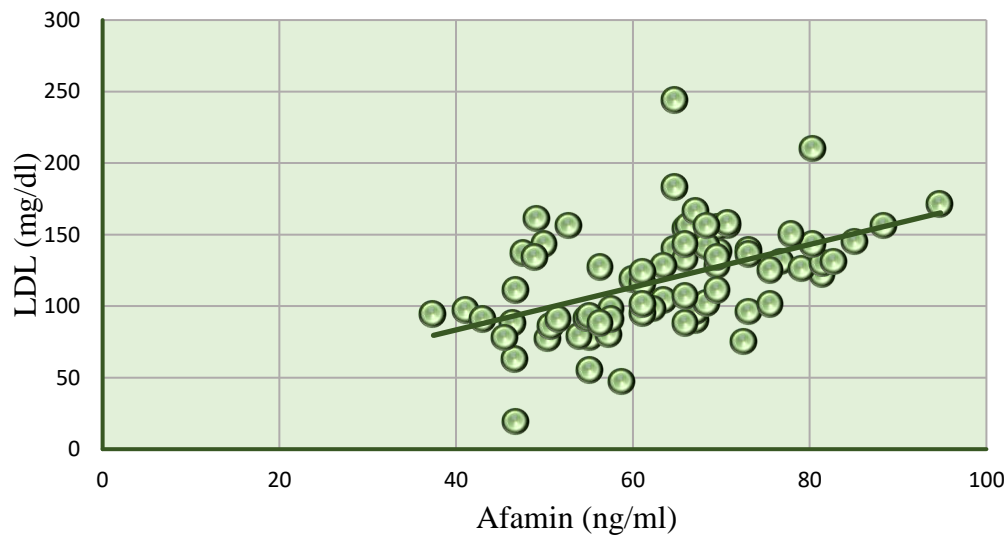


Figure 1c: The Correlation of Afamin with LDL in Type 2 Diabetes Mellitus

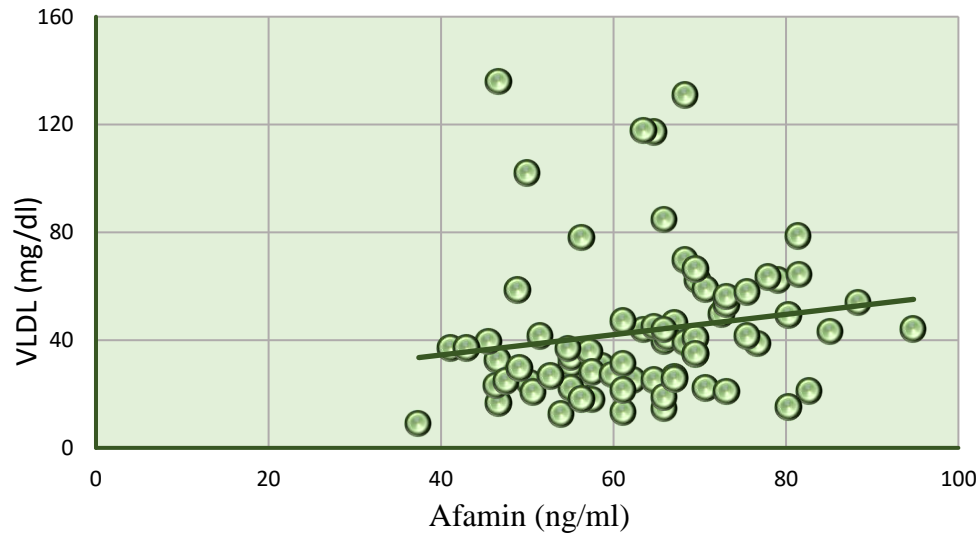


Figure 1d: The Correlation of Afamin with VLDL in Type 2 Diabetes Mellitus

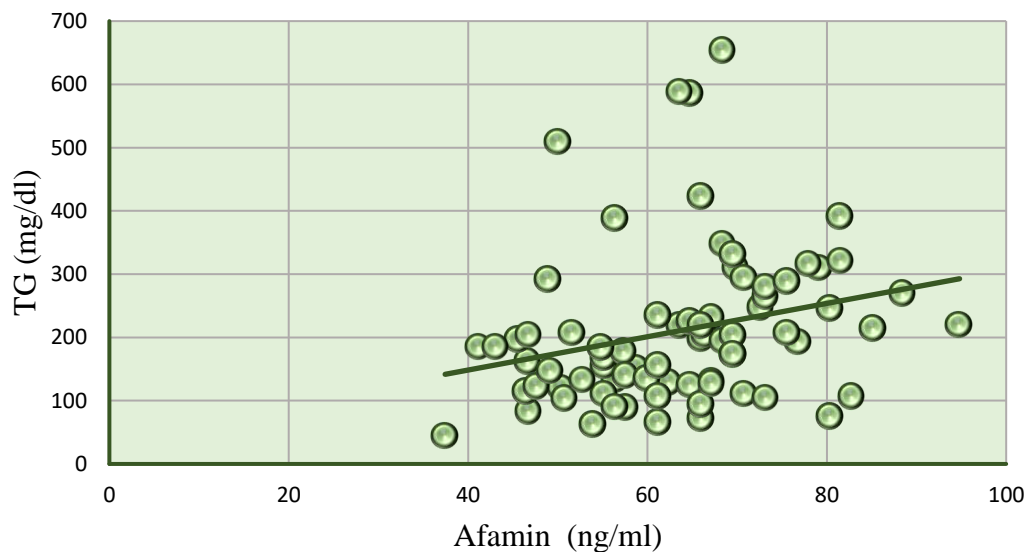


Figure 1e: The Correlation of Afamin with TG in Type 2 Diabetes Mellitus

### 3.3.2. Correlation of Afamin Level with Liver Enzymes (ALT, AST, and ALP)

The results showed in Table (7) and Figure (2a), (2b), and (2c) that afamin has a significant positive correlation with ALT ( $r = 0.326$ ;  $p = 0.01$ ), AST ( $r = 0.325$ ,  $p = 0.01$ ) and ALP ( $r = 0.427$ ;  $p = 0.01$ ) in the serum of type 2 diabetes patients; and it came agreement with [20]. These results indicate that afamin may be a good biomarker for elevated liver enzymes.

