Purification and Determination of The Arginase Enzyme Activity from Kidney Patients

Purification and Determination of The Arginase Enzyme Activity from Kidney Patients

Nuha Ali Hadi al-Samarrai1 [1*] and Nadia Ahmed Salih[2]

[1]Chemistry Department, College of Education, Samarra University.
[2]Chemistry Department, College of Education for Pure Sciences, Tikrit University.

* E-mail: Nuhaali922@gmail.com

Abstract. The current study measuring the effectiveness and purification of the enzyme (arginase) in the serum of kidney diseases samples were collected from Samarra General Hospital from October to December 2019, 60 samples were collected from the infected and 25 samples from he healthy people control group of ages 30-55 years, the effectiveness of the control group was 6.87 ± 2.95, and the patient group was 25.71 ± 8.39., the purification process was carried out using Sephadex G-200 and EDEA cellulose - using a column of separation with dimensions (32 x 1.5nm), the molecular weight of the enzyme was 245 kDa. The study included measuring the level of urea 83.7 ± 23.4mg/dl, Creatinine 2.85 ± 1.19 mg/dl, Total protein 3.8 ± 0.6 mg/dl, Albumin 4.120 ± 0.02 mg/dl, Uric acid 5.19 ± 1.52 mg/dl and Globulin was 1.29 ± 0.01 mg/dl. at P≤0.01.

Keywords: Arginase enzyme, purification, activity,Kidney.

1.Introduction

Arginase (EC 3.5.3.1, Arginine amidinase, canavanase, L-arginase, arginine transamidinase) is an enzyme containing manganese. This enzyme catalyzes the following reaction: arginine H_2O → ornithine+ urea. This enzyme is considered the last enzyme in the urea cycle. It is widely present in all areas of life. It is ubiquitous to all domains of life.Arginase stimulates the fifth step and is the final step in the urea cycle, which are chain biochemical reactions that occur in mammals that aim to rid the body of harmful ammonia. Specifically, arginine converts L-arginine to L-ornithine and urea [1,2] Mammalian arginase is active as a trimer, except that some bacterial arginases form hexmeric arginases. [3] In order to maintain proper function, the enzyme needs a two-molecule metal cluster of manganese. These ions coordinate Mn^{2+} with water, directing the molecule and fixing it. They also allow water to act as a nucleophilic antipyretic and attack L-arginine, breaking it down into ornithine and urea [4] In most mammals, there are two types of this type of enzyme; the first is Arginase I, which functions in the urea cycle, and its location is in the cytoplasm of the liver cells. The second isozyme, Arginase II, is involved in the regulation of arginine / ornithine levels within cells. And its place in the mitochondria in many different tissues of the body, and it is very abundant in both the prostate and the kidneys. They may also be found, at lower levels, in macrophages, lactating mammary glands, and the brain. [5] We can find a second isozyme even in the absence of other urea cycle enzymes [4]

Arginases catalyze the divalent cation-dependent hydrolysis of L-arginine as it synthesizes the non-protein amino acids L-ornithine and urea. This reaction is the last step in the biogenesis of urea in the liver. flux during this reaction is very large, that an adult person excretes about

10 kg of urea/year. The urea cycle arginase (arginase I or liver arginase) is considered cytosolic, and is the best characterized of the mammalian arginases. A second isozyme, arginase II or kidney arginase, it is location is mitochondria. The type I and type II arginases in human are related by 58% sequence identity [(6)], and they are immunologically distinct. Comparative properties of 2 arginase enzymes have been discussed in a number of new reviews [2-4]. With it, the activity of arginase has been detected in many non-hepatic tissues that lack the complete urea cycle; This reaction is thought to be a source of ornithine, the biosynthetic precursors of proline and polyamines. For example, in the lactating mammary glands, the activity of arginase is increased to about 25% found in the liver in order to provide proline needed for milk protein (prolactin) synthesis [5]. Ornithine is also a biosynthetic precursor of polyamine [6], the muscular activity of arginine is increased by 25-fold during pregnancy to provide the fast-growing fetus with polyamines to enable cell proliferation [7]. It has become evident that the rapidly dividing tissue requirement to enhance polyamine biosynthesis is achieved through increased arginase activity as found in gastric cancers [8-10] and in breast cancer [9]. Recently, arginase activity has been detected in some human colon cancer and human breast cancer cell lines [10].

