A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

ARGINASE ACTIVITY AND OXIDATIVE MARKES IMBALANCE IN PATIENTS WITH CHRONIC DIABETES MELLITUS COMPLICATION

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

ALA ULDDIN MOHAMMAD MAHDI AL BAYATI

ÇANKIRI

2022

ARGINASE ACTIVITY AND OXIDATIVE MARKES IMBALANCE IN PATIENTS WITH CHRONIC DIABETES MELLITUS COMPLICATION

By Ala Ulddın Mohammad Mahdı AL BAYATI

Jaunary 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor **:** Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor **:** Prof. Dr. Israa Ghassan Akram ZAINAL

Examining Committee Members:

Chairman **:** Asst. Prof. Dr. Volkan EYÜPOĞLU
Chemical
Çankırı Karatekin University

Member **:** Assoc. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kırıkkale University

Member **:** Asst. Prof. Dr. Şevki ADEM
Chemical
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Ala Ulddin Mohammed Mahdi ALBAYATI

ABSTRACT

ARGINASE ACTIVITY AND OXIDATIVE MARKES IMBALANCE IN PATIENTS WITH CHRONIC DIABETES MELLITUS COMPLICATION

Ala Ulddın Mohammad Mahdı AL BAYATI

Master of Science in Chemistry

Advisor: Prof. Dr. Volkan EYÜPOĞLU

Co-Advisor: Asst. Prof. Dr. Zehra Gülten YALÇIN

Jaunary 2022

In the world, diabetes kills both adults and children. During a two-month period, 100 diabetics and 35 healthy controls collected 135 blood samples. Patients with anemia, hepatitis, or hypertension were excluded, as sampled from women over sixty and pregnant women. After separation, the serum was kept at -200C. Total protein, albumin, globulin, amino, carbonyl, and thiol groups were isolated. All samples had arginase enzyme activity. Second, the current study included a statistical investigation of the parameter-arginzyme correlation. The research's findings Diabetics have higher levels of arginase in their blood serum than healthy controls (P0.05). Diabetes patients reported reduced levels of total protein, albumin, and globulin, all at P0.05. An increase in free amino acid content in patients relative to healthy controls (P0.05). The carbonyl group concentration decreased inpatients and increased in diabetics at the probability threshold of P0.05. NO, and MDA concentrations increased in patients compared to healthy controls.

2022, 34 pages

Keywords: Arginase, Patients, Chronic diabetes, Millets complications, oxidative,

ÖZET

KRONİK DİYABET DARI KOMPLİKASYONU OLAN HASTALARDA ARGİNAZ AKTİVİTESİ VE OKSİDATİF İŞARETLER DENGESİZLİĞİ

Ala Ulddın Mohammad Mahdı AL BAYATI

Kimya, Yüksek Lisans

Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU

Eş Danışman: Prof. Dr. Israa Ghassan Akram ZAINAL

Ocak 2022

Dünyada diyabet hem yetişkinleri hem de çocukları öldürüyor. İki aylık bir süre içinde 100 diyabet hastası ve 35 sağlıklı kontrolden 135 kan örneği alındı. Altmışın üzerindeki kadınlardan ve hamile kadınlardan örneklenen anemi, hepatit veya hipertansiyonu olan hastalar çalışma dışı bırakıldı. Ayrıldıktan sonra serum -20°C'de tutuldu. Toplam protein, albümin, globulin, amino, karbonil ve tiyol grupları izole edildi. Tüm numuneler arginaz enzim aktivitesine sahipti. İkincisi, mevcut çalışma parametre-arginzim korelasyonunun istatistiksel bir araştırmasını içeriyordu. Araştırmanın bulguları Şeker hastalarının kan serumlarında sağlıklı kontrollere göre daha yüksek arginaz seviyeleri vardır (P0.05). Diyabet hastaları, tümü P0.05'te olmak üzere toplam protein, albümin ve globulin düzeylerinin azaldığını bildirdi. Sağlıklı kontrollere göre hastalarda serbest amino asit içeriğinde bir artış (P0.05). Karbonil grubu konsantrasyonu yatan hastalarda azaldı ve diyabetiklerde P0.05 olasılık eşiğinde arttı. NO ve MDA konsantrasyonları sağlıklı kontrollere göre hastalarda arttı.

2022, 34 sayfa

Anahtar Kelimeler: Arginaz, Hastalar, Kronik diyabet, Millet komplikasyonları, oksidatif

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Prof. Dr. Volkan EYÜPOĞLU, for his patience, guidance and understanding.

Ala Ulddın Mohammad Mahdı AL BAYATI

Çankırı-2022

CONTENTS

ABSTRACT ..... **i**
ÖZET ..... **ii**
PREFACE AND ACKNOWLEDGEMENTS ..... **iii**
CONTENTS ..... **iv**
LIST OF SYMBOLS ..... **vii**
LIST OF ABBREVIATIONS ..... **viii**
LIST OF FIGURES ..... **ix**
LIST OF TABLES ..... **x**
1. INTRODUCTION **1**
2. LITERATURE REVIEW **2**
2.1 Diabetes Mellitus. **2**
2.2 Classification of Diabetes Mellitus. **2**
2.2.1 Insulin dependent diabetes mellitus (IDDM) **2**
2.2.2 NonInsulin dependent diabetes mellitus **3**
2.2.3 Gestational diabetes **3**
2.2.4 Other types of diabetes **3**
2.3 Diabetes Mellitus Symptoms **4**
2.4 Etiology of Diabetes **4**
2.5 Complications of Diabetes mellitus **5**
2.5.1 Sudden complications includes **5**
2.5.2 Chronic complications **5**
2.6 Screening, Early Detection and Treatment of Complications **5**
2.7 Arginase **6**
3. MATERIALS AND METHODS **7**
3.1 Subjects. **7**
3.2 A set of Requirements for Exclusion **7**
3.3 Samples Collection **7**
3.4 Methods. **7**
3.4.1 Estimation of total protein levels in all studied groups. **7**
3.4.2 Estimation of albumin levels by bromo cresol green method. **8**

3.4.3 Estimation of free amine level in all studied groups....8
3.4.4 Estimation of the carbonyl group level in all studied groups....8
3.4.5 Estimation of thiol groups level in all studied groups....8
3.4.6 Estimation of ischemia-modified albumin (IMA) in all studied group....8
3.4.7 Estimation of arginase activity in all studied groups. 9
3.4.8 Estimation of NO concentration in all studied groups....9
3.4.9 Estimation (MDA) in alle study groups. 9
3.4.10 Estimation of plasma thiol/disulfide homeostasis in all studied group 10
4. RESULTS AND DISCUSSION....11
4.1 Estimation of Some Biochemical Parameters in the Plasma of All Studied Groups. 11
4.1.1 Estimation of arginase activity levels in the plasma all studied groups 11
4.1.2 Estimation of total protein, albumin, globulin and albumin/globulin ratio in all Studied Groups. 11
4.1.3 Estimation of free amine levels in the plasma of all studied groups. 12
4.1.4 Estimation of carbonyl levels in the plasma of all studied groups. 13
4.1.5 Estimation of thiol levels in the plasma all studied groups. 13
4.1.6 Estimation of (IMA) levels in the plasma all studied groups. 14
4.1.7 Estimation of (thiol/disulfide homeostasis) levels in the plasma all studied group. 14
4.1.8 Estimation of (NO) levels in the plasma all studied group....15
4.1.9 Estimation of (Malondiadehyde (MDA)) levels in the plasma of all studied group 15
4.2 Correlation Analysis Study 16
4.3 Discussion....22
4.3.1 Arginase activity 22
4.3.2 Total protein, albumin, globulin and albumin/globulin ratio 22
4.3.3 Free amine 23
4.3.4 Carbonyl....23
4.3.5 Thiol 24
4.3.6 Ischemia-modified albumin (IMA) 24
4.3.7 Thiol/disulfide homeostasis....24
4.3.8 NO level....25

4.3.9 Malondiadehyde (MDA) 25
4.3.10 Correlation 26
5. CONCLUSIONS AND RECOMMENDATION 27
REFERENCES 28
CURRICULUM VITAE ..... Hata! Yer işareti tanımlanmamış.