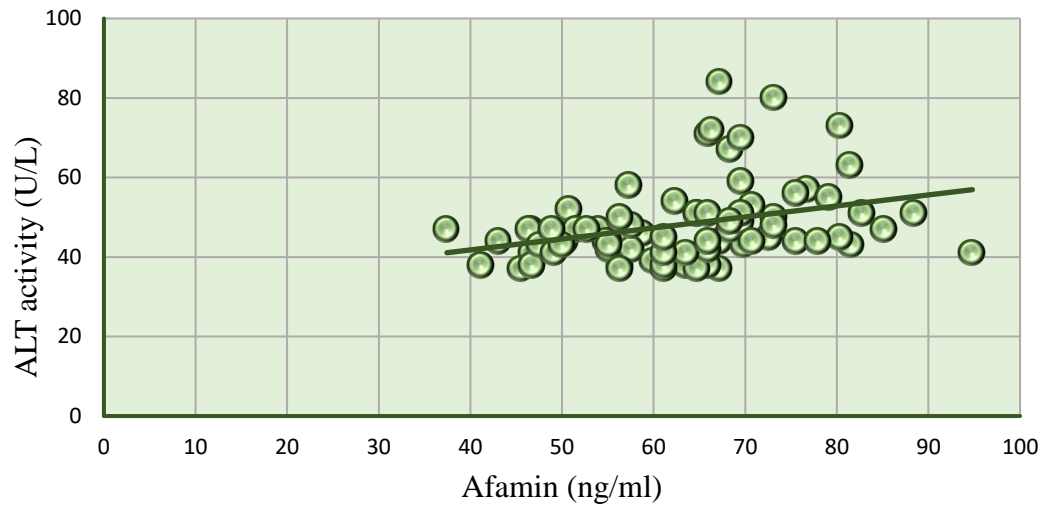


Figure 2a: The Correlation of Afamin with ALT in Type 2 Diabetes Mellitus

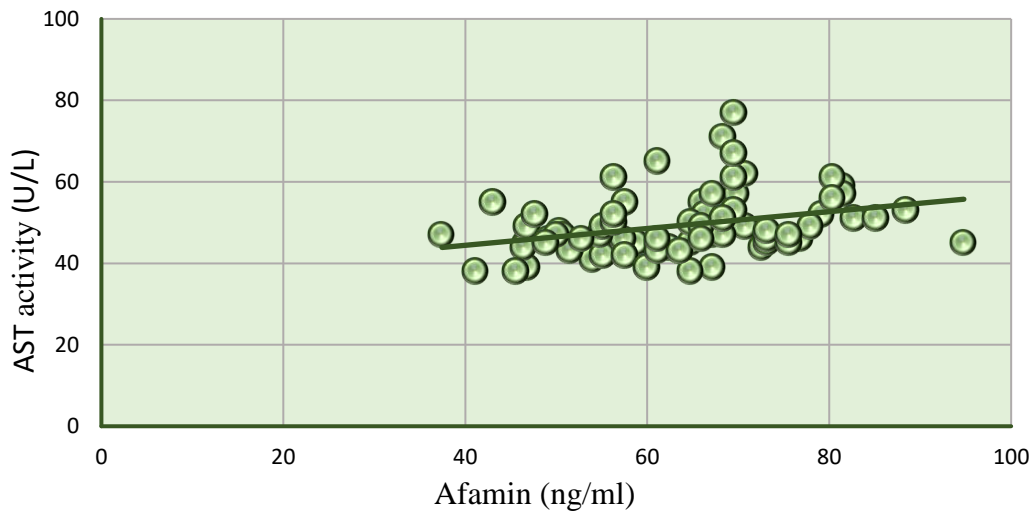


Figure 2b: The Correlation of Afamin with AST in Type 2 Diabetes Mellitus

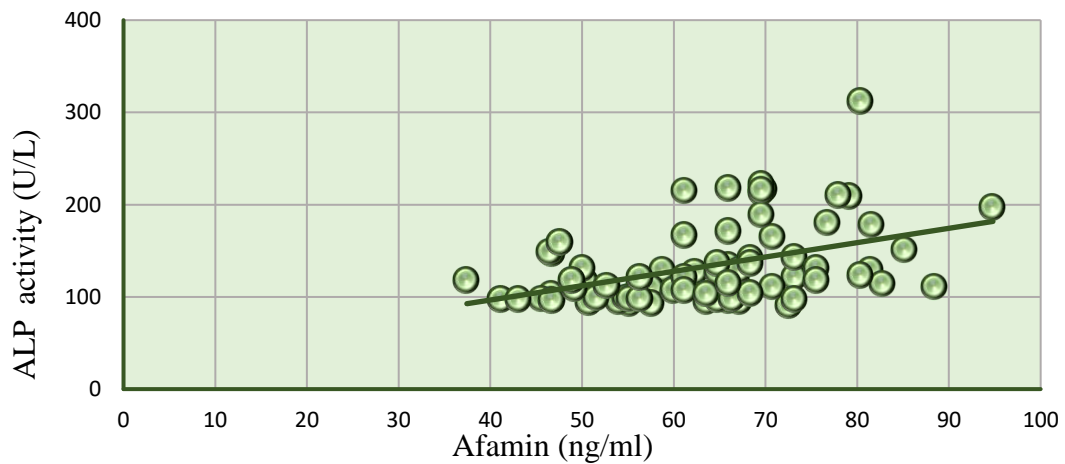


Figure 2c: The correlation of afamin with ALP in type 2 diabetes patients.

### 3.3.3. Correlation of Afamin Level with Renal Functions (creatinine and urea)

The results show in Table (7) and Figure (3a) and (3b) that there is a significant positive correlation between Afamin with Creatinine ( $r = 0.277$ ;  $p = 0.016$ ) and urea ( $r = 0.436$ ,  $p = 0.01$ ) in the serum of type 2 diabetes patients. Elevation of creatinine and urea leads to nephropathy, which is one of the complications of diabetes and can lead to serious complications if not controlled. A study showed that the afamin to creatinine ratio was significantly increased in patients with diabetic nephropathy. The researchers indicated that the afamin to creatinine ratio could be used to predict the complications of nephropathy and decreased kidney activity [29].