2.Material and Methods

25 blood samples were collected for normal cases of both sexes as control, their ages ranged between 30-55 years Samples were collected from Samarra General Hospital for the period from October to December after being diagnosed by specialized doctors on the basis of urea and creatinine examination, as 60 blood samples were collected for both sexes, whose ages ranged between 30-55 years.

• The blood was drawn the vein using a single-use 5 ml plastic syringe and the blood was placed in clean plastic tubes with a size of the blood serum separated from the coagulated part, and then the serum was withdrawn by a micropipette, after which the enzyme activity was measured directly and the sample was kept in freezing for the purpose of Make other required measurements

a- Measuring the activity of the arginase enzyme for kidney disease samples and healthy samples, using the ready-made kit for the arginase enzyme ELISA, as mentioned[11].

b- Purification of the arginase enzyme in samples with kidney disease: it was done according to the stages[12](saturation ammonium sulphate , Dialysis, Gel filtration, Ion exchange)

Enzyme unit: It is the amount of enzyme that catalyzes a reaction of 1 µl of the substrate in per minute

2.1.Saturation of ammonium sulphate

The serum proteins were precipitated using graduated concentrations of ammonium sulphate until reaching a saturation rate of 60%, as 3 gm of ammonium sulfate was added to 5 ml of serum during a period of (60 - 45) minutes by placing the serum in an ice bath with continuous stirring, usually thawing after freezing. Precipitate with 4ml of 0.1M Tris buffer - HCl pH 7.2.

2.2.Dialysis

It is one of the most important and oldest methods used in purifying enzymes, and the aim of which is to remove the remainder of the added ammonium sulfate to precipitate the proteins by placing the dissolved protein in the above step in the dialysis bag after measuring the activity (arginase enzyme) and protein concentration, and dipping the bag in 0.1M buffer solution Tris - HCl pH 7.2 The buffer solution was changed from time to time for an entire night. This step was performed at 4 ° C to maintain the enzyme effectiveness, and after the end of the membrane separation process, the effectiveness (arginase enzyme) and protein concentration were measured.

2.3.Gel filtration

The basis for the work of this technique is the difference in the molecular weight, as it was used to purify the symmetric separated by gel filtration chromatography using a Sephadex G200 gel filtration column, as 20 fraction collected the volume of each fraction was 4 ml where the effectiveness of (arginase enzyme) was measured and the total protein level of all the separated parts was measured.

2.4.Ion exchange

A separation column with dimensions (32 x 1.5) cm was used that contains DEAE cellulose-25 gel, which separates the compounds depending on the charge, and the gel material was placed at a height of 20 cm by pouring the gel on the walls of the separation column quietly and diagonally to prevent the formation of bubbles, after placing a suitable piece of glass wool at the end of the column, The blood serum was injected into the separator column in a circular motion and on the walls of the separator column, followed by ashake prosses using the buffer solution with a pH of 7.2, and the collection process was carried out at a flow rate of 0.63 ml/min, i.e. at a rate of 1.09 ml/ minute for each part, by a manual method, as was followed up. Calculation of the protein concentration by measuring the absorbance of the separated parts, where the activity of arginase enzyme was measured and the total protein level was measured for all the separated parts at 450nm wavelength.

-Estimation of the enzyme molecular weight: the molecular weight of the enzyme was estimated by filtering at [13,14].

