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Comparison Study of (urinary & serum) AST Activity from Patients with type 2 diabetes

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Abstract

Aspartate aminotransferase was purified from urine and serum of patients with type 2 diabetes in a 2 steps procedure involving dialysis bag and sephadex G-25 gel filtration (column chromatography). The enzyme was purified 346.23 fold with 1467% yield and 3.46 fold with 142.85% yield in urine and serum of patients with type 2 diabetes respectively. The purified enzyme showed single peak. The results of this study revealed that AST activity of type 2 diabetes urine and serum increased significantly (p<0.001) compared with control group.

Key word: Aspartate aminotransferase, Purification, Diabetes.

الخلاصـــــــــة

تم تنقية الأنزيم الناقل لمجموعة الأمين من ادرار وامصال المرضى المصابين بداء السكري النوع الثاني من خلال خطوتين: الاولى بأستخدام الديلزة والثانية باستخدام الترشيح بالهلام Sephadex G-25 (كروموتوغرافيا العمود). نقي الأنزيم الناقل لمجموعة الأمين AST 236.23 مرة مع الناتج 1467% و3.46 مرة مع الناتج 142.85% من ادرار وأمصال المرضى المصابين بداء السكري النوع الثاني على التوالي. أعطى الأنزيم المنقى بالترشيح الهلامي قمة واحدة. تشير نتائج هذه الدراسة الى نشاط الأنزيم AST المنقى من ادرار وامصال المرضى المصابين بداء السكري النوع الثاني أعطى تغيرا معنويا ملحوظا(P<0.001) مقارنة بالأصحاء.

Introduction:

Aspartate aminotransferase is a pyridoxal-5-phosphate dependent enzyme that catalyses the reversible transfer of an amino group from aspartate to 2-oxoglutarate in order to form oxaloacetate and glutamate(1). Aspartate: 2- oxoglutarate aminotranferase (EC 2.6.1.1, aspartate aminotransferase, AST) is the best studied of aminotransferase(2). It has been purified from a wide range of bacteria(3), fungi(4) and invertebrate(5). AST is found in many body tissue including the heart, muscle, kidney, pancreas, brain and lung. It is also present in the liver(6,7).AST is present in rich concentration in kidney and urinary tract tissues; however, reports regarding its presence with little or no activity in normal human urine is very conflicting and it has been attributed to the likely presence of some kind of urinary

inhibitors(8- 10). Elevated activity of this enzyme in urine is observed in acute renal damage or infection(8) or in conditions where serum AST activity is considerably increased(10,11) and which have been found to be highly unstable and decays within 4 to 6 hours(9,10).
Diabetes mellitus is a chronic disorder of carbohydrate, lipid and protein metabolism characterized by persistent elevations of fasting blood glucose above 200 mg/dL due to insulin insufficiency or complete ceasation of insulin synthesis or secretion and/or insulin resistance. D.M is associated with incseased risk of heart disease, stork, kidney disease, retinopathy, neuropathy, ulceration and ganarene of extremities. Thus, diabetes and it is attendant complications have significant impact on health, quality of life as well as life expectancy of sufferes(12).
Type 2 diabetes is often considered a polygenic with multiple genes located on different chromosomes being associated with this condtion(13) .
The aim of our study is to compared (urine & serum) AST activity in patients with type 2 diabetes with that of partially purified enzyme.

Experimental

Chemicals

K_2HPO_4, KH_2PO_4, were obtained from Fluka- Switzerland company, Sephadex G-25 from Sigma chemicals company and AST Kit from bio labo (France).

Patients

Two groups of patients were included in this study , first group was involved 30 (male & female) urine sample age(40-80) and 35(male & female) serum sample age (40-75) from patients with type 2 diabetes respectively. A detailed history was taken concerning the illness, age, duration of disease whether taking anydrugs, and smoking. The patients were diagnosed by specialist doctors (diabetic) in National Diabetes Center.

AST assay

The AST activity was measured colorimetrically according to the method of (Reitman & Frankel, 1957), using kit supplied by (Bio labo/France) (14).

Purification of AST from urine & serum patients with type 2 diabetes

Step 1: Dialysis

Visking dialysis tube (3/4 diameter HMC Glouchester) were used for dialysis of 10 mL of fresh urine or serum against two liters of phosphate buffer pH (7.4) inside refrigerator. The volume of urine or serum after 18 hours of dialysis was measured and enzyme activity determined in this.

Step 2: Gel filtration

The dialyzed urine or serum from step 1 was applied directly to sephadex G-25 column (20x1.5 cm) and developed at a few rate of 50mL/h and 5mL fractions were out inside a refrigerator.