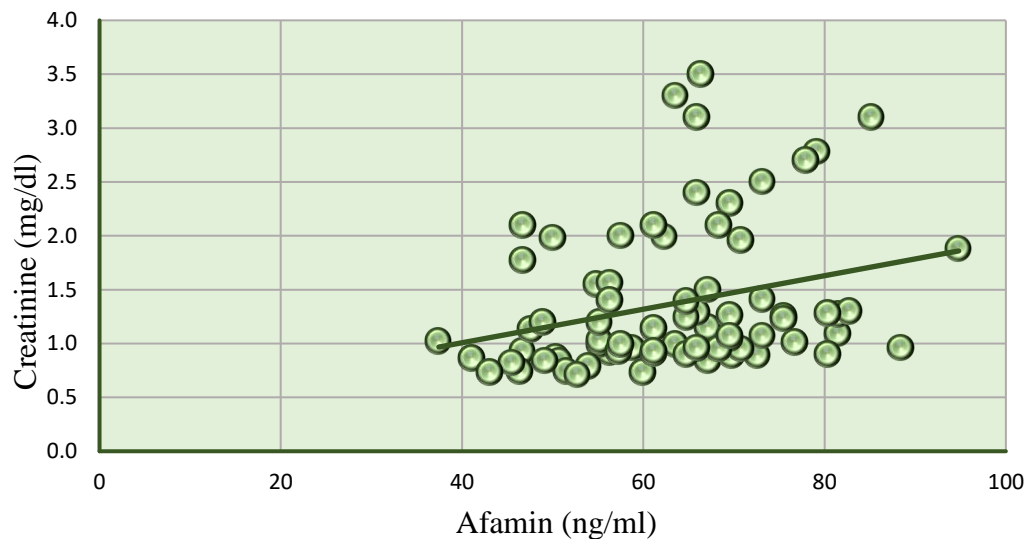


Figure 3a: The Correlation of Afamin with Creatinine in Type 2 Diabetes Mellitus

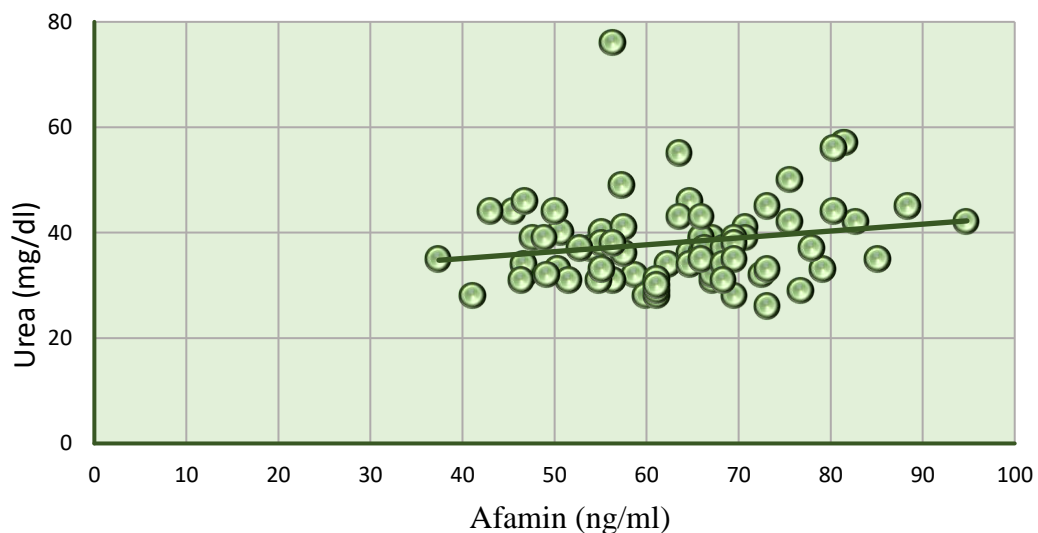


Figure 3b: The Correlation of Afamin with Urea in Type 2 Diabetes Mellitus

### 3.3.4. Correlation of Afamin Level with (Uric acid, MDA, and GSH)

The results showed in Table 7 and Figure 4a and 4b that afamin has a significant positive correlation with uric acid ( $r=0.477$ ,  $p=0.01$ ) and MDA ( $r=0.463$ ;  $p=0.01$ ) in the serum of type 2 diabetes patients. On another hand, Figure (4c) shows a significant negative correlation was found between afamin and GSH ( $r=-0.460$ ,  $p=0.01$ ) in the serum of type 2 diabetes patients. A study conducted on non-diabetic obese patients showed that afamin may be involved in the development of oxidative stress, as the results indicated a significant positive correlation of afamin with uric acid [11].

