



RESEARCH ARTICLE

Evaluating The Role of Arginine Deaminase and Its Relationship with Some Biochemical Variables in The Blood Serum of Patients with Non-Alcoholic Fatty Liver Disease

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ARTICLE INFO	ABSTRACT
Received: May 27, 2024	<p>Due to the increase in cases of liver disease, especially NAFLD, in recent years, and for the purpose of finding biochemical variables that can be used as future biomarkers for patients with NAFLD, some biochemical variables were selected and studied in the patients' serum, the most important of which is the estimation of peptidyl arginine deaminase type 4 (PADI4) levels in patients and compared with healthy people. The study included collecting 160 blood samples from adults with NAFLD and healthy people for comparison purposes for the period from November 1, 2023 to January 30, 2024. Eighty blood samples were collected from people with NAFLD their ages ranged between (25-65) years, and fasted for (12-14) hours, (41) males and (39) females and (80) blood samples from healthy people aged between (25-65) years, from (40) males and (40) females. Determination of PADI4 in the sera using kits based on the ELISA-sandwich principle, a ready-made analysis kit was used to measure T. cholesterol, high-density lipoprotein cholesterol (HDL), and triglycerides (T.G), (ALT), Urea, (GGT), Bilirubin, Protein, albumin and creatinine. Results: The results showed a significant decrease ($P \leq 7.8814 \times 10^{-47}$) in PADI4 activity in patients group compared to control group, there was a significant positive relationship between ADI4 and total protein, especially globulin, and an inverse relationship with urea, BUN, and GGT. Arginine deaminase type IV can be considered a good diagnostic indicator in patients with NAFLD to predict the severity of the disease.</p>
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INTRODUCTION

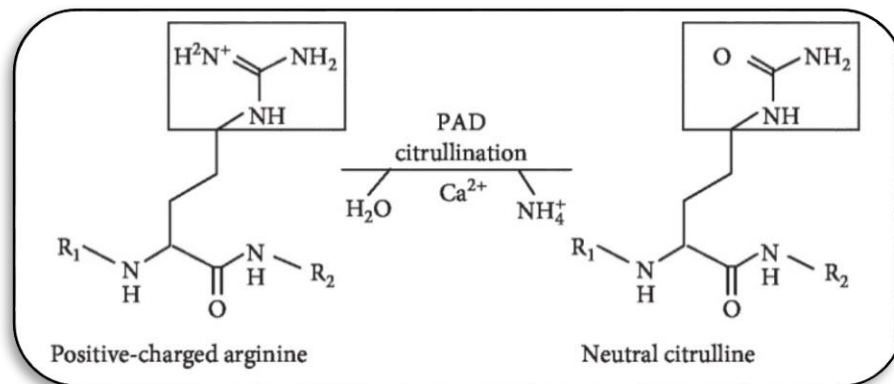
The liver is a vital organ in the human body that performs a number of functions including assisting with digestion, immunity, metabolism, detoxification, and vitamin storage. It makes up about 2% of the body weight of an adult [1].

NAFLD is a metabolic disease caused due to several factors (except alcohol) such as metabolism, expression, genetic and environmental pollution. The incidence of NAFLD has increased in many developing countries [2].

NAFLD includes simple fatty liver and non-alcoholic steatohepatitis (NASH), which can progress to fibrosis and cirrhosis, increasing the risk of hepatocellular carcinoma, NAFLD is closely associated with several metabolic diseases, including obesity, insulin resistance, type 2 diabetes, and

dyslipidemia [3] and except for lifestyle and weight loss interventions, there are no clear and effective treatments [4].

Therefore, exploring the pathogenesis of the disease may provide new insights into clinical treatments. The pathogenesis is still unknown, and NAFLD is widely discussed [5]. Given the rapidly rising global burden of nonalcoholic steatohepatitis (NAFLD), research is being done to identify appropriate nonsurgical diagnostic methods and effective treatments. Additionally, efforts must be made to find accurate noninvasive diagnostic and prognostic biomarkers to develop effective treatments for people with advanced NASH (nonalcoholic steatohepatitis) [6]. Peptidyl arginine deaminase (PADIs) (EC 3.5.3.15) are Ca^{2+} dependent enzymes [7], and they are among the enzymes of the urea cycle, as they convert peptidyl arginine to peptidyl citrulline and ammonia [8]. It can be considered a deimination reaction, producing a deaminated amino acid. This process is called citrullination [9].