LIST OF SYMBOLS

%	Percent
nm	Nanometer
mL	Milliliter
mg	Milligrams
mg/dL	Milligrams per deciliter

LIST OF ABBREVIATIONS

DM	Diabetes mellitus
IDDM	Insulin dependent diabetes mellitus
EDTA	Ethylenediaminetetraacetic acid
BSA	Bovine serum albumin
BCG	Bromo Cresol Green
IMA	Ischemia-modified albumin

LIST OF FIGURES

Figure 4.1 Regression Correlation between Argainas and Total protein.....16
Figure 4.2 Regression Correlation between Argainas and Al bumin.....17
Figure 4.3 Regression Correlation between Argainas and Globulin.....17
Figure 4.4 Regression Correlation between Argainas and Free amino.....18
Figure 4.5 Regression Correlation between Argainas and Carbonyl.....18
Figure 4.6 Regression Correlation between Argainas and Thiol.....19
Figure 4.7 Regression Correlation between Argainas and Albumin IMA.....19
Figure 4.8 Regression Correlation between Argainas thiol/disulfide homeostasis.....20
Figure 4.9 Regression Correlation between Argainas and NO concentration.....20
Figure 4.10 Regression Correlation between Argainas and MDA.....21

LIST OF TABLES

Table 4.1 The Arginase activity in the Plasma of All Studied Groups.....11
Table 4.2 Total Protein, Albumin, Globulin and Albumin/globulin ratio Levels in the Plasma of all studied Groups.....12
Table 4.3 Free Amine Levels in the Plasma of All Studied Groups.....12
Table 4.4 The Carbonyl Levels in the Plasma of All Studied Groups.....13
Table 4.5 Thiol Levels in the Plasma of All Studied Groups.....13
Table 4.6 The (IMA)Levels in the Plasma of All Studied Groups.....14
Table 4.7 The (thiol/disulfide homeostasis) Levels in the Plasma of All Studied Groups.....14
Table 4.8 The (NO concentration) Levels in the Plasma of All Studied Groups.....15
Table 4.9 The (MDA)Levels in the Plasma of All Studied Groups.....15
Table 4.10 The Correlation Data between the Studied Plasma Variables in Patients Groups. (Coefficient of Correlation/ Significance r/p).....21

1. INTRODUCTION

Diabetes mellitus (DM) refers to a set of metabolic disorders that are defined by hyperglycemia as a consequence of abnormalities in either insulin production or insulin action, or the combination thereof (Association 2008). Nearly 1.6 million people died in 2015 as a direct consequence of DM, which was predicted to impact 9 percent of the world's population in 2014 (Verhulst *et al.* 2019). (Verhulst *et al.* 2019). Diabetic consequences include retinopathy, nephropathy, neuropathy, and cardiovascular disease, which all contribute to the condition's high death rate (Zhito *et al.* 2020) (Zhito *et al.* 2020) (MJ 2008). It has become a key aspect of contemporary diabetic management to avoid and control complications. Additional diabetes outcomes, such as hyperglycemia, insulin resistance, dyslipidemia, hypertension, and immunological dysfunction, may be induced by a range of different pathogenic processes, as well. The pathogenic reasons of the typical diabetic problems are first studied in order to appreciate the biological elements that could be at play here. (Borgnakke 2019) As a consequence of diabetes, glucose is not converted into energy; Which leads to the availability of large levels of it in the blood. Carbohydrate supplies are depleted. This leads the cells of the body to depend on stored proteins and fats as alternate sources of energy (Wackett *et al.* 2002) (Wackett *et al.* 2002) Having DM. Over the years, it leads to problems, which causes significant damage to the nerves and blood vessels, and hence may lead to heart il

Study's Objectives

In the past quarter of a century, the research of antioxidant indicators in individuals with diabetic problems has not received enough attention. This is the first study of its kind to investigate the relationship between arginase activity and a number of antioxidant markers in patients with diabetes complications in comparison to healthy subjects. These parameters include total protein, albumin, globulin, albumin globulin ratio, ischemia modified albumin, native and (total thiol, amino, and carbonyl) groups, and Malondialdehydelnesses, kidney diseases, neuropathy, and diabetic foot (MJ 2008).

2. LITERATURE REVIEW

2.1 Diabetes Mellitus.

Insufficient insulin, decreased insulin sensitivity in tissues, or a combination of the two may lead to metabolic abnormalities and abnormally high glucose levels in blood. The phrase "diabetes mellitus" embraces a wide range of medical disorders (Rother 2007). Many of the body's vital organs, including the eyes, kidneys, nerves, heart, and blood arteries, are especially susceptible to long-term damage and failure as a consequence of diabetes (Care 2004). Because of their condition, diabetics have difficulty turning food into energy. Cells in the intercellular medium get glucose from meals, which is broken down into glucose and distributed throughout the body via blood. Most cell types need insulin to allow glucose to enter their cells. Because DM prevents glucose from being converted to energy, the blood glucose level rises, despite the fact that cells are still starving for energy. Deficiency or resistance to insulin by muscles and liver (Chantrapanichkul *et al.* 2020) leads to diabetes mellitus. It takes time for high blood sugar to build up in the body, and various pathogenic processes can contribute to the development of diabetes, including autoimmune destruction of beta cells in the pancreas, which results in abnormalities that lead to insulin resistance, as well as nerve and blood vessel damage that can cause complications such as heart disease, stroke, and kidney failure (Cowell 2008).

2.2 Classification of Diabetes Mellitus.

2.2.1 Insulin dependent diabetes mellitus (IDDM)

About 10% of patients with diabetes are diagnosed with this kind of the illness. Those under the age of 25 are considered to have juvenile diabetes, which is a form of the illness. This kind includes reduced or absent insulin release from pancreatic fuel cells (B cells). Autoimmunity, defined by the onslaught of immunological T cells on insulin-producing cells, is the primary cause of this breakdown(Turner *et al.* 1999) .

2.2.2 NonInsulin dependent diabetes mellitus

More than 90% of those with DM have this kind, which often affects adults over the age of (40) years and is more frequent than type 1. (Taft 1984). Glucose levels in the blood stay high with this kind of diabetes because the cells of the body are resistant to insulin's effects. The liver, muscle, and adipose tissue are particularly sensitive to the absence of insulin or a deficiency in insulin activity in this form of diabetes. Obesity and a predisposition to the disease run in families. To put it more succinctly (Jackson 1978).

2.2.3 Gestational diabetes

On par with the second type, this condition affects 2 to 5 percent of pregnant women, but medical monitoring during pregnancy can help prevent the condition or at least improve the condition of the mother long term (Association 2014). This is because insulin levels are low and tissues are less responsive to insulin's effects. Women with a low glucose tolerance are more likely to develop this kind of diabetes. During a normal pregnancy, the progesterone hormone rises; this causes an increase in growth hormone and anti-insulin activity (Lee *et al.* 2015).

2.2.4 Other types of diabetes

There are many rare causes of DM that cannot be classified as type 1 or type 2, and some cases are diabetes; Because of the lack of response to insulin receptors on the tissues of the body, even if the insulin levels are normal, and this makes this case different from the second type, and this type is very rare. Also, genetic mutations in chromosomes or in mitochondria; can lead to abnormalities in beta cell function(Wang *et al.* 2012).

2.3 Diabetes Mellitus Symptoms

When a person is diabetic, they will experience a wide range of symptoms, including (Schipper *et al.* 2021).

1.Increasing the frequency and amount of urination is necessary (Polyuria).

2. Excessive thirst and excessive water intake are caused by a considerable loss of fluids from the body.

3. Patients with type 1 diabetes lose weight for no apparent reason when the anti-insulin hormone glucagon (secreted by the pancreas) breaks down fats and proteins into sugars that are expelled in the urine. Particularly with young people, it is this way.

4.The urge to consume more and an overall sensation of weakness and weariness

2.4 Etiology of Diabetes

Type 2 diabetes is more likely to occur in people with obesity, particularly in the midsection (that is, their stomachs), than in those who are fat primarily in their limbs.Insulin is unable to bind with its receptors if a person does not engage in regular physical activity.As a result, insulin production is impaired in the pancreatic gland, which may be caused by infections, internal bleeding, or the removal of the pancreas.Because insulin sensitivity and efficacy decline with age, diabetes is becoming increasingly frequent among the elderly.One of the most important triggers for the development of an autoimmune destruction process is infection with the type 1 diabetes virus(Bluestone *et al.* 2010).

2.5 Complications of Diabetes mellitus

2.5.1 Sudden complications includes

All cells in the body have access to an increased quantity of fatty acids in their blood due to an insulin deficiency so severe that the body creates more fatty acids than it can utilize as fuel, leading to ketoacidosis. Type 1 diabetics have high blood glucose levels. Insulin deficiency or ineffectiveness prevents cells in the body from consuming glucose, resulting in a spike in blood glucose levels that exceed the body's entire reabsorption capacity and are excreted in the urine, resulting in water loss and an increased need for fluid intake(Köseoglu and Karaman 2007).