2.5. Biochemical tests

The level of urea, creatinine, albumin, total protein and uric acid was measured using a ready-made assay (Kit) supplied by Biolab Corporation. The globulin was calculated from the following equation Globulin concentration = total protein - albumin

3.Results and discussion

1- The Effectiveness of (arginase enzyme) was measured in the serum of patients have kidney diseases compared with healthy patients, according to the following table kidney and healthy patients, according to the following table

Table 1. shows the Effectiveness of the arginase enzyme in the blood serum of kidney diseases and healthy people at the probability level P≤0.01

Arginase Enzyme activity (unit)	Total and standard deviation	P-Value
Control group	6.87±2.95	≤0.01
Patient group	25.71±8.39	

It is evident from the table above that the activity of arginase enzyme in the serum of kidney patients increased compared to healthy people at the probability level P≤0.01, and this is consistent with what was mentioned (Alshamaa and. Al-Obaidi) [15], where a significant increase in the activity of the arginase enzyme was observed. In all the studied age stages, as well as the current study consistent with what was mentioned (Scalia) [16]

Those who have hypothesized that this elevation is caused by histopathology, tumors, and pathological changes such as apoptosis, kidney infection and oxidative fatigue (disruption of the balance between the production of reactive oxygen types (free radicals) and antioxidant defenses, are discussed in relation to their potential role in the production of tissue damage) that increases The nitrogen oxide ranges that block the transit of urea and creatinine throughout the liver.

The high activity of arginase associated with acute kidney disease, therefore, alters the epithelial cell function in heart disease and kidney disease. (Heisel et al.) [17] Arginase also reduces the excretion of sodium from the kidneys when consuming small amounts of salt, but stimulates sodium excretion in the case of high salt amounts (Lakowicz). [18]

Table 2. shows the stages of purification (arginase enzyme) in serum of kidney patients

Steps of purifications	Volume (ml)	Activity	Total Activity	Protein Conc (mg/ml)	Specific activity (mg)	Yield %	Folds	Total protein (mg)
Crude serum	5	10.1	50.05	7.3	1.32	100	1	36.5
Ammonium sulphate	3.5	10.19	35.36	6.2	1.64	74.2	1.2	21.7
Dialysis	4	10.3	41.2	4.1	2.5	84.9	1.8	16.4
Gel filtration	4	10.7	42.8	2.5	4.28	88	3.2	10