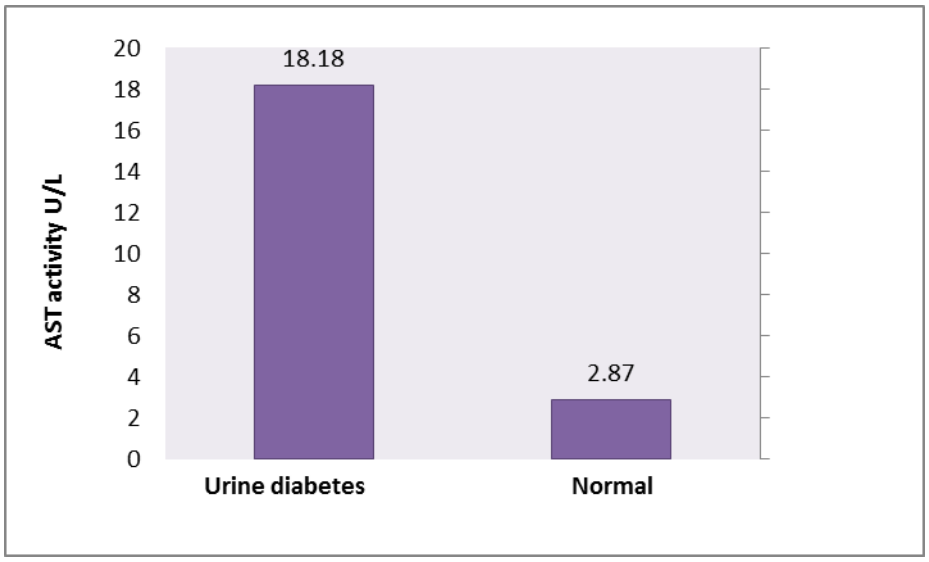
Protein determination

Protein was determined by the procedure of Lowry et al. (1951), with crystalline BSA as standard(15).

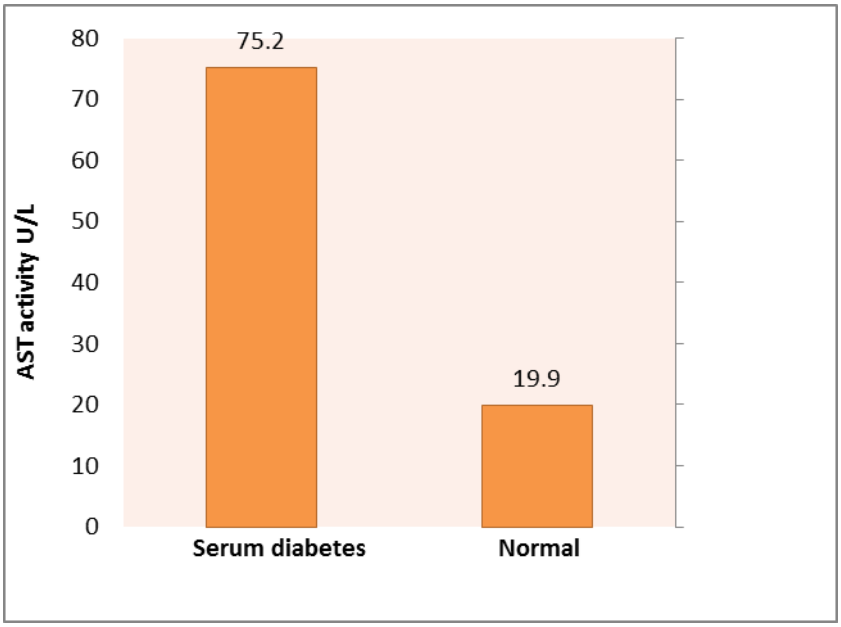
All statistical analyses in studies were performed using SPSS version 15.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated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

Results and Discussion

Fig.1 and fig. 2 showed that AST activity in (urine & serum) of patients with type 2 diabetes is higher than that of normal and they also exhibited significantly increased in p value ($p<0.001$).



Fig(1): Illustrate values of AST activity in urine of normal and patients (male& female)with type 2 diabetes.



Fig(2): Illustrate values of AST activity in serum of normal and patients (male& female)with type 2 diabetes.

The mean levels of urine AST activity (18.18±9.18)U/L and serum AST activity (75.2±11.7)U/L of the patients with type 2 diabetes revealed distinct increase ($p<0.001$) table (1),(2) with no significant difference between (male &female) patients with type 2 diabetes.In agree with our results, Debasis et al, (2009) observed increasing serum AST activity in diabetes(16). While Andallu noticed that the activity of AST was enormously elevated ($p<0.01$) by (243%) in uncontrolled diabetes from that of normals(17).

In this experiment there was an apparent rise in serum AST levels in diabetic patients, which could relate to excessive accumulation of amino acid (glutamate) in the serum of diabetic patients as a result of amino acid mobilization from protein stores (18). These excessive amino acid are then converted to ketone bodies (α-keto- glutaric) for which the enzyme AST are needed, leading to increase in enzyme activity(19).