2.5.2 Chronic complications

The eyes of many persons with DM are damaged in a variety of ways, resulting in visual impairment. The term "neuropathy," which describes diabetes-related nerve damage, is used to describe the symptoms of this condition, which may cause a person to lose some of their capacity to feel.

2.6 Screening, Early Detection and Treatment of Complications

Although diabetes management can not prevent all problems, taking action early on can reduce their severity (Gong *et al.* 2019) A frequent eye checkup is necessary for those with DM. In low- and middle-income countries, rapid laser photocoagulation and effective blood glucose management may prevent or delay irreversible vision loss. Detection of early kidney failure may be done by testing urine protein, and the development to kidney failure can be delayed by the use of critical hypertension drugs. Renal failure can only be treated by dialysis or a kidney transplant. By seeing abnormalities in the skin that may lead to gangrene and amputation, regular examinations of the legs for indications of neuropathy help control blood pressure and prevent foot ulcers. Complications on people's capacity to function may be reduced by certain rehabilitation programs including physiotherapy and occupational therapy.

2.7 Arginase

The manganese-containing enzyme urea hydrolase arginase is responsible for the urea cycle's conversion of l-arginine to ornithine and urea, which eliminates potentially toxic ammonia (Wu and Tinoco 1998). Its importance in this cycle has been well-known for quite some time. Archaea are the most prevalent microbes that produce arginase, although they are not the only ones that have developed (Dzik 2014). The mitochondria may have transported arginase from bacteria to human cells. Arginase 2 is the only arginase found in plants, bacteria, yeasts, and other invertebrates (A2). Almost all animals that produce urea have arginase 1 (A1) in their cytosols. Even while certain animals have kidneys that produce A1, the brain and retina both produce A2, as well as red blood cells. Activation of either isoform may be triggered by a variety of circumstances. A1 in humans has 322 amino acids, whereas A2 contains 354 (Dizikes et al. 1986). (Colton *et al.* 2006). Various chromosomes contain the genes that code for each isoform, and these genes are unique to each isoform. They both have comparable modes of action and create similar metabolites. More than 60% of the amino acid residues are identical, and the areas critical to enzyme action are 100% homologous (Vockley *et al.* 1996).

3. MATERIALS AND METHODS

3.1 Subjects.

To conduct this research, researchers drew blood samples from 135 participants, including 100 diabetics aged 25 to 50, as well as 35 non-diabetics in the same age range. Two months after patients arrived to Azadi Teaching Hospital in Kirkuk, Iraq, samples were taken. The Azadi Teaching Hospital's Institute, Ethical Committee received ethical clearance. It was necessary to create a questionnaire form expressly for this purpose in order to gather comprehensive information about each patient's medical history.

3.2 A set of Requirements for Exclusion

1-Patients and controls aged 60 to 90 years old
2-The term "pregnant" is used in this context.
3-Hepatitis-infected patients with high blood pressure

3.3 Samples Collection

An EDTA tube is used to separate three milliliters of blood taken using a disposable syringe into two glass tubes. The tubes are spun at 1500 xg for 15 minutes after that. Separate the cells from the plasma by removing the buffy covering. Until used, plasma samples are stored at -20 °C.

3.4 Methods.

3.4.1 Estimation of total protein levels in all studied groups.

Using BSA as a reference protein, the method is utilized to determine plasma total protein levels (Lowry *et al.* 1951).

3.4.2 Estimation of albumin levels by bromo cresol green method.

The BCG Method (Bromo Cresol Green) is used to determine albumin levels. This BCG dye has an albumin-binding property, and the blue-green color it creates is detected at 630 nm. The rise in color correlates to the amount of albumin in the sample.

3.4.3 Estimation of free amine level in all studied groups

The Bromo Cresol Green (BCG) Method is used to determine albumin concentrations. At 630 nm, an albumin-binding BCG dye creates a blue-green color, and the rise in color correlates to the albumin level.

3.4.4 Estimation of the carbonyl group level in all studied groups

Use Levine et alapproach .'s to determine protein carbonyl groups (Ellman 1959). Carbonyl group content in di-nitro phenyl hydrazine has a direct effect on the intensity of the orange hue at a wavelength of 370 nm (DNPH).

3.4.5 Estimation of thiol groups level in all studied groups

The Ellman technique was used to measure thiol groups. The amount of thiol groups connected to 5,5-dithiol-bis-(2-nitrobenzoic acid) (DTNB) is directly correlated with an increase in color intensity (Levine *et al.* 1990).

3.4.6 Estimation of ischemia-modified albumin (IMA) in all studied group

The Test incubates 95 ml of a patient sample with cobalt chloride (CoII) for five minutes. While Co (II) attaches to unaltered albumin, Ischemia-induced alterations to the N-terminus of albumin alter the capacity of albumin to bind to Co (II) during incubation. After 25 minutes of incubation, add di-thio threitol (DTT) to the mixture. The DTT-Co (II) complex, which is not linked to albumin's N-terminus, is

spectrophotometrically visible at 500 nm. Duplicate IMA data from this test were recorded, and the mean was used as the final result (Unlüer *et al.* 2010).

3.4.7 Estimation of arginase activity in all studied groups.

The Zofia and Maria technique was used to assess arginase activity. L-arginine monochloride, sodium barbitone, MgCl2, sodium barbitone, and 0.5 ml of plasma were used in a system to perform the reaction. The material was kept at 37°C for 30 minutes. To halt the process, 1.3 mL of 20% tri chloroacetic acid was employed. For the appropriate ornithine content, the incubation medium was heated for 60 minutes after the addition of 2 mL of the ninhydrin solution (250 mg ninhydrin in 4 mL of H3PO4 and 6 mL of concentrated acetic acid) and 1 mL of concentrated acetic acid. Arginase activity may be measured spectrophotometrically at a wavelength of 515 nm. Using a standard curve for calibration, the ornithine concentration was determined. Arginase activity was assessed in terms of ornithine/L. (Khaleel *et al.* 2018).

3.4.8 Estimation of NO concentration in all studied groups

50 L of distilled water was used as a blank, and 50 L of sulfanilamide (1 percent in 5 percent H3PO4) was applied to samples in microplate wells. For 10 minutes at 37 °C, the samples were incubated The sample and blank were then re-incubated for 5 minutes at 37°C with 50 L of [N-(1-naphthyl)ethylene di amine di hydrochloride (0.1 percent) in distilled water]. The enzyme, linked immunosorbent assay (ELISA) reader was used to measure the absorbance at 540 nm (Sunrise,Tecan,Austria). The 0-50 M sodium nitrate linear standard curve was used to quantify the serum NOx levels.

3.4.9 Estimation (MDA) in alle study groups.

In microplate wells, 50 microliters of plasme samples were added together with 50 L of distilled water as a blank and 50 L of sulfanilamide (1 percent in 5 percent H3PO4) to samples. Ten minutes at 37 °C were spent incubating the samples The samples and the

blanks were then re-incubated for 5 minutes at 37 °C with 50 L of [N-(1-naphthyl)ethylene di amine di hydrochloride (0.1 percent) in distilled water]. Utilizing an ELISA reader, we measured the absorbance at a wavelength of 540 nm (Sunrise,Tecan,Austria). The linear standard curve with 0-50 M sodium nitrate was used to establish the serum NOx levels (Al-Daghri *et al.* 2018).

3.4.10 Estimation of plasma thiol/disulfide homeostasis in all studied group

Total thiol and native thiol were measured using Erel et alModified .'s Ellman method. In order to generate R1, we utilized an experimentally prepared solution of sodium borohydride (NaBH4) in 1000 mL of water-methanol (at a volumetric ratio of 1/1 of 10 mM). This reducing solution was used to determine the total thiol content. It was decided to do this experiment using a freshly prepared solution of NaCl (585.5 mg) in water-methanol (1/1 volume ratio) with a final concentration of 10 mM. This solution was used to determine the amount of natural thiol present. A solution of R2 was made in 1000 mL of Tris buffer, 100 mM, with a pH of 8.2. R2 had a final formaldehyde content of 6.715 mg per 1000 mL of Tris buffer. Each working day, 3.963 grams of 5,5 dithiobis-2-nitrobenzoic acid (DTNB) were produced at a final concentration of 10 mM. This solution was used to measure the concentrations of total and native thiol.