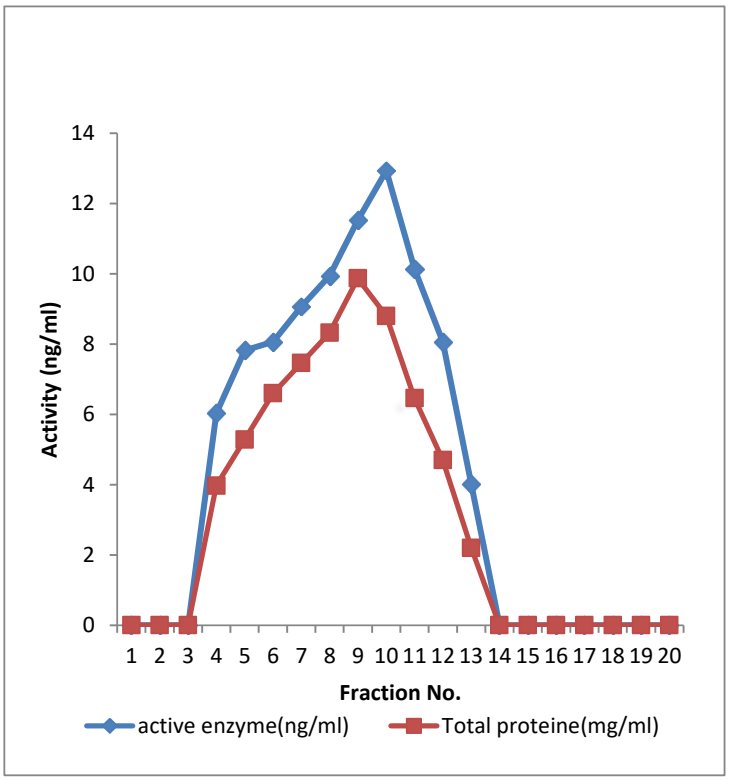

Figure 1 shows the purification of the enzyme arginase using Sephadex G-200

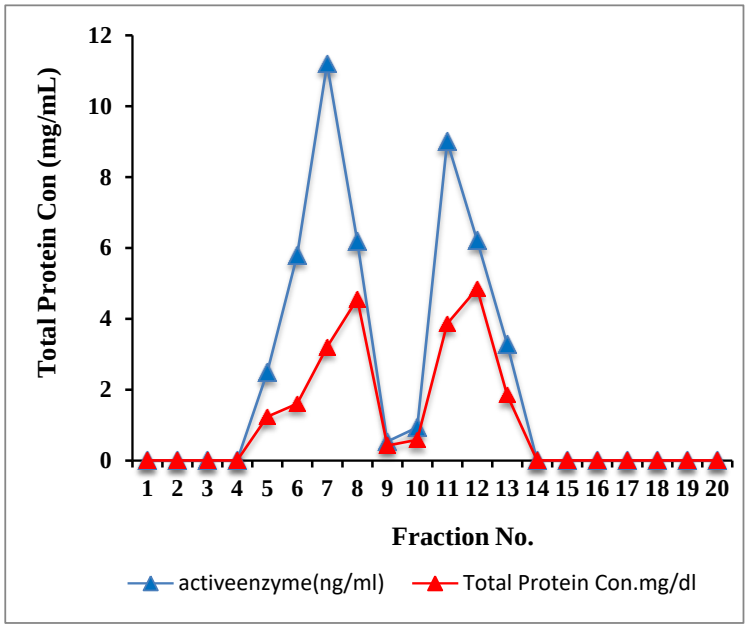

Figure 2 also shows ion exchange chromatography using EDEA-cellulose for the separated fractions versus the total protein.

The electrolysis revealed that the molecular weight of the enzyme is high and reaches 245 kDa, according to the figure below

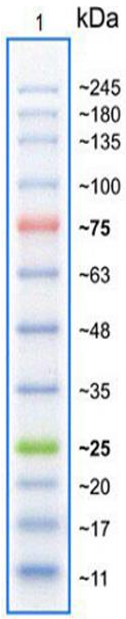

Figure 3 Electrolysis analysis showing the molecular weight of the enzyme, up to 245 kDa, using the acrylamide gel.

'Figure (3) ' shows the molecular weight of the arginase enzyme separated by electrophoresis [14,15],the electrophoresis analysis revealed the presence of two arginase peaks, according to the figure below

Figure (4) Highest effective electrophoresis of separated fractions by chromatography using Sephadex G-200 to isolate the arginase enzyme.

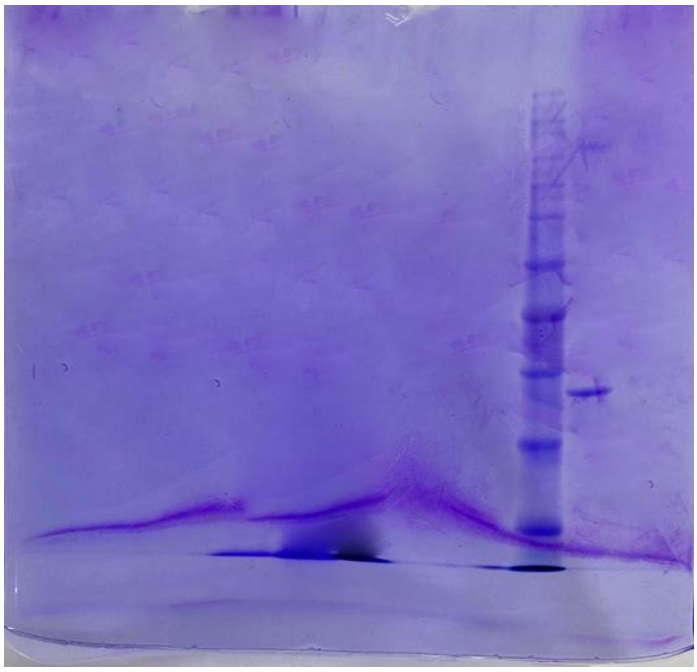

Figure 4 Electrophoresis to isolate the enzyme arginase in serum of kidney patients [13]
The literature indicates that the arginase enzyme contains two isotopes, one in red blood cells of the liver (ARGI) at a high level and the other (ARGII) at a lower level in mitochondria in many tissues [5].