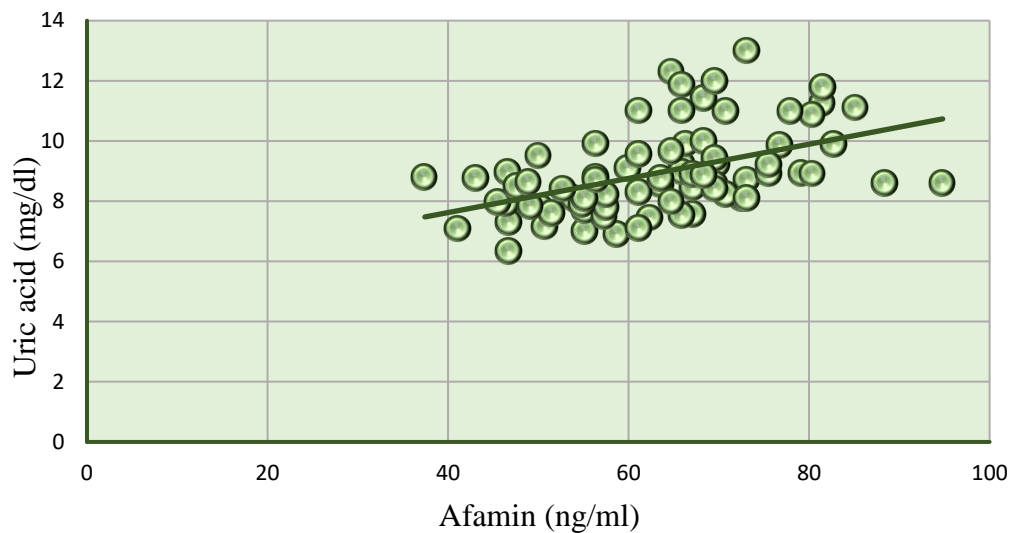


Figure 4a: The Correlation of Afamin with Uric Acid in Type 2 Diabetes Mellitus

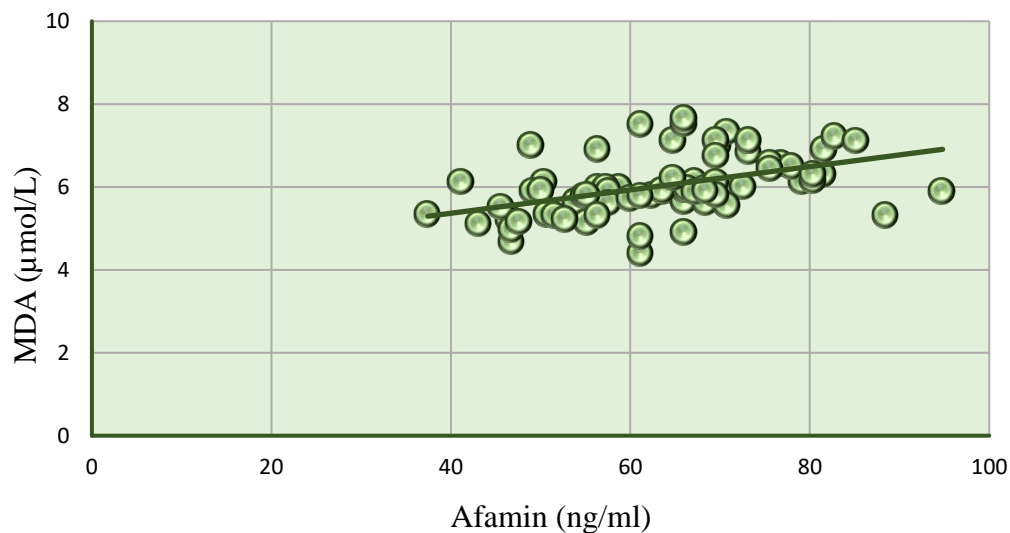


Figure 4b: The Correlation of Afamin with MDA in Type 2 Diabetes Mellitus



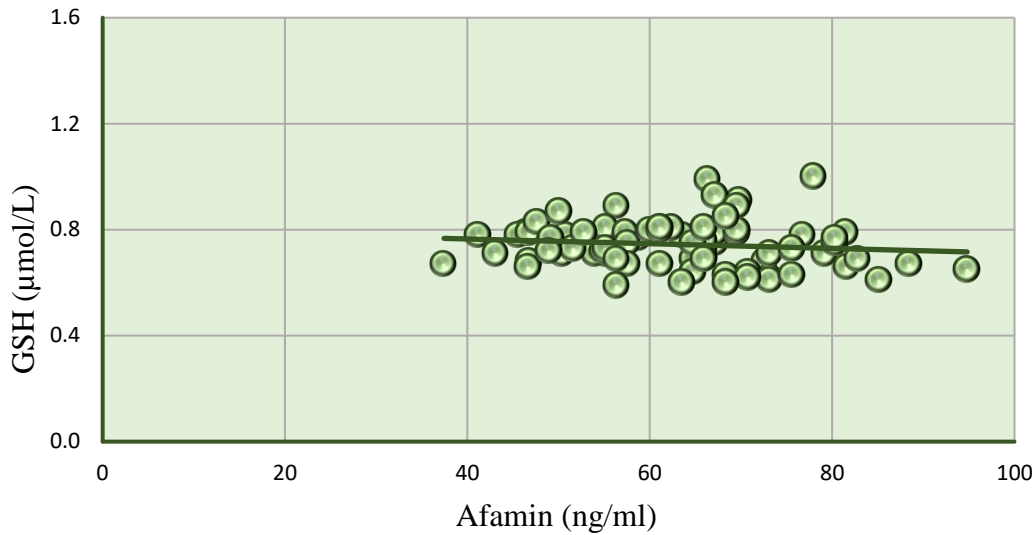


Figure 4c: The Correlation of Afamin with GSH in Type 2 Diabetes Mellitus

### 3.3.5. Correlation of Afamin Level with Calcium, Phosphorous, and Calcium /phosphorous ratio

The results show in Table 7 and Figures 5a and 5c that there is an insignificant negative correlation between afamin with calcium ( $r=-0.137$ ,  $p=0.240$ ) and calcium /phosphorous ratio ( $r=-0.031$ ;  $p=0.789$ ) in the serum of type 2 diabetes patients. But, in Figure (5b), afamin has an insignificant positive correlation with phosphorous ( $r =0.017$ ;  $p=0.886$ ) in the serum of type 2 diabetes patients.