4. RESULTS AND DISCUSSION

4.1 Estimation of Some Biochemical Parameters in the Plasma of All Studied Groups.

4.1.1 Estimation of arginase activity levels in the plasma all studied groups

There were significant differences in the activity of arginase (mean SD) (mg/dL), between diabetes complications patients and healthy volunteers, as shown in Table 4.1.

Table 4.1 The Arginase activity in the Plasma of All Studied Groups.

parameters	Diabetic compliction patients	healthy subjects	P value
Arginase (mean ± SD) (mg/mL)	5.137±1.063	2.469±0.6457	0.0015 *

The findings showed that Arginase activity levels in patients were significantly higher (p0.05) than in healthy persons.

4.1.2 Estimation of total protein, albumin, globulin and albumin/globulin ratio in all Studied Groups.

Amounts of total protein (TP), albumin (album/gloration), globulin (album), and globulin (album/gloration) are shown in Table 4.2. Radin levels in the blood of diabetes patients with complications were found to be (mean SD) (gm/dl) higher than in the blood of healthy individuals.

Table 4.2 Total Protein, Albumin, Globulin and Albumin/globulin ratio Levels in the Plasma of all studied Groups.

parameters	Diabetic complication patients (mean ± SD) (g/dl)	Healthy subjects (mean ± SD) (g/dl)	P value
TP (gm/dl)	**4.855 ± 1.135**	**7. 054± 0.6532**	**0.0005***
Albumin (gm/dl)	**1.487 ± 0.2845**	**2.469 ± 0.6457**	**0.0001***
Globulin (gm/dl)	**2.649± 0.9226**	**5.054 ± 1.203**	**0.0457***

According to the findings, the "TP," "albumin," and globulin" levels of patients were significantly lower than those of healthy people (p0.05).

4.1.3 Estimation of free amine levels in the plasma of all studied groups.

Patients with diabetes problems and their associated subgroups are represented by Tables 4.3, which show the mean (SD) (mmole/L) concentrations of free amine in their plasma as compared to healthy people.

Table 4.3 Free Amine Levels in the Plasma of All Studied Groups.

parameters	Diabetic complications patients	healthy subjects	P value
free amino (mean ±SD) mmole\L	**59.35 ± 9.477**	**31.40 ± 6.064**	**0.0041***

Compared to healthy people, diabetic Complication patients showed a substantial (p 0.05) increase in the (free amino) groups.

4.1.4 Estimation of carbonyl levels in the plasma of all studied groups.

A comparison of the carbonyl concentrations in the plasma of diabetic complications patients and the groups of diabetic complications patients separated into as compared to healthy participants is shown in Table 4.4.

Table 4.4 The Carbonyl Levels in the Plasma of All Studied Groups.

parameters	Diabetic complication major patients	healthy subjects	P value
Carbonyl (mean ± SD) nmole\mL	72.79 ± 9.613	81.29 ± 6.401	0.0085

Carbony levels in the sick groups were found to be significantly lower (p0.05) than in the healthy participants.

4.1.5 Estimation of thiol levels in the plasma all studied groups.

Table 4.5 shows the plasma concentrations of (thiol) in diabetes complications patients compared to healthy people, shown as (mean SD) (mole/L).

Table 4.5 Thiol Levels in the Plasma of All Studied Groups.

parameters	Diabetic complications patients	healthy subjects	P value
Thiol (mean ± SD) (µmole/L)	32.35 ± 7.461	21.46 ± 4.507	0.0013 *

The findings showed that (thiol) levels in patients were significantly higher (p0.05) than in healthy participants.

4.1.6 Estimation of (IMA) levels in the plasma all studied groups.

Diabetic complications patients' plasma (IMA) concentrations are shown in Table 4.6 (mean SD) (Abs unit) as compared to healthy participants.

Table 4.6 The (IMA)Levels in the Plasma of All Studied Groups.

parameters	Diabetic compliction patients	healthy subjects	P value
IMA (mean ± SD) (Abs unit)	**0.6280 ± 0.06544**	**0.4564 ± 0.1256**	0.0001*

Patients in the (IMA) inpatient groups had higher levels of (p 0.05) compared to healthy controls.

4.1.7 Estimation of (thiol/disulfide homeostasis) levels in the plasma all studied group.

Table 4.7 compares the concentrations of (thiol/disulfide homeostasis) in diabetes complications patients' plasma with the concentrations in healthy participants (mean SD) (M mole /L).

Table 4.7 The (thiol/disulfide homeostasis) Levels in the Plasma of All Studied Groups.

parameters	Diabetic compliction patients	healthy subjects	P value
thiol/disulfide homeostasis (mean ± SD) (M mole /L)	**13.61 ± 3.504**	**8.797 ± 0.9294**	**0.0001***

(Thiol/disulfide homeostasis) levels in sick groups were found to be higher than in healthy participants (p0.05).

4.1.8 Estimation of (NO) levels in the plasma all studied group.

(NO concentration) levels in plasma diabetes complications patients (mean SD) (M) and the patient groups classified into accordingly as compared to healthy participants are shown in Tables 4.8 as (mean SD) (μM).

Table 4.8 The (NO concentration) Levels in the Plasma of All Studied Groups.

parameters	Diabetic compliction patients	healthy subjects	P value
NO (mean ± SD) (μM)	31.58 ± 6.724	20.14 ± 3.695	0.0002 *

A substantial (p 0.05) rise in (NO) levels was seen in patients compared to healthy individuals.

4.1.9 Estimation of (Malondiadehyde (MDA)) levels in the plasma of all studied group

(MDA) levels in diabetes complications patients and healthy people are shown in Tables 4.9 as (mean SD)(nmol/mL).

Table 4.9 The (MDA)Levels in the Plasma of All Studied Groups.

parameters	Diabetic compliction patients	healthy subjects	P value
MDA (mean ± SD) (nmol /mL)	4.057±1.505	2.269±0.6583	0.0001*

Patients had higher levels of (MDA) compared to healthy people, according to the findings.

4.2 Correlation Analysis Study

Figures 4.1 to 4.9 and Table 4.10 show a variety of phenotypic associations between the examined biochemical variables in the inpatients group. The findings of the correlation coefficients show a substantial positive link between (Arginase - Total protein).

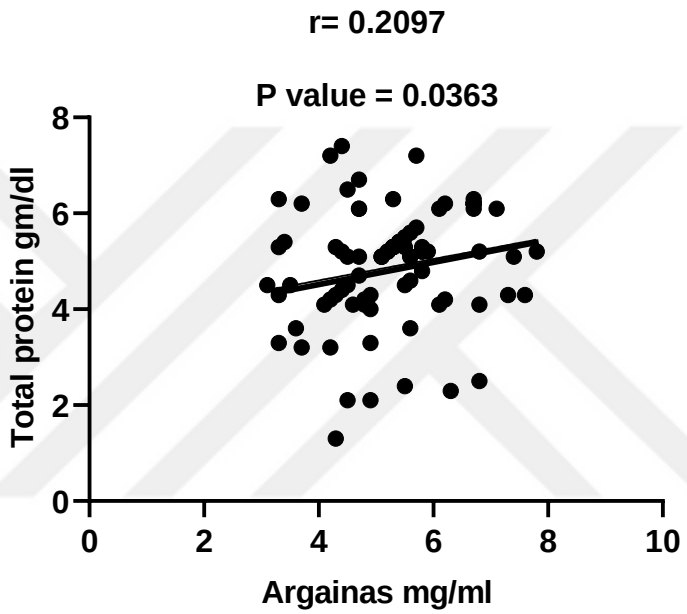

Figure 4.1 Regression Correlation between Argainas and Total protein.

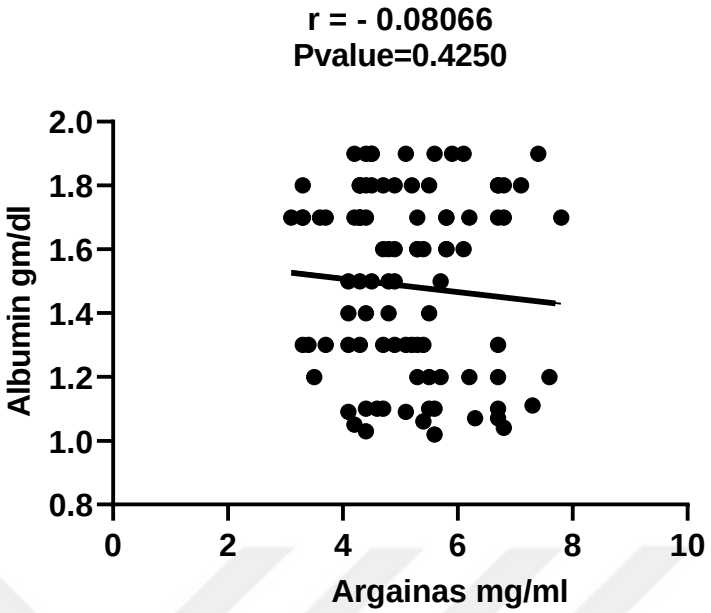

Figure 4.2 Regression Correlation between Argainas and Al bumin.