Citrullination plays important physiological roles, for example, in the central nervous system, in the skin, in the organization of Genes and various types of cancer [10], and also plays an active role by stimulating the production of antibodies in rheumatoid arthritis [11,45].

The primary organ in charge of extracting nitrogen from the blood to produce urea that the body can expel is the liver [12], and the urea cycle is very important for removing excess nitrogen that can accumulate in the form of ammonia. Decreased urea synthesis caused by a defect in urea cycle enzymes leads to hyperammonemia [13,46].

NAFLD develops as a result of the body's inability to effectively eliminate ammonia, primarily through the activation of hepatic stellate cells (Kupffer cells)

[12]. Reactive oxygen species (ROS) may be produced more readily when ammonia is present. ROS can then induce fibrosis, cell death, and inflammation, which can lead to liver damage [14,44].

The lack of effective treatments for non-alcoholic fatty liver disease (NAFLD), leads us to believe that there are still unknown pathogenic mechanisms, such as ammonia, that can influence the development of the disease, independent of lifestyle interventions [15].

Therefore, in view of the increase in cases of liver disease, especially NAFLD, in recent years, and for the purpose of finding biochemical variables that can be used as future biomarkers for patients with NAFLD, some biochemical variables were selected and studied in the patients' blood serum, which included:

- Estimating peptidylarginine deaminase 4 (PADI4) levels in patients and comparing them with healthy controls.
- Estimating the activity of GGT and ALT and their relationship with NAFLD.
- Estimating concentrations of lipid parameters, protein levels, albumin, and globulin.
- Estimating the levels of urea and creatinine in blood serum and comparing them with healthy people.
- Evaluate the

effect of age, gender, body mass index, duration of the disease, and type of treatment. Finally, • Study the correlation between peptidyl arginine deaminase 4 with the biochemical variables included in the study.

MATERIALS AND METHODS

Materials used

In this study, a ready-made analysis kit (Standard Kits) from the French company Biolabo was used to measure T.cholesterol, HDL, T.G, ALT, T. protein, albumin and creatinine, whilest, arginine deaminase4 was measured using a ready-to-measure ELISA test kit prepared by the Chinese company Bioassay Technology Laboratory.

To estimate gamma-glutamyl transferase, a ready-made analysis kit from the German company Roche was used by Cobas (c111) device, and to measure urea and total bilirubin, a ready-made analysis kit from the French company Biomerieux was used.

Criterion for inclusion and exclusion

Criterion for inclusion

This study included (80) patients ranging in their age between 25 and 69 years and (80) healthy subjects 25–65 years as control.

Criteria for exclusion

Participants outside the age range of 25–69 years old, as well as those with established pathologies including cancer, hepatitis, hypertension, or heart disease, were excluded from the study. The controls as well as the subjects were both exposed to the same exclusion criteria.

Statistical evaluation

To eliminate input mistakes, data were coded into Excel. Spreadsheets using customized Excel forms and analyzed using the Statistical SPSS package version 26 (S.P.S.S. statistics, Arm., N. Y, USA). To represent every value, the average and standard deviation were employed. To compare the averages of biochemical variables, the paired *t*-test was performed. When the possibility (*P*) was $P \leq 0.05$, the changes were judged significant [16].

Specimens used

The study included collecting 160 blood samples from adults with NAFLD and healthy people for the purpose of comparison for the period from the first of November 2023 to the thirtieth of January 2024, where a task facilitation letter was obtained from the Training and Human Development Center\Ninawa Health Department\Ministry Health and Environment (No. 55195 in 13/12/2023) While the research was approved by the Council of the University of Mosul according to Administrative Order No. 6617 dated 11-5-2023, after the approval of the Research Committee to conduct the research and provide us with the required information and samples, samples were divided into two groups: patients and healthy people (80) blood samples were collected for people with NAFLD from Nineveh Governorate. Their condition was diagnosed by specialist doctors at Ibn Sina Teaching Hospital, Al-Salam Teaching Hospital, and outpatient clinics licensed by the Ministry of Health. Their ages ranged between (25-65) years. Those who fasted for (12-14) hours of both sexes (41) males and (39) females, (80) blood samples were collected from apparently healthy people aged between (25-65) years who fasted for (12-14) hours from both sexes (40) males and (40) females.

Blood sample collection

(8-10) milliliters of venous blood were drawn using special single-use medical syringes. The blood was placed in clean, dry gel-containing tubes and left for 10 min. at room temperature, after which

centrifugation was performed at 3000 rpm for 15 minutes. The serum was separated using a micropipette and placed in clean plastic tubes. All isolated sera were frozen at -20°C until used for testing [17].