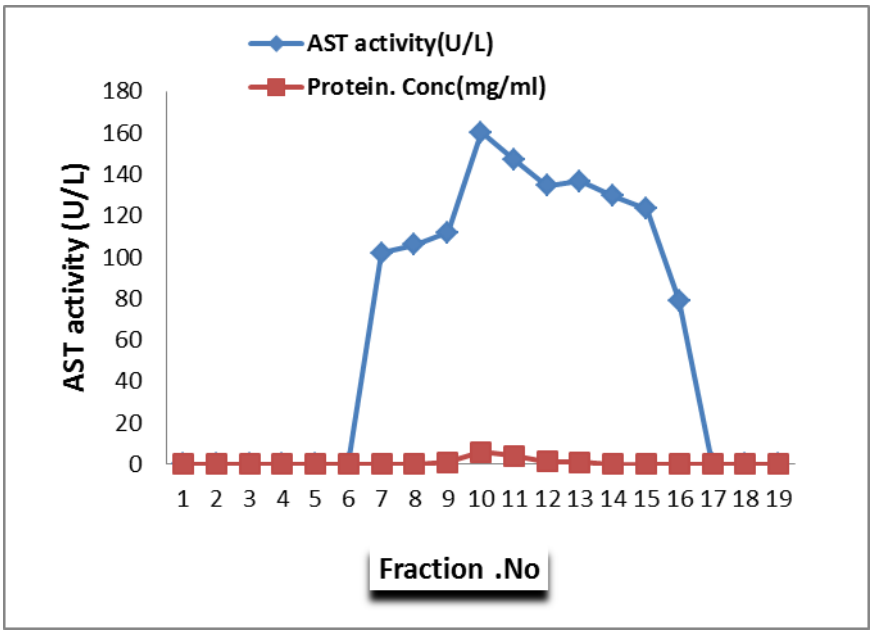
Table (1): Illustrate values of AST activity in urine of normal & patients with type 2 diabetes.

Normal				**Type 2 diabetes**			
Specimen	No. of cases	Age (years)	AST activity (U/L) mean ± S.D	No. of cases	Age (years)	AST activity (U/L) mean ± S.D	P <
Male	14	40- 75	3.07±1.93	15	40-80	17.78±12.90	0.05
Female	16	40- 80	2.67±3.40	15	40-70	18.58±6.07	0.05
Total	30	40- 80	2.87±2.69	30	40-80	18.18±9.81	0.05

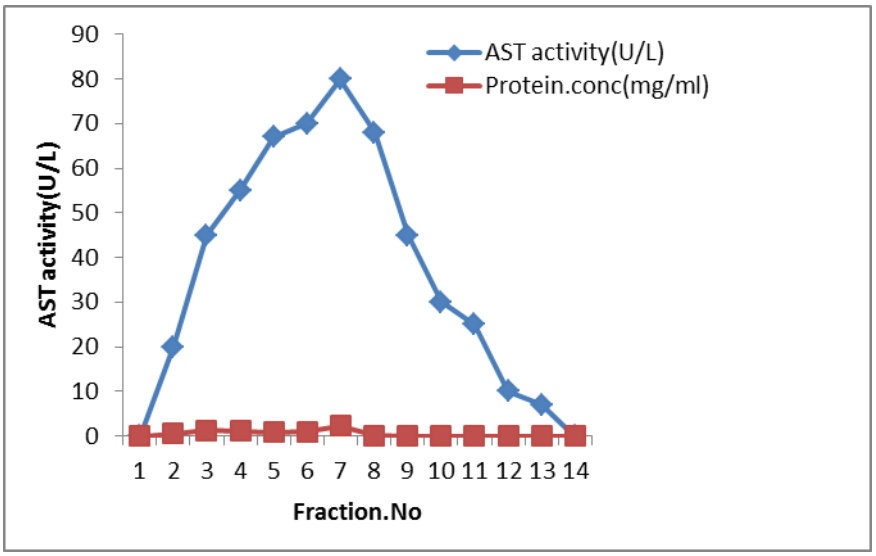
Table(2): Illustrate values of AST activity in serum of normal & patients with type 2 diabetes.