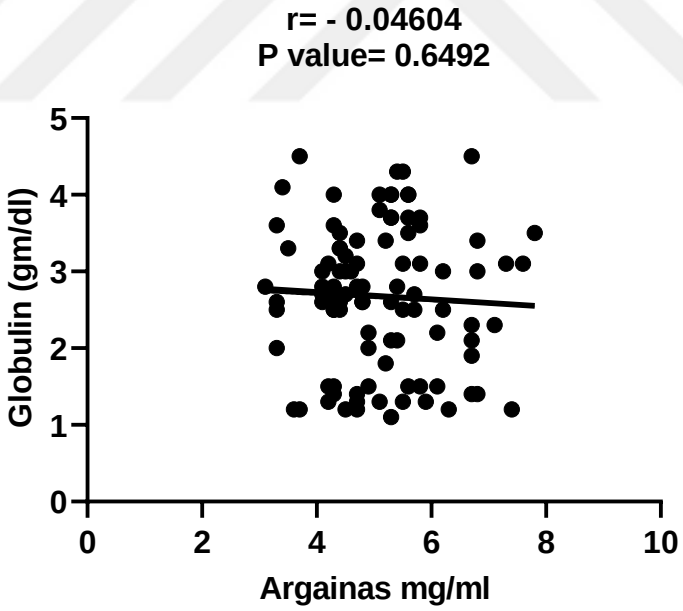

Figure 4.3 Regression Correlation between Argainas and Globulin.

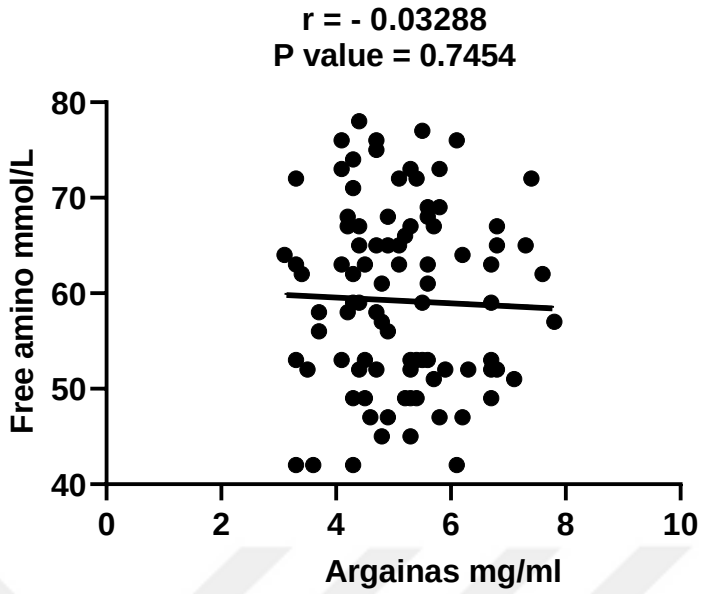

Figure 4.4 Regression Correlation between Argainas and Free amino

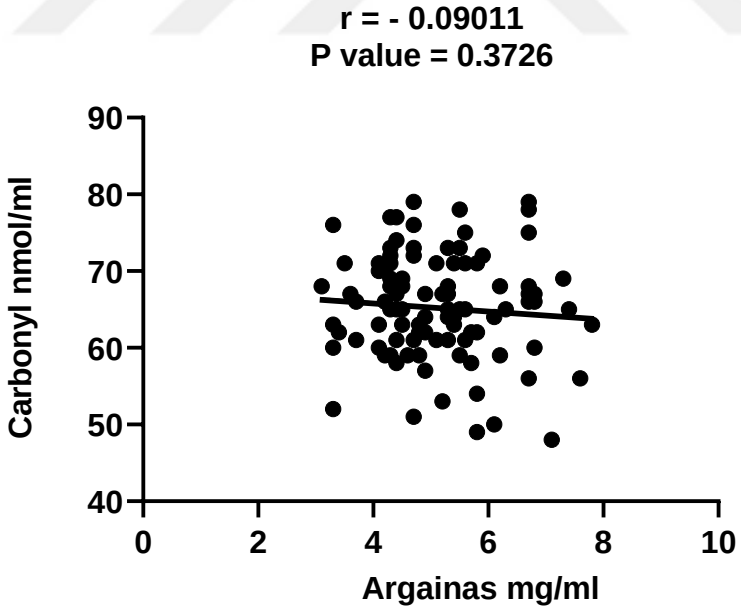

Figure 4.5 Regression Correlation between Argainas and Carbonyl

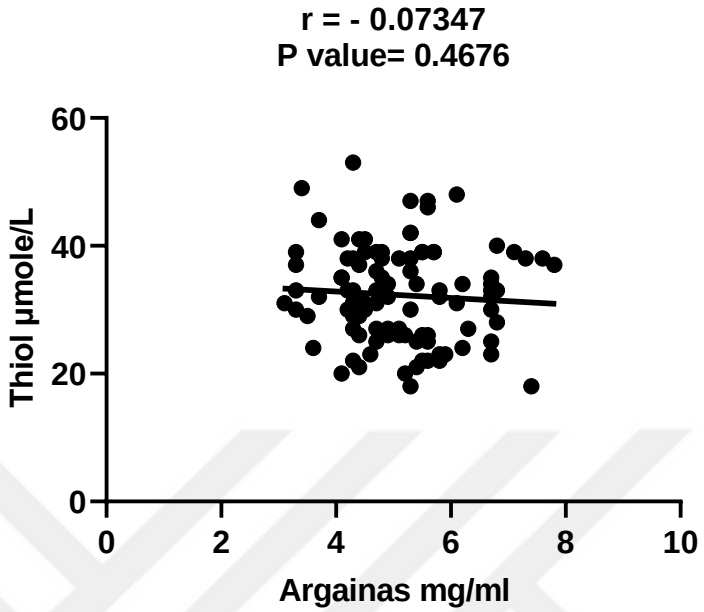

Figure 4.6 Regression Correlation between Argainas and Thiol.

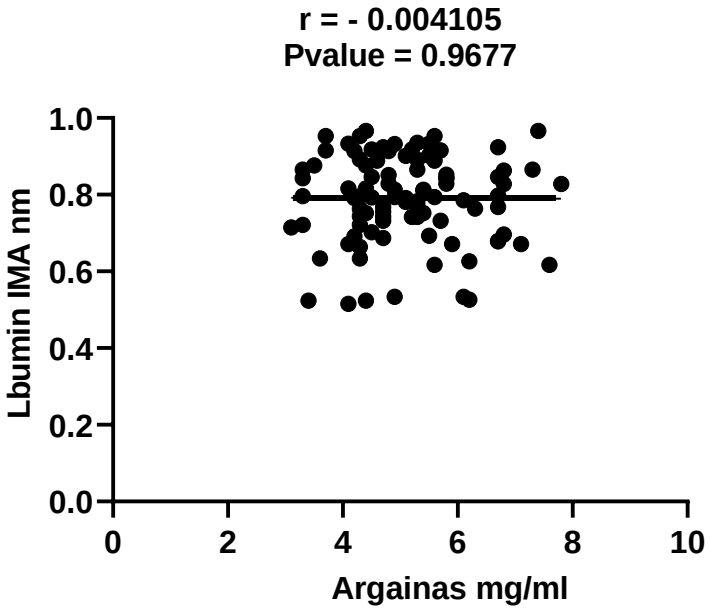

Figure 4.7 Regression Correlation between Argainas and Albumin IMA

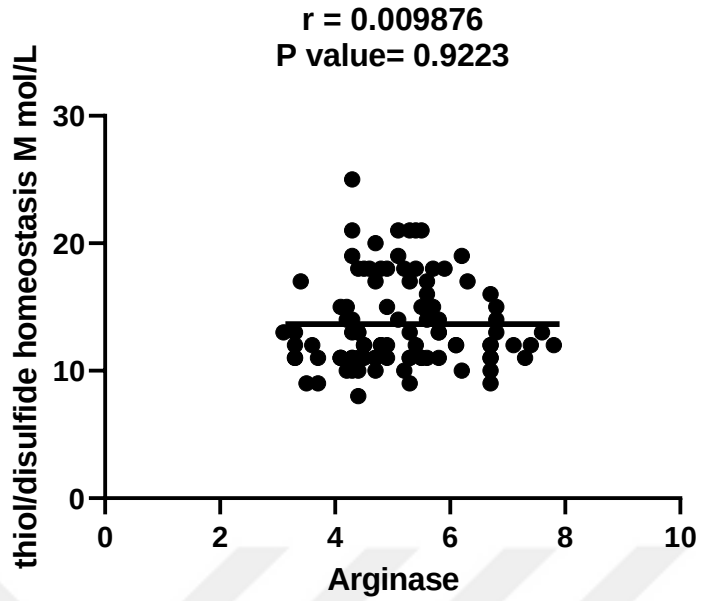

Figure 4.8 Regression Correlation between Argainas thiol/disulfide homeostasis.