Table 3. shows the level of urea in the serum of patients compared with healthy subjects measured in units (mgldL) at the probability level P≤0.01

Urea level in units (mgldL)	Mean ± standard deviation	P-Value
Control group	27.92±3.2	≤0.01
Patient group	83.7±23.4	

The above table shows a significant increase in the level of urea in the blood serum of patients with kidney disease compared to healthy people, This is in agreement with (**Hasan**) [19] Where he found a significant increase in the level of urea in patients with renal impairment, as well as the current results are consistent with the findings of (**Al-gharaghouly**) [20] that refers to the significant increase in the level of urea in patients with renal impairment. The current result is consistent with what was mentioned by Loughridge L., [21]. The reason for this increase is the weakening of kidney function with age, as this is due to the reduction of glomerular filtrate after the age of thirty.

Table 4. shows the level of creatinine in the blood serum of people with kidney disease (mgldL) compared to healthy people with the p ≤ 0.01 level.

Creatinine level In units (mg dL)	Mean ± standard deviation	P-Value
Control group	0.83±0.7	≤0.01
Patient group	2.85±1.19	

The table above shows a significant increase in the level of creatinine in the serum of kidney diseases compared to healthy people, and these results were consistent with the result of (Hababi) [22], (Philip et al.)[23]. The reasons for the increase in the level of creatinine in the blood indicate the fact that creatinine is waste of nitrogen metabolism processes, which are excreted with urine in the normal state, but in the case of a kidney disorder that may lead to a

gradual loss of kidney function and a failure in filtration, which leads to an increase in the concentration of creatinine in the body. The patients who suffer from chronic renal failure and diabetic nephropathy suffer from a decrease in the value of glomerular filtration(Hasan) [19]

Table 5. shows the level of total protein (mg/dL) in the serum of kidney patients compared with healthy people with a probability level P≤0.05

Total protein level In units (mg/ dL)	Mean ± standard deviation	P-Value
Control group	6.53±0.2	≤0.05
Patient group	3.8±0.6	

Table 5 the level of total protein in the serum of kidney patients compared with healthy people, where it was found that there are significant differences at the level of probability P≤0.05 and this is consistent with (**Al-gharaghouly**) [20], where it was mentioned that there is a decrease in the level of total protein at all ages. In patients with renal insufficiency, and also agrees with(Muthu), [24] that the reason for this decrease is inflammation in the renal glomeruli, which leads to its appearance in the urine.

Table 6. shows the level of albumin in kidney disease serum compared to healthy people with the probability level P≤0.01

The level of albumin unit (mgldL)	Mean ± standard deviation	P-Value
Control group	4.120±0.02	≤0.01
Patient group	2.51±0.39	

Table 6 The level of albumin in kidney disease serum compared to healthy people, where a significant difference was found at the probability level P≤0.01. The result of the current study is consistent with what was found by**(Hasan)** [(19),] where a significant decrease was observed compared with The result is also consistent with the findings of healthy people (Junlin et al) [25], The reasons for the decrease refer to kidney damage resulting from chronic renal failure and diabetic nephropathy, which leads to loss of albumin through damaged filter units and glomeruli, the amount of albumin in the urine increases and there is a decrease in the concentration of albumin in the blood, which ultimately leads to a state of hypoalbuminemia. Roche, M.;] [26], or that the possible cause is due to poor nutrition and the increased need for albumin in the body [Maher Borai @eta] [27].

Table 7. shows the level of globulin in serum of kidney disease compared to healthy people with the probability level P≤0.01.

Globulin level (mgldL)	Mean ± standard deviation	P-Value
Control group	2.41±0.06	≤0.01
Patient group	1.29±0.01	

Table 8. shows the level of uric acid in the blood serum of kidney patients compared with healthy people with no significant differences.NS = 0

Uric acid level in units (mg dL)	Mean ± standard deviation	P-Value
Control group	4.7±0.28	NS
Patient group	5.19±0.28	