Normal				Type 2 diabetes			
Specimen	No. of cases	Age (years)	AST activity (U/L) mean ± S.D	No. of cases	Age (years)	AST activity (U/L) mean ± S.D	P <
Male	20	40- 70	19.4±6.1	20	40-75	80.1±10.8	0.001
Female	20	40- 65	20±6.2	15	40-70	71.5±8.7	0.001
Total	40	40- 70	19.9±6.1	35	40-75	75.2±11.7	0.001

AST in urine and serum of patients with type 2 diabetes was purified with dialysis followed by sephadex G-25 gel filtration, and this enzyme showed a singl peak fig (3),(4). The purification procedures of the AST are summarized in table(3),(4). The results showed that the enzyme was purified 0.59-fold with a specific activity of 0.046 U/mg protein of urine, and 2.11-fold with a specific activity of 610.16 U/mg of serum. The enzyme was then purified with sephadex G-25 and showed 346.23-fold enzyme purification with a specific activity of 26.66 U/mg protien of urine and 3.46-fold, 1000U/mg protien of serum.



Fig(3): Aspartate aminotransferase isolated from urine of patients with type 2 diabetes by gel filtration.



Fig(4): Aspartate aminotransferase isolated from serum of patients with type 2 diabetes by gel filtration.

These results indicated the effectiveness of purification method applied in this research confirmed by the high results of purification by sephadex G-25 1467% of urine and 1142% of serum respectively table (3,4). Inspite of the low yield of purification by dialysis 52.29% of urine and 64.28% of serum respectively which it might be caused by the autolysis of the enzyme leading to loss in enzyme activity during dialysis for such along duration. Low results could also caused by the presrnce of unidentified high molecular weight inhibitors or it possibly related to the dialysis of its coenzyme(20).

Table (3): Purifiction of Aspartate aminotransferase from urine of patients with type 2 diabetes.

Purifiction step	**Volume (ml)**	**Protein (mg/ml)**	**Enzyme activity (U/ml)**	**Total enzyme activity (U)***	**Specific activity (U/mg)**	**Purifiction (fold)**	**Yield (%)**
Crude(urine)	10	141	10.9	109	0.07	1	100
Dialysis	10	122	5.7	57	0.046	0.59	52.29
Sephadex G- 25	5	6.0	160	800	26.66	346.23	1467

*One unit of AST activity was defined as the amount of enzyme producing 1µmol oxaloacetate (pyruvate) per h under standard assay condition.

Table (4): Purifiction of Aspartate aminotransferase from serum of patients with type 2 diabetes.

Purifiction step	Volume (ml)	Protein (mg/ml)	Enzyme activity (U/ml)	Total enzyme activity (U)	Specific activity (U/mg)	Purifiction (fold)	Yield (%)
Crude(serum)	10	0.194	56	560	288.6	1	100
Dialysis	10	0.118	36	360	610.16	2.11	64.28
Sephadex G- 25	5	0.08	80	6400	1000	3.46	1142.8

Previous workers have either used whole or dialysed urine in their investigations and were not aware of the presence of certain substances acting as enzyme inhibitors in these fraction(9), otherwise high molecular weight urinary inhibitors of AST have been clearly demonstrated which might have hampered the enzyme assay (21-23).

Although, elevated AST activity in the urine have been reported in acute renal demage or infections (8) or in conditions where AST activity of serum is considerably increased(10,11) but has been found to be highly unstable and decays within 4 to 6 hours. It has been suggested that little or no AST activity in the whole urine may be due to the presence of some kind of unknown enzyme inhibitors in the urine (9). Presently, we have shown the AST activity in the urine when assayed immediately after urine collection, decayed considerably within 5 to 10 hours in the patients urine irreversibly (24).

In diabetes, the causes and site of intervention in biochemical process are diverse (Larner, 1985)(25) and high serum total triglyceride level, high level of AST and urea have been implicated(4).

Observation from this study correlate with the reports from previous studies, in that, aspartate aminotransferase (AST) is released in to the serum especially when there is damage to the hepatic membrane as a result of chemical assault. Serum levels of this enzyme therefore are significant diagnostic tools in assessing the level of hepatic damage(16).

Since liver dysfunction is frequently associated with D.M, many clinical reports have indicated that serum enzyme activity derived from the liver such as AST are elevated (26). The levels of enzyme increased in D.M is ametabolic result already treatable with pancreas hormons. Befor the availability of sensitive pancreas hormone analysis, increased serum enzyme levels were considered important evidence supporting the diagnosis of D.M(27).

In the present study, the urinary AST activity increased significant compared with healthy subjucts because the raise in ketoacidosis (27), inconsideration of the fact that diabetes is the most common cause of kidney failure, accouting for nearly 44 percent of new cases(28).

Conclusion

Diabetes mellitus the major cause of renal morbidity and mortality therefore type 2 diabetes cause high levels of AST in urine and serum patients, the enzyme levels in serum higher than

that in urine, and there is a strong correlation between AST activity and ketoacidosis. This high molecular weight enzyme originates from the tubules not from glomerular filtration.

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Abbreviation:

AST: Aspartate aminotransferase
D.M: Diabetes Mellitus
BSA: Bovine Serum Albumin.