RESULTS

The totals were divided by age into two age groups: (less than 45) and (over 45) years, as shown in Table (1). The totals were also divided by gender into Females and Males, as in Table (2). The numbers were distributed in proportions. Percentiles were similar for the patient group and the control group.

Table 1: Distribution of study samples according to age

NAFLD Patients		Control group	
Greater than 45years	Less than 45years	Greater than 45years	Less than 45years
n=37	n=43	n=20	n=60
%46.3	%53.7	20%	%80

Table 2: Distribution of study samples according to gender

patients n= 80		control n = 80	
Female	Male	Female	Male
n=40	n=40	n=39	n=41
50%	50%	48.75%	51.25%

In addition to classifying patients according to their body mass index (BMI) into two groups: more than 25 and between (18-25), as shown in Table (3).

Table 3: Distribution of study samples according to BMI

Patients n = 80		control n=80	
BMI(18-25)	BMI>25	BMI(18-25)	BMI>25
n=6	n=74	n=42	n=38
%7.5	%92.5	%52.5	%47.5

Table (4) shows the results of the effectiveness of the enzyme arginine deaminase 4 (ng/ml) in the blood sera of patients with NAFLD compared with the control group. The results showed a significant decrease ($P \leq 7.8814 \times 10^{-47}$) in the activity of the enzyme in the patient group compared to the control group.

Table (4): Activity of ADI4 in the sera of patients with NAFLD compared with the control group

Groups	N	Mean	SD	SEM	P-value
"ADI4 - Control"	80	19.40875	6.07264	0.67894	7.8814 x10 ⁻⁴⁷
"ADI4 - Patient"	80	4.5175	2.13712	0.23894	

Values are expressed in ng/ml, N represents the number of samples, SD is the standard deviation, SE is the standard error.

The study also indicates that there is no effect of the duration of infection on ADI 4 rates, as shown in Table (5).

Table (5): The effect of the duration of fatty liver disease on ADI 4

Groups	N	Mean	SD	SE	p value
New Injury	25	4	1.84662	0.36932	0.14533 **
Treatment	55	4.75273	2.23291	0.30109	

** indicates that, at a degree of significance of less than 0.05, there is no discernible change ($p > 0.05$).

N represents the number of samples, SD is the standard deviation, SE is the standard error.

The results shown in Table (6) indicate that in patients with NAFLD, total bilirubin levels and the activity of the GPT and GGT enzymes begin to improve when adhering to the diet.

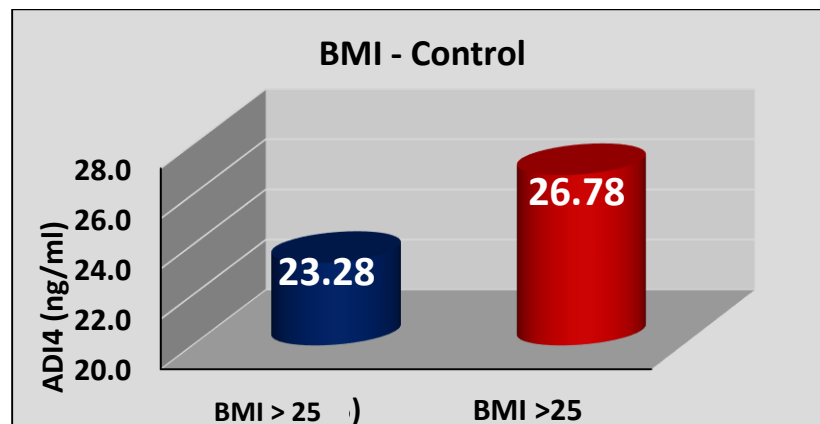
Table (6): Effect of treatment (diet or medication) on the levels of some biochemical variables

Parameters	New Injury Patients (n=25)			Treated Patients(n=55)			P value
	Mean	SD	SE	Mean	SD	SE	
T. S. B. (mg/dl)	0.614	0.17	0.01	0.659	0.15	0.02	<0.05 *
ALT(U/L)	43.57	5.71	1.24	31.09	3.35	3.612	<0.05 *
GGT(U/L)	48.11	4.06	1.53	27.55	3.06	1.367	<0.05 *

* Indicates that, at a degree of significance of less than 0.05, there is a notable variation ($p > 0.05$).