It can be seen from Table 8 uric acid is one of the non-protein organic compounds in the blood and represents in humans the final product of the metabolism of purines, a substance that is difficult to dissolve in water. The majority of it must be excreted through urine, as 20% of it is filtered through the kidneys (Salih) [28]. The formation of uric acid depends on the extent to which the food contains nucleoproteins, as well as on the extent to which the food

contains free purine bases, as is the case in tea and coffee, as well as on the process of destruction that occurs in the cells of the body, Its level in the blood increases due to a decrease in the percentage of renal filtration of uric acid as a result of renal failure as well as in the case of lytic anemia and pernicious anemia as well as gout (Fiedman@eta) [29]

References

[1] Di CCostanzo L, Pique M E and Christianson D W 2007 *J. Am. Chem. Soc.* **129** 6388 .
[2] Wu G and Morris S M 1998 *Biochem. J*. **336** 1.
[3] Dowling D P, Di Costanzo L, Gennadios H A and Christianson DW 2008. *Cell. Mol. Life Sci*. **65** 2039.
[4] Di Costanzo L, Moulin M, Haertlein M, Meilleur F and Christianson DW 2007 *Arch. Biochem. Biophys*. **465** 82.
[5] Morris S M 2002 *Annu. Rev. Nutr*. **22** 87.
[6] Reczkowski R S and Ash D E . *Arch. Biochem. Biophys*. 1994. **312** 31.
[7] Cox J D, Kim N N, Traish A M ,etal 1999 *Nat. Struct. Biol*. **6** 1043.
[8] Cama E , Colleluori D M , Emig FA, etal . 2003 *Biochemistry*. **42** 8445.
[9] Moody J A, Vernet D, Laidlaw S, etal 1997 *J. Urol.* **158** 942
[10] Iyer R K, Yoo P K and Kern R M, . 2002 *Mol. Cell .Biol*. **22** 4491.
[11] Coulomble J J and Favreau L 1963 *Clin. Chem*. 9.
[12] Pierre C , Claude B 1978 *Biochem. J.* **175** 449.
[13] Chang G G , Wang J K , Huang T M , etal, 1991 *Eur. J Biochem.* **202** 681.
[14] Hames B D and Rickwood D 1990 *Gel Electrophoresis of Proteins: A Practical Approach* . 2nd ed(Oxford University Press, Oxford) p 383.
[15] Chang G G, Wang J K and Huang T M 1991 *Eur J Biochem.* **202** 681.
[16] Alshamaa S D, Al-Obaidi S H . 2018 *Raf. J. Sci*. **27** 1.
[17] Scaglia F , Lee B 2016 . *Am J. Med Genet C Semin Med Genet*.**142** 113.
[18] Hezel M P , Liu M and Schiffer T A .2015 *J. Redox Biol*. **5** 234.
[19] Lakowicz J R *Principles of Fluorescence Spectroscopy* (Springer Publisher. New York), 2006,chapter 10.
[20] Hasan S M. , Maher F T and Kadhim N Q 2018 *Tikrit Journal of Pure Science*. **23** 10.
[21] Al-gharaghouly J M 2015. *Biochemical study of partially purified glutathione peroxidase from sera of patients with renal failure* . Master Thesis.
[22] Loughridge L and Lewis M G 2008 *Nephrotic syndrome in malignant disease , Lancet,* Willimam J Marshall. clinical chemistry 6thed p 70-82.
[23] Al-Ahbabi M Y K . *Study of Chronic Kidney Disability Patients in Salahuddin Province Biochemical and Molecular* (Master Thesis, College of Education for Pure Sciences, Tikrit University). 2018.
[24] Philip M F, David C and Richard E G 2018 *Can. J .Diabetes* .**42** S201.
[25] Muthu K M 2003 *Kidney Int Suppl*. **83** S86.
[26] Zhang J , Zhang R and Wang Y 2019 *J. Diabetes Res*.**9** 1.
[27] Roche M , Rondeau P , singh M , ,etal. 2008. **582** -1783.
[28] Maher B M B, Manar M A, Hala A A 2016 *Int. J. Curr. Adv. Res*. **4** 1337.
[29] Salih N A 2010 *Determination of Urea, Creatinine , Uric acid and Alkaline phosphatase activity , Phosphorous, Calcium and total protein in the blood of Acute Renal Failure Patients in Tikrit city*. (College of pharmacy, Tikrit University, Tikrit , Iraq).