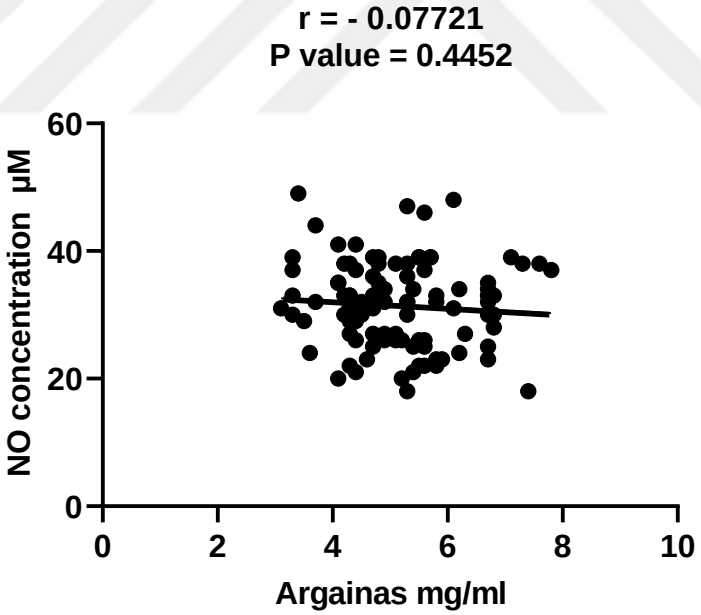

Figure 4.9 Regression Correlation between Argainas and NO concentration.

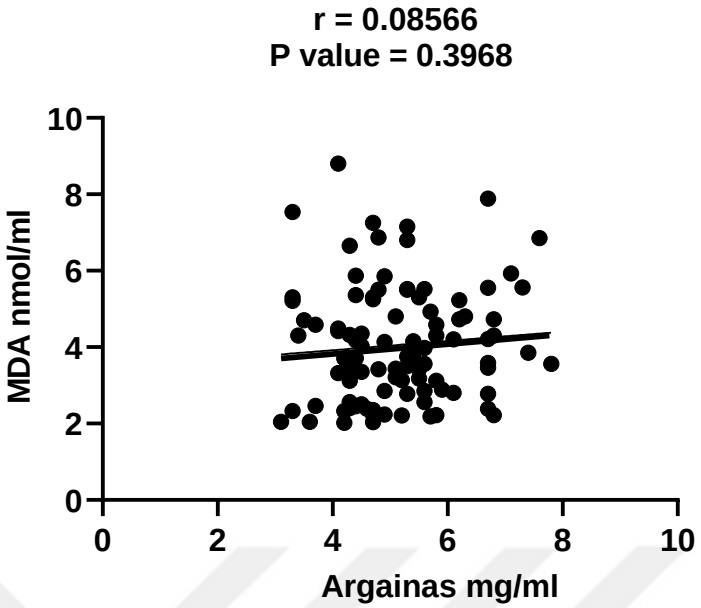

Figure 4.10 Regression Correlation between Argainas and MDA.

Table 4.10 The Correlation Data between the Studied Plasma Variables in Patients Groups. (Coefficient of Correlation/ Significance r/p).

Figures	Variables	r/p
4.1	Arginase - Total protein	0.2097/0.0363
4.2	Arginase - Albumin	-0.08066/0.4250
4.3	Arginase – Globulin	-0.04604/0.6492
4.4	Arginase – Free amine	-0.03288/ 0.7454
4.5	Arginase - Carbonyl	-0.09011/ 0.3726
4.6	Arginase- Thiol	-0.07347/ 0.4676
4.7	Arginase - Albumin IMA	-0.004105/0.9677
4.8	Arginase - thiol/disulfide homeostasis	0.009876/0.9223
4.9	Arginase- NO concentration	- 0.07721/0.4452
4.10	Arginase- Albumin MDA	0.08566/0.3968

IndicatedArgenase Cornelated with TP and mgbe effective for monitoring diabetes complications, according to the study's correlation data.

4.3 Discussion

4.3.1 Arginase activity

Arrange activities in a way that makes it difficult for others to find it.Patients with diabetes had greater levels of arginine activity than healthy controls, according to the data above Taple 4.1. Analogues of arginine are present in two places in the body: the liver and other tissues, including tiny amounts in the kidneys, prostate, pituitary, thyroid, small intestine and muscles of the skeleton as well as the heart and lung. The liver contains the first analogue virtually entirely. An increased activity of arginase as well as an increase in the metabolism of arginine in certain brain cells are both associated with an increased risk of neurodegenerative disease and aging when arginine levels are elevated (Shosha *et al.* 2020). A lot of arginine arginase is found in bone marrow cells and red blood cells because it impairs lymphocyte function by leaking from red blood cell membrane cells and from white blood cells, particularly neutrophils, during blood transfusion (Huang *et al.* 2016) due to this rise A diabetic patient's increased glucose levels are to blame, according to this research. Kashyap et al. (Kashyap et al. 2008) evaluated 12 diabetic individuals and found similar findings.

4.3.2 Total protein, albumin, globulin and albumin/globulin ratio

Taple version 4.2 Protein-containing molecules, such as "enzymes, hormones, and antibodies," keep the blood's osmotic pressure in balance. There is a belief that human plasma has the highest proportion of albumin (Pieniazek *et al.* 2018). A buildup of toxic waste is accumulated in the kidneys due to diabetes, leading in the loss of total protein (TP), albumin, and globulin in urine. This causes low levels of albumin and globulin to be seen in the bloodstreams of individuals with diabetes (Kawata *et al.* 2021). Albumin and globulin, two common transport proteins, may act as early warning signs of diabetic problems. In 2012, (Santos *et al.* 2012) The albumin component accounts for more than half of plasma protein. Osmotic pressure control, delivery of nutrients, and waste

disposal are just a few of albumin's various functions in the body. Among the other things it moves are harmful substances like bilirubin and cholesterol. According to Ahmed et al. A study of the prognosis of protein oxidation in patients with diabetes mellitus, the findings of the TP, albumin, and globulin levels are in accord.

4.3.3 Free amine

It has been shown that the presence of fatty acid-bound branch-chain amino acids (BCAAs) and central nervous system amino acids (Acyl-CNSO) is a disease sign for persons with diabetes (Mihalik *et al.* 2012). The faster BCAA gating mechanism may have led to a rise in diabetes millet propionyl carnitine Taple 4.3 (C3) and isovalerylcarnitine (C5) concentrations in plasma (Libert *et al.* 2018) Patients with diabetes were shown to have comparable amino/TP levels as those with thalassemia, breast cancer and diabetes, according to an investigation of protein oxidation.

4.3.4 Carbonyl

Table 4.4 th time around Stress and inflammation of the airways in patients may be to blame for an elevated carbonyl level (Odetti *et al.* 1999). Protein carbonyls have been detected in a number of human disorders, including chronic kidney disease and diabetes mellitus. Overhydration, sarcopenia, and low plasma prealbumin levels may all contribute to a drop in plasma protein carbonyl levels (Trombetta *et al.* 2006). There is some disagreement with Karam Y.Noah on the effect of some biopolymer derivatives on the level of free radicals in the sera of diabetics, but the results of this study are in agreement with Mateen et al (Mateen *et al.* 2016) and Odetti et al (Odetti *et al.* 1999), who studied the above parameters in rheumatoid arthritis and type 2 diabetes patients, respectively (Wang *et al.* 2020).