N represents the number of samples, SD is the standard deviation, SE is the standard error.

The results shown in Figure (1) and Figure (2) show a significant decrease in ADI4 activities with increasing body mass index percentage, meaning that the relationship is inverse between the enzyme and BMI for both groups (control and patients), respectively.

**Figure (1) Relationship of enzyme activities with BMI in the control group**

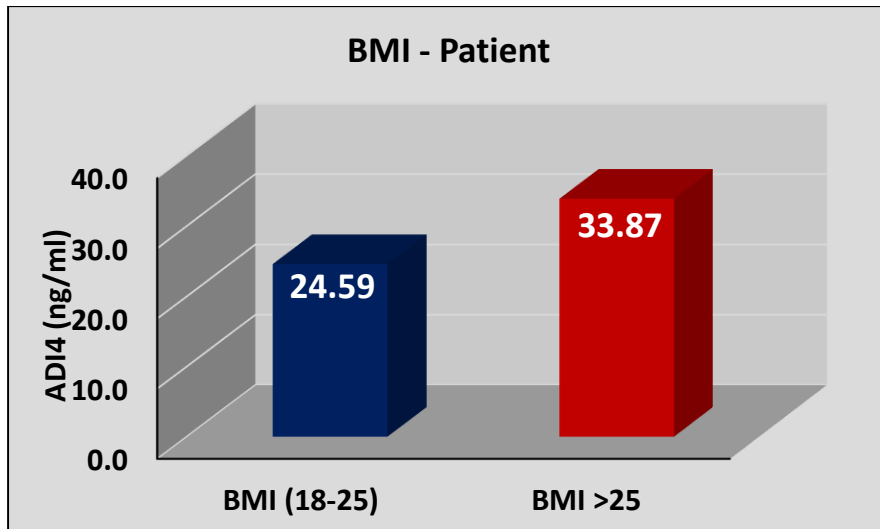


Figure (2) Relationship of enzyme activities with BMI in NAFLD group

The results in Tables 7 and 8 indicate ADI4 activity decreased with aging regardless of person health condition.

Table (7): The effect of age on ADI 4 in the sera of a group of patients with NAFLD

Groups	N	Mean	SD	SEM	P-value
"ADI4 -Patient (<45)"	43	4.94884	2.3902	0.3645	0.00 *
"ADI4 -Patient (>45)"	37	4.01622	1.69616	0.27885	

* Indicates that, at a degree of significance of less than 0.05, there is a notable variation (p > 0.05).

N represents the number of samples, SD is the standard deviation, SE is the standard error.

Table (8): The effect of age on ADI 4 in the sera of control group

Groups	N	Mean	SD	SEM	P-value
"ADI4 -Control (<45)"	60	20.17833	6.11691	0.78969	0.00 *
"ADI4 -Control (>45)"	20	17.1	5.4462	1.21781	

* Indicates that, at a degree of significance of less than 0.05, there is a notable variation (p > 0.05).

N represents the number of samples, SD is the standard deviation, SE is the standard error.

The results shown in Figure (3) show that the the enzyme is higher in males compared to females and for both groups (patients and healthy people), (ng/ml) for males compared to 4.18 ng/ml for females (in the patient group),4.86 ng/ml for males compared to 17.52 ng/ml for females (in the control group).21.21

