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Study of partially purification AST activity in sera of Iraqi patients with diabetic nephropathy

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ABSTRACT

Diabetic nephropathy is one of the major causes of chronic renal failure.After many years of diabetes the delicate filtering system in the kidney becomes gradually destroyed.Initially becoming leaky to larger blood proteins such as albumin which are then lost in urine. Both serum urea and creatinine are widely used to assess the function of kidney.The aim of the study is to determinethe effect of diabetic nephropathy on serum AST level, and purification of enzyme in Diabetic Nephropathy and control.Laboratory investigations including fasting blood glucose, serum AST, ALT, Total protein, serum urea, uric acid, and creatinine had been measured in patients with Diabetic Nephropathy and control. In addition purification of AST from serum patients with Diabetic Nephropathy and control by using ammonium sulphate (64-72 g)/ 100 ml and gel filtration (Sephacryl S-300). Blood samples were obtained from the patients age [mean±SD: (49.00± 5.20)] and the control [mean±SD :(39.20±5.38)]age matched normal healthy individuals who came to the hospital for health checkup.fasting blood glucose, AST, Serum urea, and creatinine, significant difference between the patients and control group. AST with he highest specific activity afterPurificationchromatography on Sephacryl S-300.

KEYWORDS

Aspartate aminotransferase (AST);
Diabetic nephropathy.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that can lead to severe cardiovascular, renal, neurologic and retinal complications[1]. The classical definition of diabetic nephropathy is a progressive rise in urine albumin excretion, coupled with increasing blood pressure, leading to declining glomerular filtration and eventually end stage kidney failure. Patients generally have diabetic nephropathy Recently, greater appreciation of the close links between nephropathy and cardiovascular disease have led to the inclusion of premature cardiovascular disease, cardiovascular risk increasing in parallel with albuminuria. Diabetic nephropathy is now the single commonest cause of end-stage kidney failure worldwide and is acknowledged as an independent risk factor for cardiovascular disease[2]. A number of risk factors have been associated with the metabolic syndrome, including hypertension, poor glycemic control, central obesity, smoking, dyslipidemia and glycated end product[3].

Aspartate aminotransferase (L-aspartate-2-oxoglutarate aminotransferase,(EC2.6.1.1) comprises anionic and cationic isoenzymes, (AST) also known as

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glutamic oxalo acetic transaminase (GOT)[4]. The biochemical function of amino transferase is to transfer an amino group from an alfa - aminoacid to an Alfa- keto acid with pyridoxal phosphate (vitamin B_6) asa cofactor[5]. AST is present in high concentrations in cytoplasm of the liver kidney and myocardial and skeletal muscle and occur in other organs at lower activities[6,7]. Low levels of (AST) are normally found in the blood, when body tissue or an organ such as the heart or liver is diseased or damaged, additional (AST) as relealsed into the bloods stream[8]. The enzyme has been is isolated from avariety of sources including mammalianorgans, plants, a yeast, agreenalgo and bacteria[9].

Serum Aspartate aminotransferase (AST) activity which is increased in diabetes[10]. increasing incidence of DM has made it a health problem. Diabetic patients are at a increased risk of developing specific complications including: nephropathy, retinopathy,neuropathy and atherosclerosis[11,12].

The aim of the study is to determine the effect of diabetic nephropathy on serum AST level, and Partially purification of enzyme in Diabetic Nephropathy.

MATERIALSAND METHODS

Five ml have been collected from each subject by vein puncture, centrifuged at 3000 rpm for 5 min after allowing the blood to clot at room temperature.

Fourty serum sample obtained from type II diabeticnephropathy (twenty) males age (40-60) years and (twenty) females age (40-75) years. The medical history has been taken, body weight and height have been measured. The patient has been diagnosed by specialist doctors in AL-Yarmokhospital (National Diabetes Center).

For comparison, Fourty apparently healthy [(twenty) males age (40-60) years] and[(twenty) females age (40-60) years].

All patients were subjected to a detailed history taking, thorough clinical examination, and laboratory investigations including Fasting blood glucose (FBG),urea and Creatinine were measured by spectrophotometeric methods supplied by BiomaghrebDiagnostic. The activity of AST and ALTactivity were assayed at pH 7.5 and 25°C by method of [13].