4.3.5 Thiol

Taple's 4.5 Thyol groups at physiological pH levels are more powerful nucleophiles because of their lysine and arginine side chains (Zeng and Davies, 2005, Thorpe and Baynes 2003). A recent research in people with type 2 diabetes examined the aforementioned factors (Wang *et al.* 2020). Total thiols have been used as an indicator of oxidative stress to study a wide range of diseases, including ischemic heart disease, diabetes and pulmonary diseases as well as preeclampsia and renal illness (Gumusyayla et al. 2016).

4.3.6 Ischemia-modified albumin (IMA)

Taple 4.6 Ischemia-Modified Albumin is a sign of ischemia and is most often studied in cardiac pathology. IMA has been proven to be a key marker of ischemia in a variety of diseases related with oxidative stress. Albumin's N-terminus undergoes a shift and loses its metal bonding activity in a hypoxic state. When ROS are released as a result of ischemia, albumin undergoes a modification known as IMA. Compared to the control group, the serum IMA levels in the patients group were considerably higher (Sbarouni et al. 2008).

4.3.7 Thiol/disulfide homeostasis

Taple 4.7 is the current version. Prooxidants and antioxidant defense mechanisms are dynamically balanced in the human body. As a result of an imbalance in the body's internal chemistry, oxidative stress, lipid peroxidation, DNA damage, and cell death ensue. Intracellular and extracellular regions include several oxidative and antioxidative compounds. The non-enzymatic antioxidant system of the human body includes the oxidation-reduction processes of dynamic thiol disulfide homeostasis. Oxidation reactions with free radicals may damage sulfur-containing amino acids' thiol groups. As oxidative stress occurs, the disulfide bonds of proteins' thiol groups are changed to SH groups (Erel 2004b). This is a two-way response. Disulfide linkages may revert to thiols

in an oxygen-rich environment. Thiol-disulfide is thus a dynamic equilibrium that changes in response to the oxygenation level in the environment. In fact, thiols account for half of all available antioxidant capability. Because of this, it may serve as a reference point for determining serum levels in clinical settings (Erel 2004a).

4.3.8 NO level

Table 4.8. The metabolic, circulatory, and cellular actions of nitric oxide make it a critical regulator molecule in biology. In type 2 diabetes, insulin regulates the activation of NO synthase (NOS) via the Akt pathway, which regulates NO metabolism. Insulin resistance has been linked to changes in NO production, which may influence the vascular response (Muniyappa and& Quon 2007, Erel 2004a).

4.3.9 Malondiadehyde (MDA)

The latest version of Taple is 4.9 It is a naturally occurring endogenous byproduct of the cellular lipid peroxidation process. Lipid peroxidation happens when the antioxidant defense systems' capacity to scavenge free radicals or eliminate their products is outstripped by the creation of free radicals themselves. When searching for high oxidative stress in tissues, malondiadehyde is one of the most useful indications since it is formed by the oxidation of unsaturated fats, particularly those with two double or three triple bonds. Lipid peroxidation results in the production of an impregnated membrane that allows fluids and materials from and to it to pass through without restriction, i.e., the membrane loses its capacity to selectively let certain substances to pass through (Peerapatdit and Sriratanasathavorn 2010). There are two types of malone aldehyde that may be discovered in a person's diet: the free form and the protein-bound form. When fatty acids are oxidized, lipid hydroperoxide is generated, which subsequently undergoes fragmentation to produce (MDA) molecules. Glutathione peroxidase (GPX glutathione peroxidase) has been shown to have a negative correlation with the quantity of MDA in blood due to oxidative stress or the presence of certain disorders.

Because hemodialysis accelerates the creation of free radicals that may pass from dialysis fluid to blood components, the MDA content in the blood serum of patients with renal failure rises, which is a sign of oxidation and infection of certain illnesses. It has an OH root. Breaks down cell membranes by interfacing directly with unsaturated fatty acids, causing fat peroxidation. This results in the production of a number of compounds, each having therapeutic value (Niedernhofer *et al.* 2003). DNA and proteins have been shown to interact with MDA under conditions of oxidative stress, and this interaction has been shown to play a significant role in the incidence of genetic abnormalities and the subsequent development of malignant tumors (Miyata *et al.* 2001).

4.3.10 Correlation

Tape 4.10 The other metrics evaluated in this research show a weak and non-significant "positive and negative" correlation. According to the study's findings, there is a substantial positive association between Arginase - Total protein, Arginase - Albumin MDA, and Arginase - Thiol/Di-Sulfide homeostasis, which may be useful in the assessment of diabetes complications. (Arginine-Alcohol and Arginase-Globular) are shown in the table 4.10 to have strong negative correlations with each other. There is a significant positive correlation between (TP – globulin) and (Albumin/globulin ratio – Albumin) as well as a significant negative correlation between these two variables (Albumin/globulin ratio –Albumin). However, these findings do not agree with those of Ismail K. Abd (Ferrand *et al.* 2021).

5. CONCLUSIONS AND RECOMMENDATION

1. Comparing the efficiency of arginase enzyme in hepatitis patients to diabetes

2. Purification of arginase enzyme and investigation of its kinetic and thermodynamic characteristics in diabetic blood serum

3. Conducting a biochemical investigation for additional factors such as CRP and discovering the enzyme arginase in diabetes patients' blood serum.

4. Effect of heavy chemical components and elements on arginase enzyme activity in diabetes patients' blood serum

REFERENCES

Alt, N., Carson, J. A., Alderson, N. L., Wang, Y., Nagai, R., Henle, T., Thorpe, S.R., and Baynes, J. W. 2004. Chemical modification of muscle protein in diabetes. Archives of Biochemistry and Biophysics., 425: 200–206.

Association, A. D. 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care., 37: S81–S90.

Association, A. D., 2008. Diagnosis and classification of diabetes mellitus. Diabetes Care., 31: S55–S60.

Bluestone, J. A., Herold, K., and Eisenbarth, G. 2010. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature., 464: 1293–1300.

Borgnakke, W. S. 2019. IDF Diabetes Atlas: Diabetes and oral health–A two-way relationship of clinical importance. Diabetes Research and Clinical Practice., 157: 107839.

Buege, J. A., and Aust, S.D. 1978. Microsomal lipid peroxidation. In Methods in enzymology., 52: 302–310.

Care, D., 2004. Position statements and ADA statements. Diabetes Care., 27: S106–S109.

Chantrapanichkul, P., Indhavivadhana, S., Wongwananuruk, T., Techatraisak, K., and Dangrat, C. 2020. Prevalence of type 2 diabetes mellitus compared between lean and overweight/obese patients with polycystic ovarian syndrome: a 5-year follow-up study. Archives of Gynecology and Obstetrics., 301: 809–816.

Colton, C. A., Mott, R. T., Sharpe, H., Xu, Q., Nostrand, W. E., and Vitek, M. P. 2006. Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD. Journal of Neuroinflammation., 3: 1–12.

Cowell, K. M., 2008. Type 2 diabetes mellitus. Pediatrics in Review., 29: 289.

Dizikes, G. J., Grody, W. W., Kern, R. M., and Cederbaum, S. D. 1986. Isolation of human liver arginase cDNA and demonstration of nonhomology between the two human arginase genes. Biochemical and Biophysical Research Communications., 141: 53–59.

Dzik, J. M. 2014. Evolutionary roots of arginase expression and regulation. Frontiers in Immunology., 5: 544.

Ellman, G. L. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics., 82: 70–77.

Erel, O. 2004a. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry., 37: 277–285.

Erel, O. 2004b. A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical Biochemistry., 37: 112–119.

Ferrand, J., Plessier, A., and Polo, S. E. 2021. Control of the chromatin response to DNA damage: Histone proteins pull the strings. In Seminars in cell and developmental biology., 113: 75–87.

Gong, Q., Zhang, P., Wang, J., Ma, J., An, Y., Chen, Y., Zhang, B., Feng, X., Li, H., and Chen, X. 2019. Morbidity and mortality after lifestyle intervention for people with impaired glucose tolerance: 30-year results of the Da Qing Diabetes Prevention Outcome Study. The Lancet Diabetes and Endocrinology., 7: 452–461.

Gumusyayla, S., Vural, G., Bektas, H., Deniz, O., Neselioglu, S., and Erel, O. 2016. A novel oxidative stress marker in patients with Alzheimer's disease: dynamic thiol–disulphide homeostasis. Acta Neuropsychiatrica., 28: 315–320.

Huang, J., Rajapakse, A., Xiong, Y., Montani, J. P., Verrey, F., Ming, X. F., and Yang, Z. 2016. Genetic targeting of arginase-II in mouse prevents renal oxidative stress and inflammation in diet-induced obesity. Frontiers in Physiology., 7: 560.