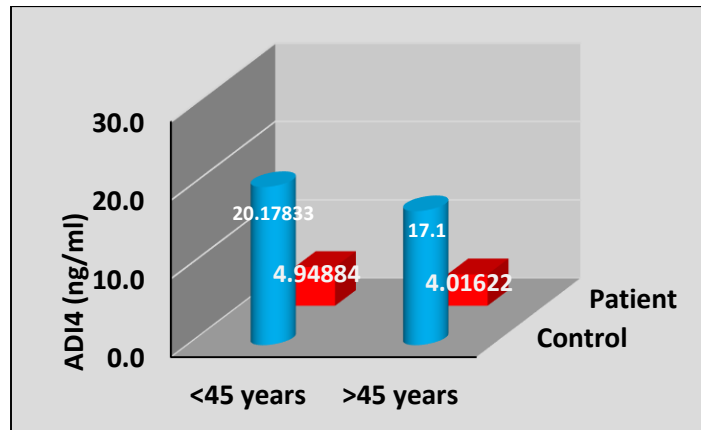


Figure (3): The effect of age on ADI4 activities

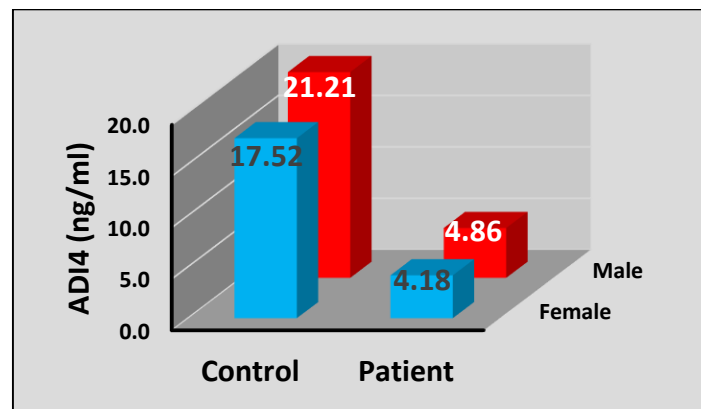


Figure (4): The effect of gender on ADI4 activities

The results in Table (9) show a substantial rise ($P < 0.05$) in the amounts of T. cholesterol, T.G, LDLC, and VLDL-C, and a reduction in the levels of HDL-C in the blood serum of people with fatty liver compared with the control group

Table (9): Biochemical variables of fats measured and studied in the control groups and patients with NAFLD

Parameters	Control				Patient			
	Mean	SD	SE	P value	Mean	SD	SE	P value
Cholesterol (mg/dl)	168.263	22.671	2.535	<0.05*	195.325	40.107	4.484	<0.05*
Triglyceride (mg/dl)	94.225	22.251	2.488	<0.05*	212.788	102.207	11.427	<0.05*
HDL (mg/dl)	51.538	9.551	1.068	<0.05*	39.438	9.258	1.035	<0.05*
LDL (mg/dl)	97.880	26.215	2.931	<0.05*	113.330	42.116	4.709	0.006*
VLDL (mg/dl)	18.845	4.450	0.498	0.50**	21.518	2.561	0.286	<0.05*

* Indicates that, at a degree of significance of less than 0.05, there is a notable variation ($p > 0.05$).

** indicates that, at a significance level of less than 0.05, there is no discernible change ($p > 0.05$).

N represents the number of samples, SD is the standard deviation, SE is the standard error

The results in Table (10) indicate a significant decrease ($P < 0.05$) in urea, creatinine, and BUN in the blood serum of people with fatty liver compared to the control group.

Table (10): Some indicators of kidney function measured and studied in the control groups and patients with NAFLD

Parameters	Control				Patient			
	Mean	SD	SE	P value	Mean	SD	SE	P value
Urea (mg/dl)	31.075	4.846	0.542	<0.05 *	25.725	6.366	0.712	<0.05 *
Creatinine (mg/dl)	0.77875	0.184	0.021	<0.05 *	0.737	0.172	0.019	<0.05 *
BUN (mg/dl)	14.51	2.266	0.253	<0.05 *	11.988	2.975	0.333	<0.05 *

* Indicates that, at a degree of significance of less than 0.05, there is a notable variation ($p > 0.05$)., N represents the number of samples, SD is the standard deviation, SE is the standard error.

The results in Table (11) showed a significant increase ($P < 0.05$) in the levels of liver enzymes (ALT and GGT), a slight increase in albumin levels, a decrease in the levels of total protein and globulin, and a slight decrease in the levels of total bilirubin in the blood serum of people with NAFLD compared to the control group.

Table (11): Liver function indicators measured and studied in the control groups and NAFLD patients

Parameters	Control				Patient			
	Mean	SD	SE	P value	Mean	SD	SE	P value
T.S.Bilirubin (mg/dl)	0.667	0.173	0.019	<0.05 *	0.609	0.175	0.020	<0.05 *
ALT (U/L)	18.000	5.578	0.624	0.128 **	43.575	32.305	3.612	<0.05 *
GGT (U/L)	13.800	4.856	0.543	<0.05 *	44.862	39.061	4.367	<0.05 *
Total protein (g/L)	72.388	3.267	0.365	<0.05 *	69.575	4.304	0.481	<0.05 *
Albumin (g/L)	42.113	2.930	0.328	<0.05 *	44.200	3.033	0.339	<0.05 *
Globulin (g/L)	30.563	3.412	0.381	<0.05 *	25.363	4.355	0.487	<0.05 *

* Indicates that, at a degree of significance of less than 0.05, there is a notable variation ($p > 0.05$).