AST activity was determined by measurement of oxaloacetate produced duringthe assay according to the method of[13]. Thesamples were incubated at 37°C for 1 h in tubes containing 100 μM asparticacid, 2μM α-ketoglutaric acid, 20mM sodium phosphate buffer, pH 7.5 andappropriate amount of enzyme to give a final volume of 1.0ml. The reactionwas stopped by the addition of 1ml 2,4-dinitrophenylhydrazine reagentand allowed to incubate 20min at room temperature and 10ml 0.4N sodiumhydroxide was added. The absorbance was recorded at 505 nm. Theenzymatically liberated oxaloacetate was calculated from a standard curve. One unit of enzyme activity was definedas the amount of enzyme producing 1 μmol oxaloacetate (pyruvate) per hourunder the standard assay conditions.

Purification of AST

Preparation of crude extract

1-Crude extract was prepared by mixedforty sample and carried out (10 ml) and determined AST activity, and dialyzed overnight against the 20mMsodium phosphate buffer, pH 7.5.

2-Ammoniumsulphate

The dialyzed solution from Step 1 was further purified by $(NH_4)_2SO_4$ precipitation. Optimum precipitation occurred between 64 and 72 % saturation[14].

3- Sephacryl chromatography

AST was applied to a Sephacryl S-300 column (95×1.6 cm) equilibrated with 50mM sodium phosphate buffer, pH 7.5 and developed at a flowrate of 60ml/h and 3ml fractions were collected.

Protein determination

Protein was determined either by measuring the absorbance at 280 nm byLawery method of usingbovine serum albumin as a standard[15].

All statistical analyses in studies were performed using SPSS version 15.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $P< 0.05$ = significant, $P> 0.05$ = non-significant.

RESULTS AND DISCUSSION

There is no significant different in age between diabetic nephropathy and normal subjects.FBG,urea, creatinine,ASTand ALT, uric acid were found to be significantly increased with p value ($p<0.001$), ($p<0.05$) and ($p<0.01$) respectively.total serum protein were found to be significantly decrease with P value < 0.05 in patients when compared to control group as shown in TABLE 1.

The increased level of serum AST in blood indicates liver failure. Diabetes mellitus also disturbs the liver function, due to which the levels of serum AST is increased in the blood[16,17]. In this study there was a significant rise in serum AST level in diabetic nephropathy patients,which could relate to excessive accumulation of amino acids (glutamate) in the serum of patients as result of amino acids mobilization from protein stores[18,19].These excessive amino acids are converted to ketone bodies for the AST is needed, leading to increased enzyme activity[20].

An increase in urea level is seen when there is damage of the kidney or the kidney is not functioning properly. increment of blood urea leval with the increment of blood glucose leval clearly indicates that the increase blood sugarlevel causes damage to the kidney[17].Serum creatinine and urea are established markers of Glomerular Filtration Rate (GFR). Though serum creatinine is a more sensitive index of kidney function compared to plasma urea level. This is because creatinine fulfills most of the requirements for a perfect filtration marker[13]. The purification of AST is summarized in TABLE 2. purification was restricted to AST A Sephacryl S-300 column (Figure 1) was used to obtain AST with the highest possible specific activity (30.6) units/mg protein in patients and 6 units/mg in control at 37°C (pH 7.5)). observed activity of AST increase in serum patients having diabetic nephropathy after process of purification because removed inhibitors which decrease AST activity.

One form of AST has been identified in *Trichomonas vaginalis*[21], *Chalmydomonas reinhardtii*[22] and *Leishmania maxicana*[23]. Some *Trypanosoma* spp. produce at least two isoenzymes[24] and in *Toxoplasma gondii*two isoenzyme were described[25]. Also, two peaks of AST were observed during DEAE-cellulose chromatography of protein extract from alkalophilic Bacillus[26].

These result had been agreement with other research (specific activity 217 units/mg) from sheep liver[27], and from *Hyalomma dromedarii* (specific activity 3.63 units/mg)[28], And from(specific activity 1533

TABLE 1 : The mean and standard deviation of Age, FBG. Urea, Creatinine, Uric acid, AST, ALT and Total protein in patients group and control group

Characteristic	Patients [mean±SD]	Control [mean±SD]	*P*Value
Age year	49.00±5.20	39.20±5.38	N.S
FBG[mg/dl]	255±64.12	90.60±7.20	<0.001
Creatinine[mg/dl]	1.73±0.38	0.78±0.25	<0.001
Uricacid [mg/dl]	6.09±1.58	4.91±1.05	<0.005
AST[U/L]	74.6±7.87	19.9±6.1	<0.01
ALT[U/L]	156±11.46	22.42±11.37	<0.01
Total protein [mg/dl]	5.9±0.9	7.1±0.63	<0.05

TABLE 2 : Partial purification of AST in serum of diabetic nephropathy patients and control group using ammonium sulphate and gel filtration chromatography