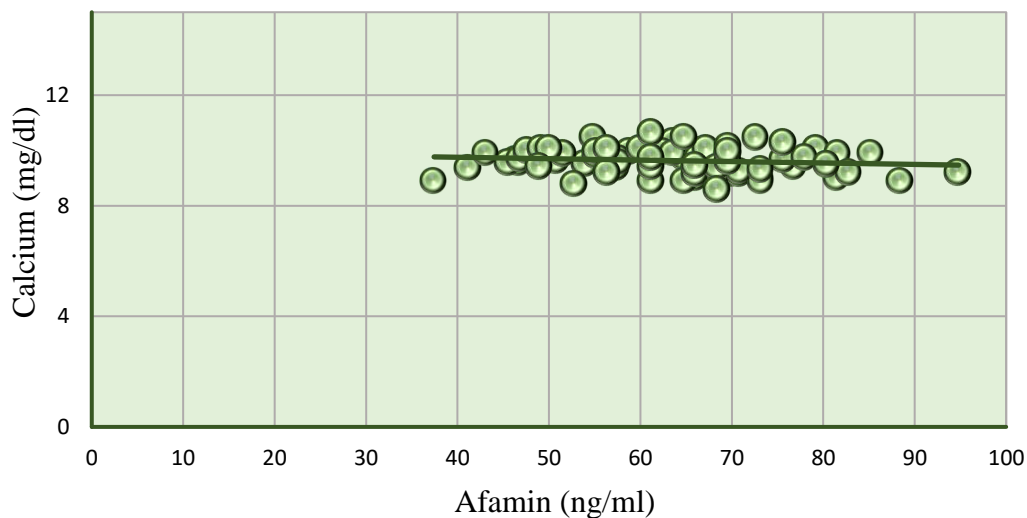


Figure 5a: The Correlation of Afamin with Calcium in Type 2 Diabetes Mellitus

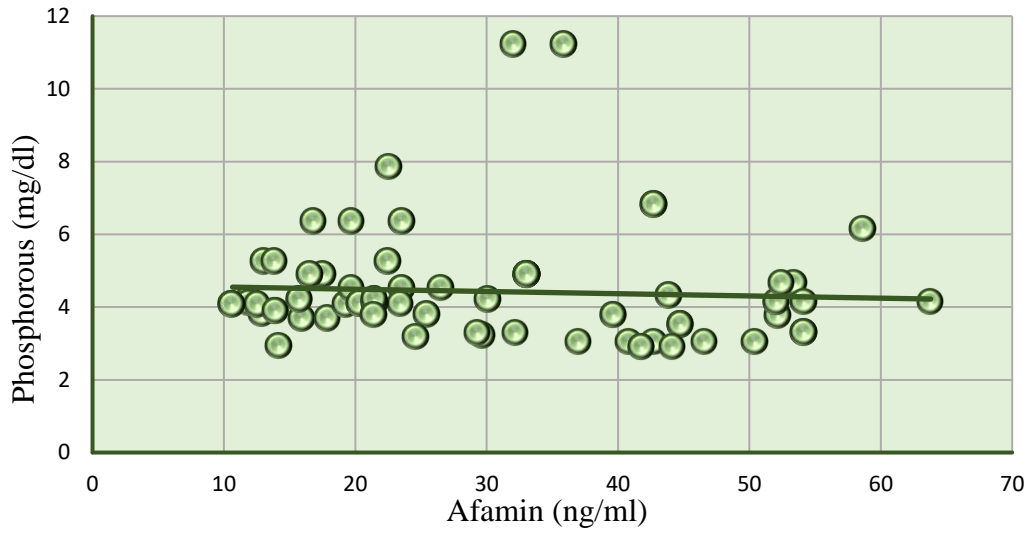


Figure 5b: The Correlation of Afamin with Phosphorous in Type 2 Diabetes Mellitus

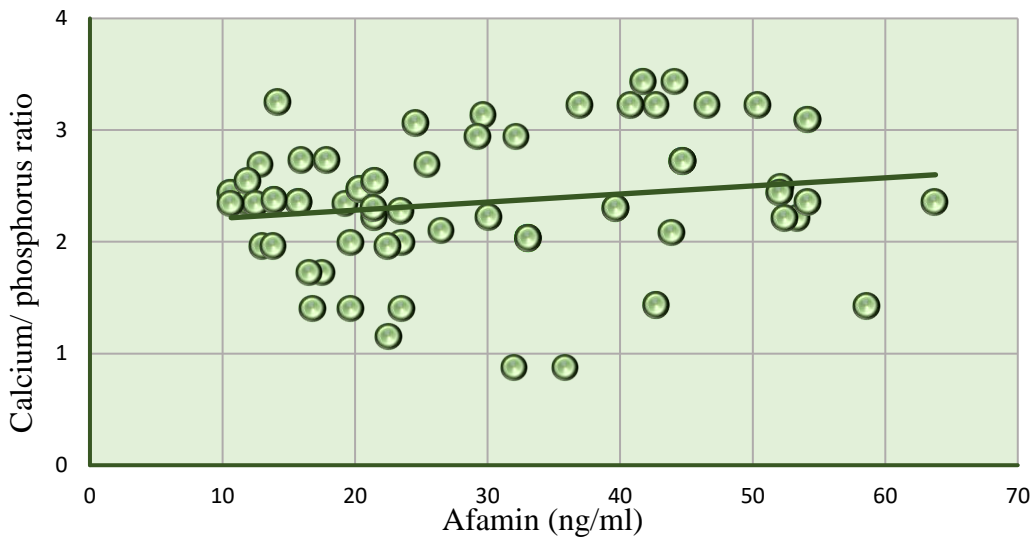


Figure 5c: The Correlation of Afamin with Calcium/phosphorous ratio in Type 2 diabetes Mellitus



Table 7: The Correlation of Afamin Level with Biochemical Parameters Study in Patients with Type 2 Diabetes Mellitus

Parameters	Afamin, Pearson correlation (r), P °
Cholesterol(mg/dl)	-0.036; 0.761
HDL (mg/dl)	-0.072; 0.539
LDL (mg/dl)	**0.479; 0.01
VLDL (mg/dl)	0.167; 0.151
TG (mg/dl)	*0.256; 0.027
ALT (U/L)	**0.326; 0.01
AST (U/L)	**0.325; 0.01
ALP (U/L)	**0.427; 0.01
Creatinine (mg/dl)	*0.277; 0.016
Urea (mg/dl)	**0.436; 0.01
Uric acid (mg/dl)	**0.477; 0.01
MDA (µmol/L)	**0.463; 0.01
GSH (µmol/L)	** -0.460; 0.01
Calcium (mg/dl)	-0.137; 0.24
Phosphorous (mg/dl)	0.017; 0.886
Calcium /Phosphorous ratio	-0.031; 0.789
mg: Milligram; dl: Deciliter; U: unit; ml: Milliliter; µmol :Micromole; L: Liter; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; VLDL: Very low-density lipoprotein; MDA: Malonaldehyde; GSH: Glutathione; ; P°: refer to P –value; * Correlation is significant at the 0.05 level (2-tailed ); ** Correlation is significant at the 0.01 level (2-tailed)	