** indicates that, at a significance level of less than 0.05, there is no discernible change ($p > 0.05$).

The statistical relationship between the ADI4 enzyme and the measured biochemical variables

The results shown in Figure (9) indicated that there is a significant direct relationship between the activity of the ADI4 enzyme and total protein ($r=0.529$, $p=0.011$), as well as between the activity of the enzyme and serum globulin ($r=0.613$, $p=0.023$) Figure (10). There was an inverse relationship between the activity of the ADI4 enzyme and urea ($r = -0.532$, $p = 0.021$), and the relationship was inverse between the activity of the ADI4 enzyme and BUN ($r = -0.520$, $p = 0.016$), as well as inverse and significant with GGT ($r = -0.518$, $p = 0.015$) as shown in Figures (11), (12) and (13), respectively.

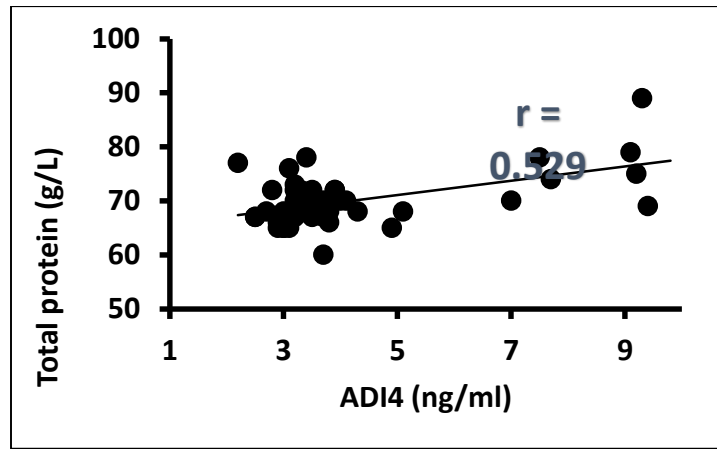


Figure (9) The direct relationship between arginine deaminase 4 and total protein