	Group	Volume (ml)	Activity (Unit)	Total activity (units)	Total Protein (mg./ml)	Specific Activity (units/mg.)	Fold purification	Recovery (%)
patients	Crude	10	74.6	746	58.6	1.27	1	100
	Ammonium sulphate (64-72g)/100 ml	5.6	20.5	120.95	68.0	0.30	0.23	16.21
	SephacrylS-300	5	15.3	76.5	0.5	30.6	24.09	10.25
control	Crude	10	18	180	7	2.57	1	100
	Ammonium sulphate (64-72g)/100 ml	5.6	16.7	85.17	6	2.78	1.08	47.31
	Sephacryl S-300	5	14	70	0.6	23.3	9.07	38.88

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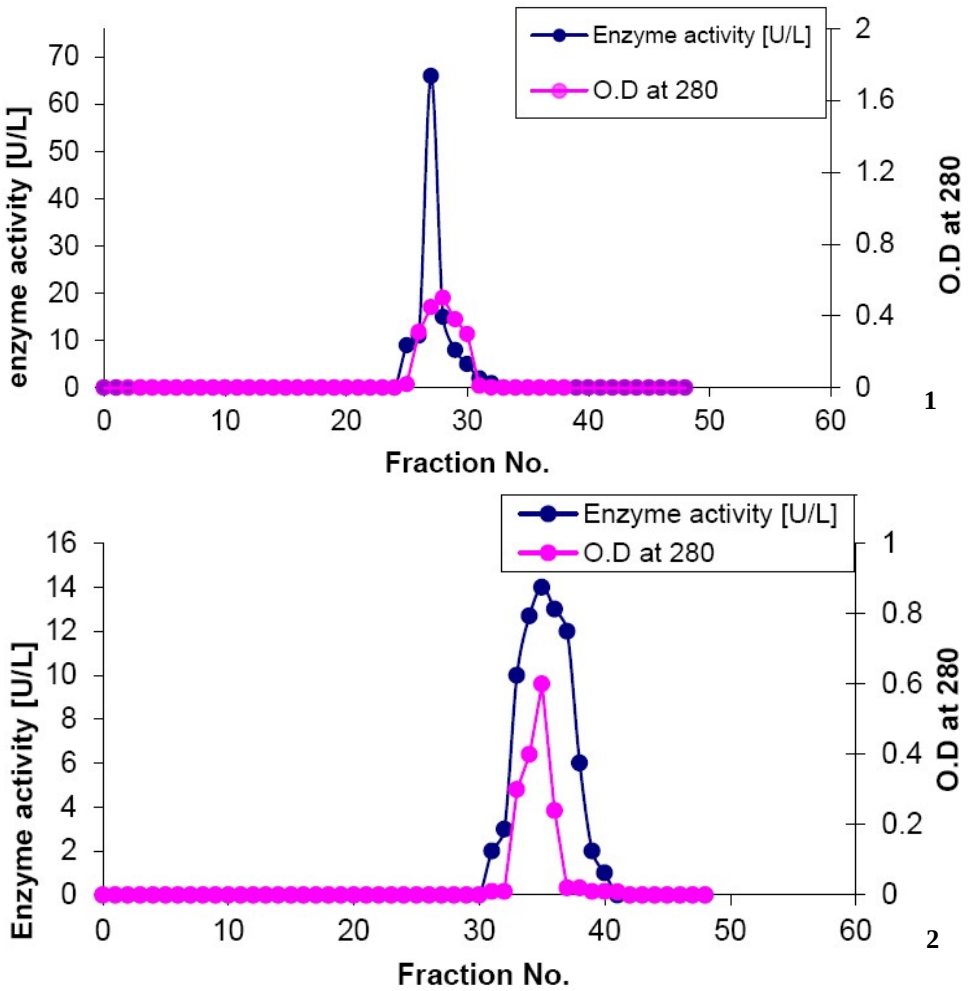

Figure 1 : A typical elution profile for the chromatography of AST[1] patientsand[2] control on Sephacryl S-300 column (95×1.6 cm) previously equilibrated with 50mM sodium phosphate buffer, pH 7.5 at a flow rate of 60ml/h and 3 ml fractions

units/mg) fromthe halophile archaebacteriu *Haloferax mediterranei*[29].

CONCLUSION

AST is could be considered as an indicator of liver damage or injury from different types of diseases. Therefore an elevation of its level was found in the sera of diabetic patients with nephropathy complications.the increased of enzyme activity after purification compare with activity enzyme without purified, my be refer to existence inhibitors which limited activity of AST. This study will help clinicians to used purified enzyme in kinetics study as inhibition.

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