Jackson, W. P. U. 1978. Epidemiology of diabetes in South Africa. In Advances in metabolic disorders., 9: 111–146.

Kashyap, S. R., Lara, A., Zhang, R., Park, Y. M., and Fronzo, R. A. 2008. Insulin reduces plasma arginase activity in type 2 diabetic patients. Diabetes Care., 31: 134–139.

Kawata, A., Taguchi, A., Baba, S., Miyamoto, Y., Tanikawa, M., Sone, K., Tsuruga, T., Mori, M., Oda, K., and Kawana, K. 2021. A low preoperative albumin-to-globulin ratio is a negative prognostic factor in patients with surgically treated cervical cancer. International Journal of Clinical Oncology., 26: 980–985.

Khaleel, F. M., Noor, N. O., and Abed, B.A. 2018. Disturbance of Arginase Activity and Nitric Oxide Levels in Iraqi Type 2 Diabetes Mellitus. Baghdad Science Journal., 15: 2.

Köseoglu, E., and Karaman, Y. 2007. Relations between homocysteine, folate and vitamin B12 in vascular dementia and in Alzheimer disease. Clinical Biochemistry., 40: 859–863.

Lee, H. J., Li, C. W., Hammerstad, S. S., Stefan, M., and Tomer, Y. 2015. Immunogenetics of autoimmune thyroid diseases: a comprehensive review. Journal of Autoimmunity., 64: 82–90.

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S., and Stadtman, E. R. 1990. Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymology., 186: 464–478.

Libert, D. M., Nowacki, A. S., and Natowicz, M. R. 2018. Metabolomic analysis of obesity, metabolic syndrome, and type 2 diabetes: amino acid and acylcarnitine levels change along a spectrum of metabolic wellness. PeerJ., 6: e5410.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry., 193: 265–275.

Mateen, S., Moin, S., Khan, A. Q., Zafar, A., and Fatima, N. 2016. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PloS One., 11: e0152925.

Mihalik, S. J., Michaliszyn, S. F., Las Heras, J., Bacha, F., Lee, S., Chace, D. H., DeJesus, V. R., Vockley, J., and Arslanian, S. A. 2012. Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: evidence for enhanced mitochondrial oxidation. Diabetes Care., 35: 605–611.

Miyata, T., Saito, A., Kurokawa, K., and Strihou, C. V. Y. 2001. Advanced glycation and lipoxidation end products: reactive carbonyl compounds-related uraemic toxicity. Nephrology Dialysis Transplantation., 16:8–11.

MJ, F. 2008. Microvasculares e macrovasculares do diabetes. Clin Diab., 26: 77–82.

Müller, K., and Brunnberg, L. 2010. Determination of plasma albumin concentration in healthy and diseased turtles: a comparison of protein electrophoresis and the bromcresol green dye-binding method. Veterinary Clinical Pathology., 39: 79–82.

Muniyappa, R., and Quon, M. J. 2007. Insulin action and insulin resistance in vascular endothelium. Current Opinion in Clinical Nutrition and Metabolic Care., 10: 523–530.

Niedernhofer, L. J., Daniels, J. S., Rouzer, C. A., Greene, R. E., and Marnett, L. J. 2003. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. Journal of Biological Chemistry., 278: 31426–31433.

Odetti, P., Garibaldi, S., Noberasco, G., Aragno, I., Valentini, S., Traverso, N., and Marinari, U.M. 1999. Levels of carbonyl groups in plasma proteins of type 2 diabetes mellitus subjects. Acta Diabetologica., 36: 179–183.

Peerapatdit, T., and Sriratanasathavorn, C. 2010. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients. J. Med. Assoc. Thai., 93: 682–693.

Pieniazek, A., Gwozdzinski, L., Zbrog, Z., and Gwozdzinski, K. 2018. Alterations in conformational state of albumin in plasma in chronic hemodialyzed patients. Plos One., 13: e0192268.

Rother, K. I. 2007. Diabetes treatment—bridging the divide. The New England Journal of Medicine., 356: 1499.

Santos, A. F. S., Argolo, A. C. C., Paiva, P. M. G., and Coelho, L. C. B. B. 2012. Antioxidant activity of Moringa oleifera tissue extracts. Phytotherapy Research., 26: 1366–1370.

Sbarouni, E., Georgiadou, P., Kremastinos, D. T., and Voudris, V. 2008. Ischemia modified albumin: is this marker of ischemia ready for prime time use. Hellenic J Cardiol., 49: 260–266.

Schipper, S. B. J., Veen, M. M., Elders, P. J. M., Straten, A., Der Werf, Y. D., Knutson, K. L., and Rutters, F. 2021. Sleep disorders in people with type 2 diabetes and associated health outcomes: a review of the literature. Diabetologia., 64: 2367–2377.

Shosha, E., Fouda, A.Y., Narayanan, S. P., Caldwell, R. W., and Caldwell, R. B. 2020. Is the Arginase Pathway a Novel Therapeutic Avenue for Diabetic Retinopathy? Journal of Clinical Medicine., 9: 425.

Thorpe, S. R., and Baynes, J. W. 2003. Maillard reaction products in tissue proteins: new products and new perspectives. Amino Acids., 25: 275–281.

Trombetta, D., Gangemi, S., Saija, A., Minciullo, P. L., Cimino, F., Cristani, M., Briuglia, S., Piraino, B., Isola, S., and Salpietro, C. D. 2006. Increased protein

carbonyl groups in the serum of patients affected by thalassemia major. Annals of Hematology., 85: 520–522.

Turner, A. P. F., Chen, B., and Piletsky, S. A. 1999. In vitro diagnostics in diabetes: meeting the challenge. Clinical Chemistry., 45: 1596–1601.

Unlüer, E. E., Kiliç, T. Y., Akgöl, E., İşgüven, D., Vardar, E., Bayol, U., Yilmaz, O., Erkan, N., and Gökmen, N. 2010. The role of cobalt-albumin binding analysis in the diagnosis of experimental abdominal compartment syndrome in rabbits. Ulus Travma Acil Cerrahi Derg., 16: 491–496.

Verhulst, M. J. L., Loos, B. G., Gerdes, V. E. A., and Teeuw, W. J. 2019. Evaluating all potential oral complications of diabetes mellitus. Frontiers in Endocrinology., 10: 56.

Vockley, J. G., Jenkinson, C. P., Shukla, H., Kern, R. M., Grody, W. W., and Cederbaum, S. D. 1996. Cloning and characterization of the human type II arginase gene. Genomics., 38: 118–123.

Wackett, L., Sadowsky, M., Martinez, B., and Shapir, N. 2002. Biodegradation of atrazine and related s-triazine compounds: from enzymes to field studies. Applied Microbiology and Biotechnology., 58: 39–45.

Wang, F., Long, S., Zhang, J., Yu, J., Xiong, Y., Zhou, W., Qiu, J., and Jiang, H. 2020. Antioxidant activities and anti-proliferative effects of Moringa oleifera L. extracts with head and neck cancer. Food Bioscience., 37: 100691.

Wang, Y., Chen, L., Horswell, R., Xiao, K., Besse, J., Johnson, J., Ryan, D. H., and Hu, G. 2012. Racial differences in the association between gestational diabetes mellitus and risk of type 2 diabetes. Journal of Women's Health., 21: 628–633.

Wills, E. D. 1969. Lipid peroxide formation in microsomes. General considerations. Biochemical Journal., 113: 315–324.

Wu, M., and Tinoco, I. 1998. RNA folding causes secondary structure rearrangement. Proceedings of the National Academy of Sciences., 95: 11555–11560.

Zaia, D. A. M., Barreto, W. J., Santos, N. J., and Endo, A. S. 1993. Spectrophotometric method for the simultaneous determination of proteins and amino acids with p-benzoquinone. Analytica Chimica Acta., 277: 89–95.

Zeng, J., and Davies, M. J. 2005. Evidence for the formation of adducts and S-(carboxymethyl) cysteine on reaction of α-dicarbonyl compounds with thiol

groups on amino acids, peptides, and proteins. Chemical Research in Toxicology., 18: 1232–1241.

Zhito, A. V, Iusupova, A.O., Kozhevnikova, M. V, Shchendrygina, A. A., Privalova, E. V, and Belenkov, Y. N. 2020. E-Selectin as a Marker of Endothelial Dysfunction in Patients with Coronary Artery Disease Including Those with Type 2 Diabetes Mellitus. Kardiologiia., 60: 24–30.