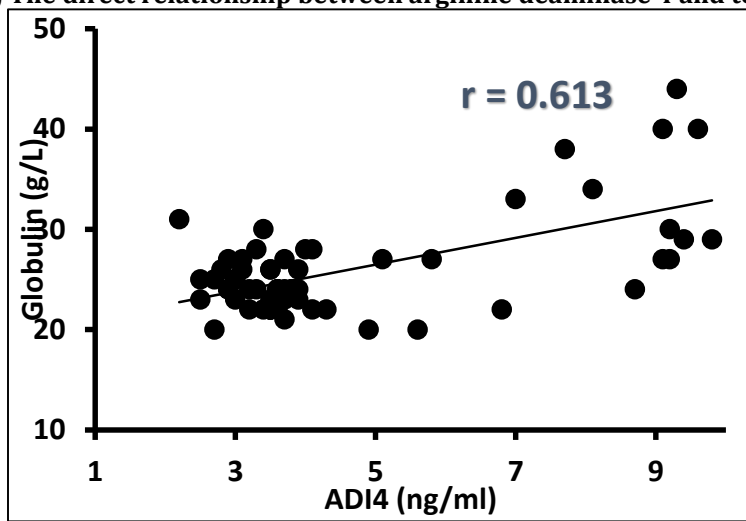


Figure (10) The direct relationship between arginine deaminase 4 and globulin

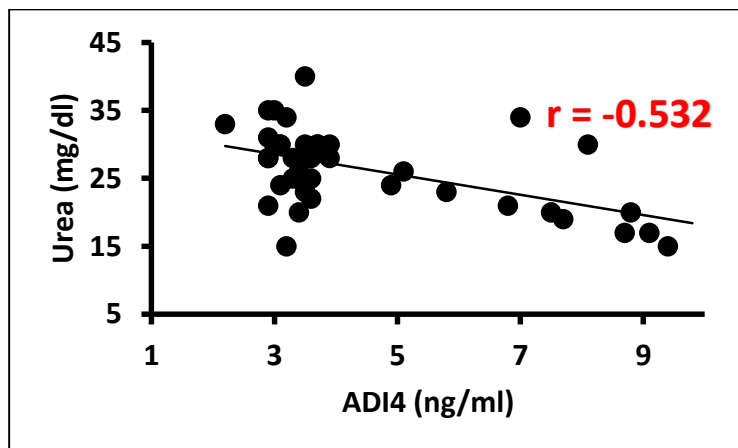


Figure (11) The inverse relationship between arginine deaminase 4 and urea

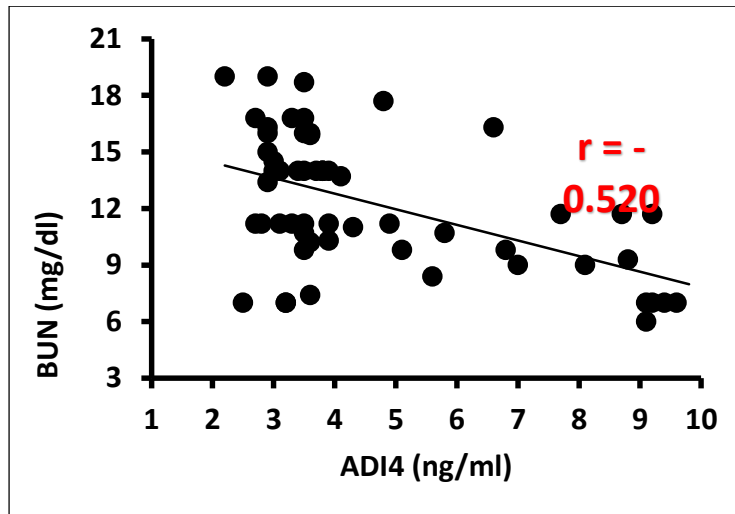


Figure (12) The inverse relationship between arginine deaminase 4 and blood urea nitrogen

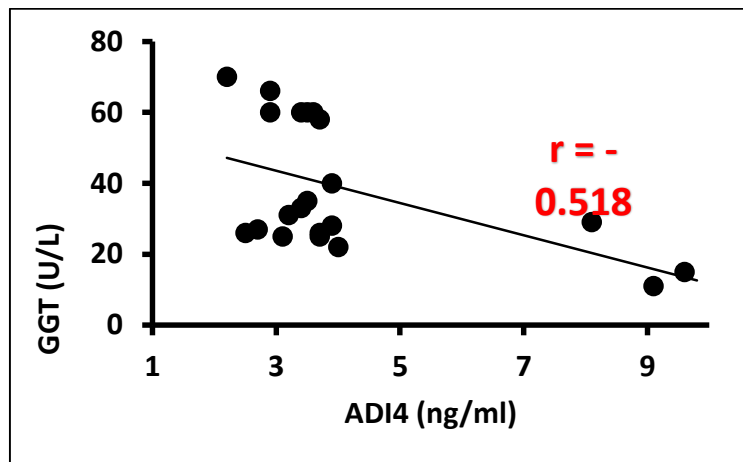


Figure (13) The inverse relationship between arginine deaminase 4 and GGT

DISCUSSION

The low levels of ADI 4 in patients' blood serum are due to the build-up of free fatty acids in the liver, which are either oxidized by mitochondria [18] or used to form triglycerides and cholesterol. If the amount of free fatty acids exceeds the oxidation capacity of mitochondria, build-up of triglycerides occurs in the liver and thus decreased urea synthesis. brought about by a urea cycle flaw enzymes and low levels of ADI 4 this leads to hyperammonemia [13].

The study also indicates that there is no effect of the duration of infection on ADI 4 rates, as shown in Table (5). The reason may be attributed to the fact that the activity of the enzyme is essentially low in patients, because the enzyme is crucial in removing toxins from the liver, whether the person is recently infected or is suffering from fatty liver for a longer period, as it is noted that the effectiveness of the enzyme begins to gradually increase, which is statistically imperceptible to the therapists, which may indicate the benefit of using the treatment (Liveramin). A healthy diet with exercise, taking the drug Liveramin, or both, compared with their levels in untreated patients and those newly diagnosed with fatty liver. The reason for this may be that exercise activates the process of metabolic reprogramming in many types of cells [19], including Kupffer cells, which prefer the anti-inflammatory type [20]. It also improves cognition by improving increased glycolysis in

macrophages in the central nervous system [21]. There is some evidence of exercise-mediated adaptation of liver mitochondria [22]. A previous study showed that exercise for 3 months led to a decrease in the percentage of fat in the liver of people with NAFLD and improved the function of liver cells, but there were no changes in hepatic ATP [23]. The use of treatment (Liveramin) with vitamin E prescribed by the doctor to patients has its beneficial therapeutic medical effects by improving liver functions and modifying the effectiveness of the enzymes studied because it contains the active ingredient silymarin, which is insoluble in water [24], as it rejuvenates liver cells, anti-inflammatory action, reduction of liver enzymes, and improvement in lipid parameters [25]. Also, no significant differences or influence of the variables studied were observed by the type of treatment. Studies have indicated that weight gain in patients with NAFLD is caused by the build-up of triglycerides in liver cells [26].