#### 4. Conclusions

The findings of the study indicate a significant positive relationship between afamin and many physiological markers, including lipid profile (specifically LDL and TG levels), liver function (as indicated by ALT, AST, and ALP levels), kidney function (as indicated by creatinine and urea levels), and oxidative stress (as indicated by uric acid and MDA levels). The afamin protein exhibits a notable inverse relationship with glutathione (GSH). In contrast, the relationship between afamin and the calcium/phosphorous ratio lacks statistical significance. Hence, based on



our findings, higher levels of afamin could lead to the onset of type 2 diabetes and its associated problems, including disturbances in lipid metabolism that are linked to the development of atherosclerosis and subsequent cardiovascular disorders. Moreover, an elevated concentration of afamin has been associated with potential disruptions in hepatic enzymes, kidney efficiency, and an increase of oxidative stress.

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## تقييم علاقة مستوى الأفامين مع بعض المتغيرات الكيموحيوية في أمصال المرضى المصابين بداء السكري من النوع الثاني

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### المستخلص

**الخلفية:** يلعب الأفامين دورًا مهمًا في داء السكري من النوع 2. **الهدف من الدراسة:** هدفت الدراسة إلى دراسة ارتباط الأفامين مع خصائص الدهون، ووظائف الكبد، ووظائف الكلى، والإجهاد التأكسدي، ونسبة الكالسيوم / الفوسفور في مرضى السكري من النوع الثاني. **المواد وطرق العمل:** اشتملت الدراسة على 150 بالغًا مقسمين إلى مجموعتين: 75 مريضًا يعانون من داء السكري من النوع 2 و 75 شخصًا سليمًا من كلا الجنسين، تراوحت أعمارهم بين (35-65 عام). تم قياس المتغيرات البيوكيميائية التي تضمنت الأفامين، الدهون (Cholesterol, HDL, LDL, TG and VLDL)، ووظائف الكبد (ALT, AST and ALP)، ووظائف الكلى (Creatinine and urea)، الإجهاد التأكسدي (Uric acid, MDA and GSH) ونسبة الكالسيوم / الفوسفور. **النتائج:** وجد ارتباط إيجابي معنوي بين مستوى AFM مع LDL ( $r=0.479$ ;  $p=0.01$ ) و TG ( $r=0.256$ ;  $p=0.027$ ) ووظائف الكبد (ALT ( $r=0.326$ ;  $p=0.01$ ) و ALP ( $r=0.427$ ;  $p=0.01$ ) و AST ( $r=0.325$ ;  $p=0.01$ ) ووظائف الكلى (كرياتينين ( $r=0.277$ ;  $p=0.016$ ) واليوريا ( $r=0.436$ ;  $p=0.01$ ) و الإجهاد التأكسدي (MDA ( $r=0.477$ ;  $p=0.01$ ) و Uric acid ( $r=0.463$ ;  $p=0.01$ ) (على النقيض من ذلك، يرتبط الأفامين سلبًا مع GSH ( $r=-0.460$ ;  $p=0.01$ ). **الاستنتاج:** يرتبط الأفامين بشكل إيجابي مع LDL و TG، ووظائف الكبد (ALT، و AST، و ALP)، ووظائف الكلى (الكرياتينين واليوريا)، والإجهاد التأكسدي (حمض البوليك و MDA). أفامين يرتبط سلبًا ب GSH. أفامين لا يؤثر على نسبة الكالسيوم / الفوسفور. وبالتالي، فإن المستويات العالية من الأفامين تحفز مرض السكري من النوع 2 وما يرتبط به من عواقب التمثيل الغذائي للدهون والتي تتعلق بأمراض القلب وتصلب الشرايين ووظائف الكبد، ووظائف الكلى، والإجهاد التأكسدي.