The main role of liver mitochondria is to produce energy by oxidizing amino acids and fatty acids. The close coupling between substance oxidation and ATP generation is precisely controlled by hepatocytes [27]. Fat accumulation leads to fatty liver resulting from impaired mitochondrial fatty acid oxidation and decreased ADI4 activity, but may also result from other mechanisms, e.g. Activate lipogenesis and reduce low-density lipoprotein secretion [28]. The results showed a decrease in ADI4 activities in patients and when compared to lower age groups, the control group was older (over 45), as shown in Table (7) for the patient group and Table (8) for the control group. The reason for this may be due to a lack of protein synthesis (Construction processes decrease with age) and therefore ADI4 activities decrease [29]. In general, men are more likely than women to have fatty liver due to differences in the structure of chromosomes and levels of sex hormones, as well as differences in lifestyle. In men, the typical prevalence of fatty liver is 26%, which is twice that in women (13%) [30].

The effectiveness of the enzyme is higher in males compared to females. This may be due to some hormonal changes in females. Estrogen deficiency reduces the effectiveness of the ADI4 enzyme, especially in women with polycystic ovary syndrome. Progesterone can change the activity of ADI4, as it inhibits the activity of the enzyme. It increases intracellular calcium, allowing ADI4 to move into the nucleus [31]. The results in Tables 7 and 8 indicate ADI4 activity decreased with aging regardless of person health condition. . It is possible that increased cholesterol and hyperlipidemia in the blood serum of people with fatty liver result from inhibition of Lipoprotein lipase [32, 33]. The decrease in high-density lipoprotein could be due to

This lipoprotein has protective, antioxidant and anti-inflammatory properties [34]. Hyperlipidemia and high levels of triglycerides in the blood are due to abnormal formation of triglycerides [35], a defect in their metabolism and how to remove them, which are associated with very low-density lipoprotein (VLDL), and decreased fatty acid oxidation [36]. The primary organ in charge of extracting nitrogen from the blood to produce urea that the body can expel is the liver [12]

The urea cycle is also very important to remove excess nitrogen that can accumulate in the form of ammonia [37]. Decreased urea synthesis caused by a defect in urea cycle enzymes leads to hyperammonemia [13]. There is a negative correlation between NAFLD and the skeletal muscle index, which is calculated as the total skeletal muscle excess relative to body weight. The body excretes creatinine at a fairly constant rate, which is mostly influenced by total muscle mass. Recently, an interesting new index called the ratio of Cr to body weight (Cr/BW) has been shown to be very useful for predicting NAFLD [38]. Elevated levels of ALT are a sign of NAFLD, which is associated with obesity, insulin resistance, and genetic markers ([39]., confirming the relationship between alanine metabolism and the liver [40]. GGT contributes to the synthesis and metabolism of glutathione in a number of bodily tissues [41]. Albumin levels are affected in patients with fatty liver disease. The results showed a slight increase in albumin levels, as it prevents damage to liver cells and protects mitochondria from oxidative stress caused by cytokines. Mitochondrial damage is caused by high-fat diets in patients with NAFLD and in inflammatory conditions [42]. The findings

indicated a minor drop in bilirubin levels, which is typically brought on by a malfunction in liver secretion. Since bilirubin is first coupled to albumin in the blood serum and then expelled in bile, its secretion and conjugation are strongly linked to the liver's conjugation and excretion function, although bilirubin alone is not a sign of the structural function of the liver. The findings revealed a considerable drop in globulin levels; individuals with NAFLD have a malfunction in their liver cells, which explains this decline [43].

CONCLUSION

Through the results of the research study on patients with NAFLD, a number of important conclusions were reached: A decrease in ADI4 enzyme activities in the NAFLD patient group compared with the control group, an increase in the activity of ALT, GGT, total cholesterol, triglycerides, LDL-C, and VLDL-C in the NAFLD group in contrast to the control group, most patients with NAFLD have a high BMI, there is a significant direct relationship between ADI4 and total protein, especially globulin, and an inverse relationship with urea, BUN, and GGT. The literature has shown that Arginine deaminase type IV can be considered a good diagnostic indicator in patients with NAFLD to predict the severity of the disease.

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